ZINC COMPLEXES OF CARBONIC ANHYDRASE INHIBITORS. CRYSTAL STRUCTURE OF [Zn(5-AMINO-1,3,4-THIADIAZOLE-2-SULFONAMIDATE)$_2$(NH_3)].H_2O. CARBONIC ANHYDRASE INHIBITORY ACTIVITY

P. Borja[1], G. Alzuet[1], J. Casanova[1], J. Server-Carrió[1], J. Borrás*[1], M. Martinez-Ripoll[2] and C.T. Supuran[3]

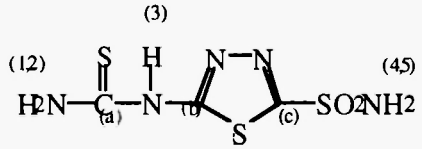
[1]University of Valencia, Faculty of Pharmacy, Department of Inorganic Chemistry, Vicent Andrés Estelles s/n, E-46100, Burjassot (Valencia), Spain
[2]CSIC, Institute of Physics Chemistry "Rocasolano", Department of Crystallography, Serrano 119, E-28006, Madrid, Spain
[3]University of Florence, Laboratory of Inorganic and Bioinorganic Chemistry, Via Gino Capponi 7, I-50121, Firenze, Italy

Abstract: Zn(II) complexes of acetazolamide derivatives, inhibitors of Carbonic Anhydrase enzyme, are synthesised and characterised. The crystal structure of [Zn(5-amino-1,3,4-thiadiazole-2-sulfonamidate)$_2$(NH_3)].H_2O complex, obtained by hydrolysis of 5-thioureido-1,3,4-thiadiazole-2-sulfonamide, was solved by X-ray diffraction method. The complex crystallizes in the triclinic P1 space group with a = 7.662(1), b = 7.830(1), c = 7.964(1) Å, α = 88.54(1), β = 61.46 (1), γ = 65.68(1)°, and Z = 1. The structure was refined to R = 0.048 (R_w = 0.053). The structure is formed by infinite chains ligand-Zn-ligand. The Zn(II) ion is tetrahedrally coordinated to four nitrogen atoms from the sulfonamidate ligands and from the ammonia molecule. It is noteworthy that one of the sulfonamidate ligands acts as a bridging ligand through the Nthiadiazole and Nsulfonamidate atoms while the other binds the Zn(II) in a monodentate form through the Nsulfonamidate atom. The other Zn(II) complexes are characterized by IR and ^{1}H and ^{13}CNMR spectra. EHMO calculations applied to fractional coordinates of the [Zn(5-amino-1,3,4-thiadiazole-2-sulfonamidate)$_2$(NH_3)].H_2O complex indicate that the atomic orbitals of the Zn(II) ion are not involved in the HOMO and LUMO of the complex. The Carbonic Anhydrase inhibitory activity of the ligands and their Zn(II) complexes has been tested and has shown that the complexes reported here behave as very strong CAI and CAII inhibitors.

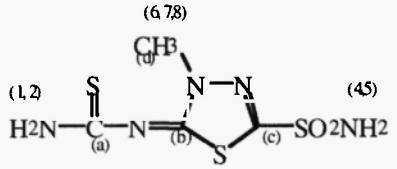
Introduction

Carbonic Anhydrases (CA) constitute a family of zinc enzymes (nine isozymes are presently known) containing one metal ion bound to a single polypeptide chain of 260 amino acids.[1] The conical active site of HCAII, the best studied enzyme, is about 15-Å deep, and it is roughly divisible into a hydrophobic and hydrophilic cleft. The catalytically required zinc ion resides at the bottom of this cleft, and it is here where the hydration of the carbon dioxide into bicarbonate ion and a proton is catalysed at a rate close to that of diffusion control. Although this ubiquitous enzyme and its isozymes are present in a variety of tissues, CA plays an important role in the eye, since electrolyte secretion processes in the aqueous humor in which CA intervenes are linked to elevated intraocular pressure and glaucoma.[2] Importantly, it has been demonstrated that the inhibition of CA by sulfonamides such as acetazolamide is effective in glaucoma therapy, and this has highligted the pharmaceutical importance of CA as a target for rational drug design.[3] Given the pharmaceutical interest in CA inhibitors, it is desirable to explore new compounds which may ultimately lead to differing specificities with regard to carbonic anhydrase isozymes and tissue localizations. Novel types of CA inhibitors are constantly designed and extensively studied in order to elucidate the mechanism of CA catalysis and inhibition. Inhibitors of the sulfonamide type are used therapeutically since 1950 in the management of diverse disorders.[4]

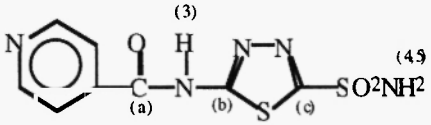
In a previous work, we have reported numerous metal complexes of acetazolamide and its derivatives with high inhibitory CA activity.[5] As a continuation of this research we have synthesised acetazolamide derivatives such the 5-amino(thioxo)methylamino-1,3,4-thiadiazole-2-sulfonamide (H_2uacm), its N-methyl thiadiazole derivative, [2-amino(tioxho)methylimino-3-methyl-2,3-dihydro -1,3,4-thiadiazole-5-sulfonamide] (Hmuacm) and the pyridyl derivative of acetazolamide [2-(3-pyridylcarboxamido)-5-sulfamoyl-1,3,4-thiadiazole] (H_2pyacm) (Figure 1)



H_2uacm



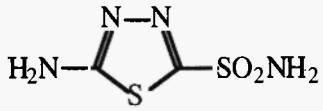
Hmuacm



H_2pyacm

Figure 1. 5-amino(thioxo)methylamino-1,3,4-thiadiazole-2-sulfonamide (H_2uacm), [2-amino(thioxo)methylimino-3-methyl-2,3-dihydro -1,3,4-thiadiazole-5-sulfonamide] (Hmuacm) and [2-(3-pyridylcarboxamido)-5-sulfamoyl-1,3,4-thiadiazole] (H_2pyacm).

In this paper we report Zn(II) complexes of these sulfonamide derivatives and we test their inhibitory CA ability against the isozymes CAI and CAII. Furthermore, we report the crystal structure of a Zn(II) complex of the hydrolytic product of the H_2uacm, the 5-amino-1,3,4-thiadiazole-2-sulfonamide (Hats) (Figure 2), ligand previously reported and tested as CA inhibitor by Supuran.[6]



Hats

Figure 2. 5-amino-1,3,4-thiadiazole-2-sulfonamide (Hats).

Materials and Methods

All the reagents were of analytical grade. Analytical data (C, H, N) were determined in a Perkin Elmer model 2400 spectrometer; S was determined in a Carlo Erba MOG 1106. The zinc content was determined by atomic absorption spectroscopy. IR spectra were obtained in KBr pellets with a Perkin Elmer 843 spectrometer, in the range 400 - 4000 cm^{-1}. ^{1}HNMR and ^{13}CNMR spectra were registered in a Varian 400 MHz instrument;^{13}CNMR spectrum was recorded on a Varian Unity 300 CP/MAS. Sulfonamides were synthesised as described in the literature,[7,8,9] starting from 5-amino-1,3,4-thiadiazole-2-sulfonamide (obtained by acid hydrolysis from acetazolamide) or 5-imino-δ^2-4-

methyl-2-sulfonamido-1,3,4-thiadiazoline (obtained from methazolamide). Acetazolamide and methazolamide were purchased from Sigma.

Synthesis of $Zn_3(uacm)_2(NH_3)_2Cl_2$ **(1)** $Zn_3(uacm)_2(CH_3CH_2NH_2)_2Cl_2$ **(2)** *and* $Zn(muacm)(NH_3)(H_2O)(Cl)$ **(3)**.- A solution containing 1 mmol of $ZnCl_2.2H_2O$ in 20 ml of methanol was added to 20 ml of a methanolic solution of the ligand (0.5 mmols) (H_2uacm for the complexes **1** and **2** and Hmuacm for complex **3**). To the resulting mixture addition of concentrated ammonia, (30%), 0.5 ml. for complex **1** and 2 ml. for complex **3** or, 2 ml of concentrated ethylamine (70 %) for complex **2** gave inmediately a white solid that was filtered, washed with methanol and dried to constant weight. *Anal.* Found for $C_6H_{12}N_{12}O_4S_6Cl_2Zn_3$ (**1**): C 10.2; H 1.7; N 21.1; S 24.3; Zn 24.9%. Calc.: C 9.3; H 1.6; N 21.7; S 24.8; Zn 25.2%. Found for $C_{10}H_{20}N_{12}O_4S_6Cl_2Zn_3$ (**2**): C 15.7; H 2.9; N 19.6; Zn 23.3%. Calc.: C 14.4; H 2.4; N 20.2; Zn 23.4%. Found for $C_4H_8N_6O_3S_3ClZn$ (**3**): C 12.7; H 2.2; N 20.9; Zn 17.1%. Calc.: C 12.5; H 2.1; N 21.8; Zn 17.0%.
Synthesis of $[Zn(pyacm)(CH_3OH)_2]$ **(4)**.-The synthesis procedure is similar to that previously described for the complex **3** using 0.5 mmols. of Hpyacm instead of Hmuacm. *Anal.* Found for $C_{10}H_{13}N_5O_5S_2Zn$ (**4**): C 29.8; H 2.9; N 17.1; Zn 15.9%. Calc.: C 29.1; H 3.1; N 16.9; Zn 15.8%.

Crystallographic Data.- Intensity data for a prismatic colourless well-formed crystal with dimensions 0.20 x 0.23 x 0.30 mm were measured on a four circle diffractometer SEIFERT XRD3000S, using CuKα radiation with a graphite oriented monochromator. Unit cell dimensions were obtained from the least squares fit of 62 reflections ($\theta < 35°$). The space group was determined from the structure solution. Intensity measurements by $\omega/2\theta$ scans, width $(1.5 + 0.3\tan\theta)°$, detector apertures 2x2° and θ limits ($1°<\theta<65°$) were performed in the hkl range (-7 7, -8 8, 0 8) plus Friedel. Two standard reflections were checked every 100 reflections without variation. Absorption correction was applied using Ψ-scan (transmission factors between 1.00-0.71). The structure was solved by Direct Methods using the program SIR92.[10] All non-hydrogen atoms were anisotropically refined by least-squares on Fobs using the XRAY76 System.[11] The hydrogen atoms, kept fixed in the refinement, were located by difference synthesis, except one of the hydrogen of the water molecule. Crystal data are shown in Table I.

The structural chirality was determined by Bijvoet methods[12] and η-refinements for the Zn dispersors.[13] Considering reflections with $Fo>3\sigma(Fo)$ there are N=132 Friedel pairs with $\Delta F>0.70$. The discrepancy indices are $R1 = \Sigma|[Fo(+h)-Fo(-h)] - [Fc(+h)-Fc(-h)]/N = 0.333$ (2.114 for the wrong enantiomer) ; $R2 = 1 + \Sigma|| Fo(+h)/Fo(-h)| / | Fc(+h)/Fc(-h) | -1 | / N = 1.020$ (1.173) and $R3 = \Sigma | [Fo(+h)^2-Fo(-h)^2] - [Fc(+h)^2-Fc(-h)^2] | /\Sigma | Fo(+h)^2-Fo(-h)^2 | = 0.327$ (1.848). The η-refinements were done with the (+x,+y,+z) model starting at $\Delta f'' = -0.678$, 0.00 and +0.678. The three refinements converged to a η value of +0.97(4).

EHMO calculations.- All calculations were performed by using the Package of Programs for Molecular Orbital Analysis by Mealli and Proserpio,[14] based on CDNT (atom Cartesian coordinate calculations), ICON (extended Hückel method with the weighted *Hij* formula) and FMO (fragment molecular orbital), including in the drawing program CACAO (computer aided composition of atomic orbitals). The extended Hückel parameters are as follows: *Hii*: Zn 4s, -12.41 eV; Zn 4p, -6.53 eV; N 2s, -26.00 eV; N 2p, -13.40 eV; S 3s, -20.00 eV; S 3p, -13.30; O 2s, -32.30 eV; O 2p, -14.80 eV ; C 2s, -21.40 eV; C 2p, -11.40 eV; H 1s, -13.60 eV. Orbital exponents: Zn 4s, 2.01; Zn 4p, 1.70; N 2s, 2p, 1.950; S 3s, 3p, 1.817; O 2s, 2p, 2.275; C 2s, 2p, 1.624; H 1s, 1.300.

Inhibitory test.- Human CAI and CAII cDNAs were expressed in Ion^- Escherichia coli strain SG20043 from the plasmids described by Forsman et al.[15] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by Lindskog's group[16] and enzymes were purified by affinity chromatography according to the method of Khalifah et al.[17] Enzyme concentrations were determined spectrophotometrically at 280 nm, utilizing a molar absorptivity of 49 $mM^{-1}.cm^{-1}$ for CAI and 54 $mM^{-1}.cm^{-1}$ for CAII, respectively, based on M_r = 28.85 kDa for CAI, and 29.3 kDa for CAII, respectively.[18,19]

Electronic spectra were recorded with a Cary 3 spectrophotometer interfaced with an IBM PC. Initial rates of 4-nitrophenyl acetate hydrolysis were monitored spectrophotometrically, at 400 nm and 25°C. Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied between 10^{-2} and 10^{-4} M. A molar absorption coefficient $\varepsilon = 18400\ M^{-1}.cm^{-1}$ was used for the 4-nitrophenolate formed by hydrolysis, in the conditions of the experiments (pH 7.80), as reported by Pocker and Stone.[20] Non-enzymatic hydrolysis rates were always subtracted from the observed rates. Stock solutions of inhibitors (10 mM) were prepared in DMSO and dilutions up to 0.1 nM were done with distilled deionized water. Duplicate experiments were done for each inhibitor, and the values reported throughout the paper are the averages of such results. IC_{50} values represent the molarity of inhibitor producing a 50% decrease of enzyme specific activity for the investigated reaction.

Table I. Experimental Data and Structure Refinement Parameters and Procedures

Formula	$ZnC_4H_{11}N_9O_5S_4$
Crystal System, Space Group	Triclinic P1
a, b, c (Å)	7.662 (1), 7.830(1), 7.964(1)
α, β, γ (°)	88.54(1), 61.46(1), 65.68(1)
V(Å^3), Z	373.6(1), 1
Dc(g·cm^{-3}), M, F(000)	2.039, 458.81, 232
μ(cm^{-1})	78.9
Number of reflections :	
Independent	2326
Observed	2309 (3σ(I) criterion)
Value of Rint	0.005
Number of variables	205
Max. final shift/error	0.004 [z of O(1)]
w-scheme	Empirical as to give no trends in $<w\Delta^2F>$ vs $<Fo>$ or $<\sin\theta/\lambda>$
Max. thermal value	0.086 [U_{22} of O(1)]
Final ΔF peak	0.6 e·Å^{-3}
Extinction coefficient	0.0019(2)[a]
S, unit weight standard deviation	1.05
Final R and Rw	0.048, 0.053
Computer and programs	VAX 6410, PESOS[b], CSU[c]
Scattering factors and anomalous dispersion	Int. Tables for X-Ray Crystallography[d]

[a] A. C. Larson, *Acta Cryst.*, **23**, 664,(1967); [b] M. Martínez-Ripoll and F. H. Cano, PESOS, a computer program for the automatic treatment of weighting schemes, Instituto Rocasolano, C.S.I.C., Madrid, Spain, 1975; [c] I. Vickovic, CSU, Crystal Structure Utility, Faculty of Science, Univesity of Zagreb, Yugoslavia, 1988; [d] International Tables for X-ray Crystallography, Vol.4, Kynoch Press, Birmingham, England, 1974.

Results and Discussion

A first aspect to be underlined is the dideprotonated nature of the H_2uacm and H_2pyacm ligands. This behaviour contrasts to that found for acetazolamide which, in the same conditions, binds the Zn(II) as a monodeprotonated ligand. As for acetazolamide,[21] at the pH of working conditions it must be expected the presence of several species in solution due to the two acid protons of the ligands. The Hmuacm only presents one acid group and as consequence behaves as monodeprotonated form in a similar way of the methazolamide.[22]

When the filtrate obtained in the synthesis of the $Zn_3(uacm)_2(NH_3)_2Cl_2$ was allowed to stand for two to three weeks, few single crystals suitable for X-ray diffraction measurements were formed. The X-ray analysis indicates that the crystals have the following composition: [Zn(5-amino-1,3,4-

thiadiazole-2-sulfonamidate)$_2$(NH_3)].H_2O. The 5-amino-1,3,4-thiadiazole-2-sulfonamido ligand is obtained as consequence of the hydrolysis reaction of the H_2uacm, according to the following scheme (Figure 3):

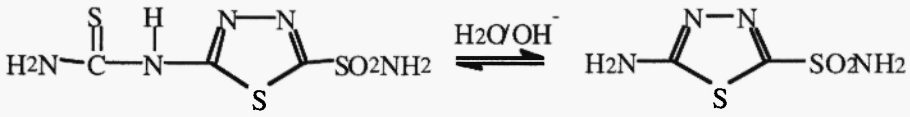


Figure 3. Hydrolysis reaction of H_2uacm.

Crystal Structure of [Zn(5-amino-1,3,4-thiadiazole-2-sulfonamidate)$_2$(NH_3)].H_2O.

The final X-Ray model showing its absolute chirality and the atomic labeling is shown in Figure 4. Atomic parameters for non-H atoms are listed in Table II. Bonds lengths and angles are given in Table III.

Table II. Atomic Coordinates and Thermal Parameters for Non H-Atoms Thermal Parameters as U_{eq}=1/3:Σ[U_{ij} :a*:a*:a_i :a_j:cos(a_i ,a_j)] 10^3 for the [Zn(5-amino-1,3,4-thiadiazole-2-sulfonamidate)$_2$(NH_3)].H_2O complex

Atom	x	y	z	U_{eq}
Zn	-0.1510 (-)	0.4700 (-)	0.7650 (-)	22 (1)
S(11)	0.6589 (2)	0.3676 (2)	0.3331 (2)	29 (1)
S(12)	0.1715 (2)	0.6689 (1)	0.5716 (1)	20 (1)
S(21)	0.6909 (2)	0.0794 (2)	0.5502 (2)	34 (1)
S(22)	0.1938 (2)	0.1605 (1)	0.8339 (1)	23 (1)
O(1)	0.4166 (7)	0.3447 (7)	1.1052 (6)	62 (1)
O(11)	0.2198 (5)	0.7578 (4)	0.4057 (5)	31 (1)
O(12)	0.0306 (5)	0.7931 (4)	0.7627 (4)	30 (1)
O(21)	0.2034 (6)	0.3299 (5)	0.8839 (5)	36 (1)
O(22)	0.2310 (5)	0.0174 (4)	0.9474 (4)	35 (1)
N(1)	-0.4018 (6)	0.6905 (5)	0.9856 (5)	32 (1)
N(11)	0.7063 (5)	0.4309 (5)	0.6179 (5)	23 (1)
N(12)	0.4857 (6)	0.5556 (5)	0.6785 (5)	24 (1)
N(13)	1.0298 (6)	0.1876 (5)	0.3554 (5)	32 (1)
N(14)	0.0884 (5)	0.5233 (5)	0.5585 (5)	24 (1)
N(21)	0.6094 (6)	-0.0509 (6)	0.2697 (5)	34 (1)
N(22)	0.4054 (6)	0.0474 (5)	0.4405 (5)	29 (1)
N(23)	0.9897 (6)	-0.2334 (7)	0.1664 (6)	44 (1)
N(24)	-0.0218 (5)	0.2184 (4)	0.8291 (5)	25 (1)
C(11)	0.8185 (6)	0.3215 (6)	0.4428 (6)	23 (1)
C(12)	0.4389 (6)	0.5362 (6)	0.5474 (6)	22 (1)
C(21)	0.7751 (7)	-0.1270 (6)	0.3029 (6)	30 (1)
C(22)	0.4243 (6)	0.0436 (6)	0.5937 (6)	22 (1)

The structure consists of infinite chains ligand-Zn-ligand along the **a** direction that are interconnected by a hydrogen bonding system involving the nitrogen atoms, the water molecule and the sulfonamido oxygen atoms as shown in figure 5. These hydrogen bonds together with other hydrogen contacts, listed in table IV, mainly determine the crystal packing.

The coordination about the zinc ions is tetrahedral being the metal ion surrounded by four nitrogen atoms, three from the sulfonamide ligands and the other one from the ammonia molecule.

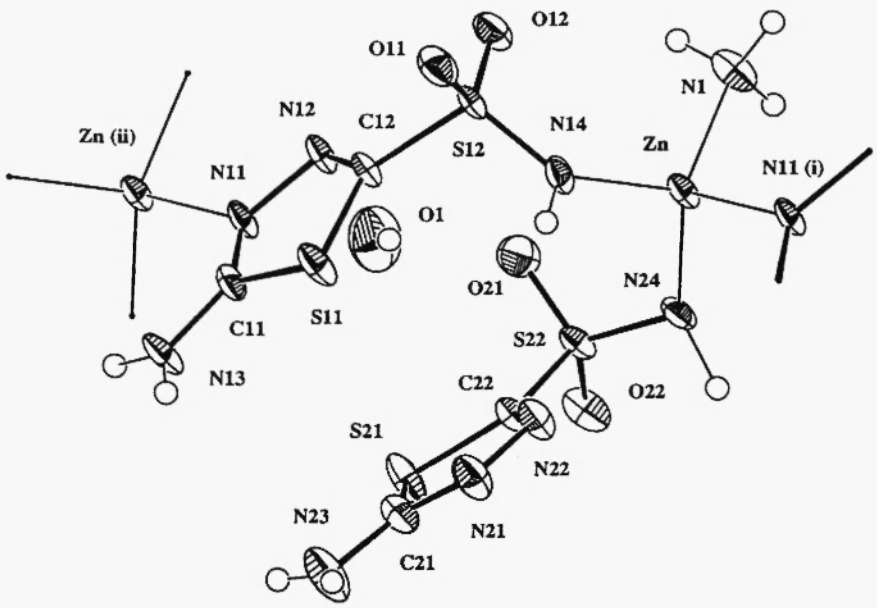


Figure 4. ORTEP drawing of the asymmetric unit showing the absolute configuration, the connectivities between two different units through the Zn atoms and the labelling scheme. Symmetry operators are listed in Table IV.

Two of the N atoms of the ligands linked to the zinc ion are derived from the sulfonamidate groups while the other one is from the thiadiazole ring. The Zn-Nsulfonamido and Zn-Nammonia bond distances are the same (1.989(4) Å) while the Zn-Nthiadiazole is slightly larger (2.045(4) Å).These distances compare well with those reported for similar bonds in the $ZnL_2(NH_3)_2$ (L=acetazolamidato and methazolamidato) complexes[22,23] and in related sulfonamide complexes: the [Zn(8-quinolinsulfonamidato)$_2$(NH_3)].NH_3[24] and the tosylamido Zn(II) compound with the tridentate ligand, 1,5,9-triazacyclododecane, reported by Kimura.[25] The bond angles of the coordination polyhedron range from 101.2(2)° to 117.9(1)° indicating a distorted tetrahedron. This distortion is higher than that observed for the CA-acetazolamide complex (104° and 113°)[26] and for the $ZnL_2(NH_3)_2$ (L= acetazolamidato and methazolamidato) complexes (108.3° and 115.5°, and 105.3° and 113.1°, respectively)[22,23] and lower than that found for the above cited Kimura's compound (99.5° and 123.2°).[25]

Table III. Bond Lengths (Å) and Angles (°) for the [Zn(5-amino-1,3,4-thiadiazole-2-sulfonamidate)$_2$(NH_3)].H_2O complex

Bond Lengths			
Zn–N(1)	1.989 (3)	S(22)–O(21)	1.435 (5)
Zn–N(14)	1.989 (4)	S(22)–O(22)	1.444 (3)
Zn–N(24)	1.987 (3)	S(22)–N(24)	1.542 (5)
Zn–N(11) (i)	2.045 (4)	S(22)–C(22)	1.774 (3)
S(11)–C(11)	1.741 (6)	N(11)–N(12)	1.389 (5)
S(11)–C(12)	1.741 (3)	N(11)–C(11)	1.314 (5)
S(12)–O(11)	1.437 (4)	N(12)–C(12)	1.286 (8)
S(12)–O(12)	1.438 (3)	N(13)–C(11)	1.329 (5)
S(12)–N(14)	1.544 (5)	N(21)–N(22)	1.384 (4)
S(12)–C(12)	1.787 (5)	N(21)–C(21)	1.314 (7)
S(21)–C(21)	1.749 (5)	N(22)–C(22)	1.294 (7)
S(21)–C(22)	1.715 (5)	N(23)–C(21)	1.338 (5)

Bond Angles			
N(1)–Zn–N(14)	115.8 (2)	N(12)–N(11)–C(11)	114.0 (4)
N(1)–Zn–N(24)	117.9 (1)	N(12)–N(11)–Zn (ii)	119.9 (3)
N(1)–Zn–N(11) (i)	103.2 (2)	C(11)–N(11)–Zn (ii)	124.4 (3)
N(14)–Zn–N(24)	110.9 (1)	N(11)–N(12)–C(12)	111.3 (4)
N(14)–Zn–N(11) (i)	101.2 (2)	Zn–N(14)–S(12)	130.3 (2)
N(24)–Zn–N(11) (i)	105.4 (2)	N(22)–N(21)–C(21)	112.1 (4)
C(11)–S(11)–C(12)	86.7 (2)	N(21)–N(22)–C(22)	112.4 (4)
O(11)–S(12)–O(12)	117.4 (2)	Zn–N(24)–S(22)	118.0 (2)
O(11)–S(12)–N(14)	111.9 (2)	S(11)–C(11)–N(11)	112.6 (4)
O(11)–S(12)–C(12)	103.0 (2)	S(11)–C(11)–N(13)	122.2 (4)
O(12)–S(12)–N(14)	110.7 (2)	N(11)–C(11)–N(13)	125.2 (4)
O(12)–S(12)–C(12)	105.6 (2)	S(11)–C(12)–S(12)	119.8 (3)
N(14)–S(12)–C(12)	107.4 (2)	S(11)–C(12)–N(12)	115.4 (4)
C(21)–S(21)–C(22)	86.4 (2)	S(12)–C(12)–N(12)	124.8 (4)
O(21)–S(22)–O(22)	115.1 (2)	S(21)–C(21)–N(21)	113.6 (4)
O(21)–S(22)–N(24)	109.4 (2)	S(21)–C(21)–N(23)	121.1 (4)
O(21)–S(22)–C(22)	106.3 (2)	N(21)–C(21)–N(23)	125.3 (5)
O(22)–S(22)–N(24)	114.0 (2)	S(21)–C(22)–S(22)	121.2 (3)
O(22)–S(22)–C(22)	103.2 (2)	S(21)–C(22)–N(22)	115.5 (4)
N(24)–S(22)–C(22) .	108.1 (2)	S(22)–C(22)–N(22)	123.2 (4)

(i) +x-1,+y,+z

The sulfonamide displays two coordination behaviours, on one hand, it acts as monodentate ligand through the N-sulfonamido atom and on the other hand it behaves as a bridging ligand linking the metal ions through the N-sulfonamido and the N-thiadiazole atoms. It is noteworthy that the N-thiadiazole atom binds the metal. Until now in the crystal structures of the metal acetazolamide complexes previously reported[27,28] this atom acts as donor one only when the contiguous acetamido group linked to the thiadiazole is deprotonated.

Table IV. Hydrogen Contacts (Å, °) for the [Zn(5-amino-1,3,4-thiadiazole-2-sulfonamidate)$_2$(NH_3)].H_2O.

X–H...Y	X–H	X...Y	H...Y	<X–H...Y
O(1)–H(01)...O(21)	0.44	2.95 (1)	2.54	161
N(1)–H(0103)...O(1) (i)	0.85	3.45 (1)	2.67	152
N(13)–H(1301)...N(22) (ii)	0.96	3.03 (1)	2.11	160
N(1)–H(0101)...N(21) (iii)	0.97	3.13 (1)	2.26	148
N(1)–H(0102)...O(22) (iv)	0.99	3.07 (1)	2.08	173
N(13)–H(1302)...O(22) (v)	0.92	2.91 (1)	2.00	167
N(14)–H(14)...O(1) (vi)	0.96	3.16 (1)	2.25	159
N(23)–H(2301)...O(11) (vii)	0.77	3.14 (1)	2.43	153
N(23)–H(2302)...O(12) (viii)	0.77	3.09 (1)	2.32	172
N(1)–H(0103)...O(11) (ix)	0.85	3.07 (1)	2.58	118
N(24)–H(24)...O(12) (x)	1.13	3.21 (1)	2.09	177

(i) +x-1,+y,+z; (ii) +x+1,+y+z; (iii) +x-1,+y+1,+z+1; (iv) +x-1,+y+1,+z; (v) +x+1,+y,+z-1, (vi) +x,+y,+z-1; (vii) +x+1,+y-1,+z; (viii) +x+1,+y-1,+z-1; (ix) +x-1,+y,+z+1; (x) +x,+y-1,+z.

The coordination of the sulfonamide as a bridging ligand has been also observed in the [Cu(acetazolamidato)(NH_3)$_2$(H_2O)]. $2H_2O$,[28] but to our knowledge is the first time that a thiadiazole sulfonamide derivative links the Zn ion to the N-thiadiazole atom; usually in the Zn thiadiazole sulfonamido derivatives complexes, the metal is linked to the N-sulfonamido atom. Another important observation is the absence of the coordination of O-sulfonamido atoms such as it was

proposed from molecular mechanics calculations for CA-acetazolamide and CA-methazolamide adducts. Effectively, the shortest Zn-Osulfonamido bond distance is 3.07(4) Å near to that found in the CA-acetazolamide complex,[26] in the model complexes of acetazolamide and methazolamide and in the above cited Kimura's compound.[25] This is according to the results obtained by Liang and Lispcomb from SCF MO calculations[29] that had shown that the coordination of N atoms to Zn(II) avoid the bond between the O-sulfonamido and the Zn(II) ion.

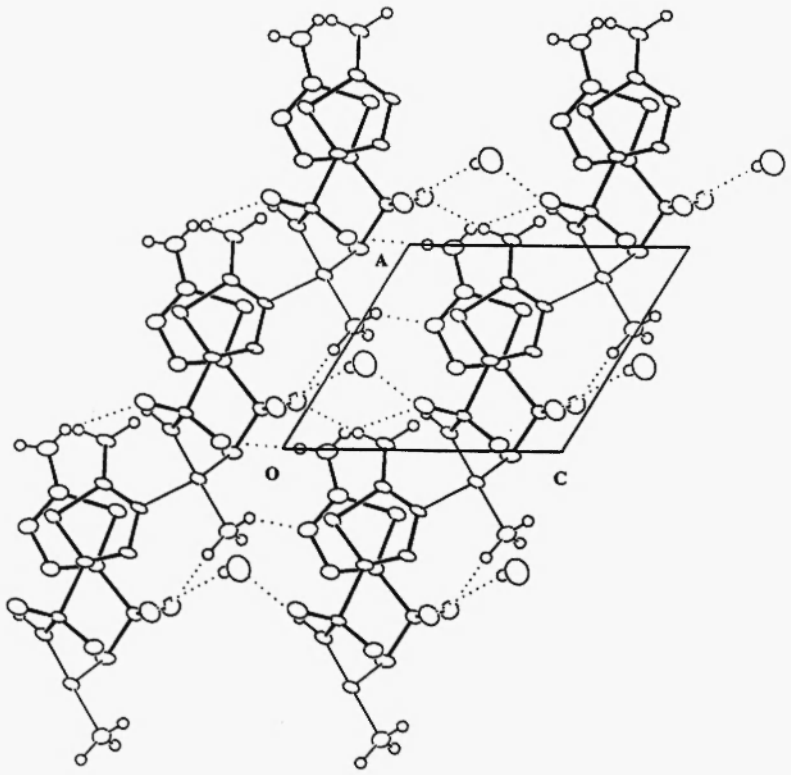


Figure 5. Crystal packing projected along **b** axis.

Upon coordination bond lengths of the ligand are slightly modified. The most significant difference has been found in the S-N bond length of the sulfonamido group that is shortened respect to that of the ligand (S-Nav 1.543 Å in the complex, 1.569 Å in the ligand[30]). This effect, that has been observed in the acetazolamide and methazolamide Zn(II) complexes was attributed to the change from the amido to the imido form of the sulfonamido moeity upon deprotonation. As in the Ni(acetazolamidate)$_2$(NH$_3$)$_4$ compound[27] the coordination of N-thiadiazole atom to metal ion does not involve modification of the N-N bond length.

A comparative study of the IR spectral data of the complexes with those of the ligands and the previously reported complexes[22,24] may give positive information regarding the binding sites of the ligands molecules. Table V shows the most characteristic frequencies of the IR spectra of the ligands and the complexes.

In the IR spectra of all complexes except for Zn(pyacm)(CH$_3$OH)$_2$ the characteristic $\nu(SO_2)$ vibrations are shifted with respect to those of the ligands.

In the [Zn(ats)$_2$(NH$_3$)].H$_2$O compound the coordination through the N-thiadiazole atom can be inferred from the modifications of the bands corresponding to ν(C=N) and the thiadiazole ring vibrations. The band assigned to ν(S-N) is shifted to higher frequencies in agreement with the shortening of the S-N bond distance observed in the crystal structure.

Table V.- Selected IR Vibrations (cm^{-1}) for the ligands and their complexes.

Compound	ν(N-H)	ν(C=N)	thiadiazole	$\nu(SO_2)_{asym}$	$\nu(SO_2)_{sym}$	ν(S-N)
H_2uacm	3420[a] 3300[a] 3190[a] 2900[b] 2610[b]	1610	1500	1340	1170	980
Hmuacm	3400 3100	1610	1540	1360	1170	920
H_2pyacm	3390	1610	1525	1340	1140	890
Hats	3430 3250	1610	1510	1340	1170	940
1	3350	1610	1500	1290	1140	940
2	3350	1610	1500	1290	1140	940
3	3260	1580	1570	1300	1150	980
4	3500	1620	1540	1340	1140	900
$[Zn(ats)_2(NH_3)].H_2O$	3600[c] 3480-3430d[e] 3300 3190	1630-1600d	1520-1500d	1280	1150	980

a= free; b= associated; c= water; d= doublet; e= ammonia

In the IR spectrum of the complexes **1** and **2** the typical ν(C=N) and thiadiazole ring vibrations remain unchanged while significant modifications are found in the bands attributed to the free and associated ν(N-H) vibrations and in the ν(S-N) band which appears at 980 cm^{-1}. From this data the coordination of the ligand through the amino and deprotonated sulfonamido nitrogen atoms can be suggested.

For complex **3** the modifications of the bands assigned to the ν(C=N) and the thiadiazole ring vibrations and the shifts of the bands attributed to the characteristic vibrations of the sulfonamido group indicate that the ligand interacts with the metal through the thiadiazole ring and/or the sulfonamido moiety.

The IR spectrum of the $[Zn(pyacm)(CH_3OH)_2]$ exhibits significant differences compared with that of the H_2pyacm in the vibrations of the pyridyl and the thiadiazole rings while the bands of the SO_2 group remain practically unchanged. According to that, we propose that the ligand is coordinated to the Zn(II) ion through the pyridyl and thiadiazole rings.

The ^{1}HNMR spectrum of the deuterated DMSO solutions of H_2uacm (for labelling of the H atoms see Figure 1) shows a signal at 8.2 ppm that must correspond to all the H of the ligand. The ^{1}HNMR spectrum of deuterated DMSO solutions of $[Zn_3(uacm)_2(NH_3)_2Cl_2]$ exhibits a doublet at 4.4 ppm corresponding to the proton signals of the ammonia ligand, a doublet at 7.1 and a broad signal at 7.8 ppm. Due to the partial dissociation of the complex in solution these signals can be attributed to the H bonded to N atoms of the sulfonamide in the complex and to those of the free ligand. The ^{1}HNMR spectrum of the $[Zn_3(uacm)_2(CH_3CH_2NH_2)_2Cl_2]$ shows signals at 0.9 and 1.1 ppm assigned to the methyl and methylene H of the ethylamine, a doublet at 4.3 ppm attributed to the H of N coordinated ethylamine and a doublet at 7 ppm corresponding to the H of N of coordinated $uacm^{2-}$ and free $uacm^{2-}$ anion.

The ^{13}CNMR of the H_2uacm in deuterated DMSO presents (for labelling of C atoms see Figure 1) signals at 159, 177 and 193 ppm that, according to the literature,[31] can be attributed to the C(b), C(c) and C(a) respectively. The ^{13}CNMR spectrum of the $[Zn_3(uacm)_2(NH_3)_2Cl_2]$ exhibits two doublets

at 158.3 and 177.9 ppm, suggesting that the complex is partially dissociated in deuterated DMSO solution.

The ^{13}CNMR spectrum of deuterated DMSO solutions of $[Zn_3(uacm)_2(CH_3CH_2NH_2)_2Cl_2]$ shows signals at 16.0, 35.9, 157.9 (doublet) and 177.4 (doublet) ppm. The first two signals can be attributed to the Cmethyl and Cmethylene of the ethylamine and the two doublets are similar to those observed in the above described Zn complex. We conclude from these ^{13}CNMR spectra that the $uacm^{2-}$ has the same coordination behaviour in both complexes in agreement to the interpretation of the IR spectra. The ^{13}CNMR in the solid state shows signals at 16.8, 40.4, 157, 173 and two singlets at 181 and 183 that are attributed to Cmethyl, Cmethylene, C(b), C(c) and C(a) respectively. It is interesting to observe that the C(a) give rise two singlets that may be a consequence of a different conformation of the two sulfonamidato ligands.

The ^{1}HNMR spectrum of DMSO solutions of Hmuacm ligand shows signals at 1.2, 7.7 and at 8.8 (broad) ppm attributed to H(6,7,8) and H(4,5) and H(1,2) respectively. The ^{13}CNMR spectrum presents signals at 38.7 ppm [C(d)], at 156 ppm [C(b)], at 168 ppm [C(c)] and at 130 ppm [C(a)]. The last signal that appeared at 193 ppm in the H_2uacm is shifted downfield in the Hmuacm probably due to the strong delocalization between the C=S and N-C=N bonds of the ligand. The ^{1}HNMR spectrum of the **3** exhibits signals to 1.4, 4.1, 6.1 and 7.2 ppm corresponding to the H(6,7,8), Hammonia, H(4) and H(1,2), respectively. The ^{13}CNMR spectrum shows signals at 38, 153 and 154 ppm assigned to C(d), C(b) and C(c) respectively. The shifts of the signals are in agreement with the IR data.

The ^{1}HNMR spectrum of the H_2pyacm exhibits two signals at 7.8 and 8.1 ppm attributed to H(3,4,5) and to the pyridine protons. The ^{13}CNMR spectrum presents signals at 150.5 ppm corresponding to the C of the pyridine ring, at 157.8 ppm [C(b)] at 166.2 ppm [C(c)] and at 171.6 ppm [C(a)]. As the $[Zn(pyacm)(CH_3OH)_2]$ is only slightly soluble in DMSO, it was not possible to record its NMR spectrum.

From the analysis based on extended Hückel calculations, using the computational parameters given in the Experimental Section, and the geometrical coordinates deduced from a idealised model derived from the crystal structure (Figure 6), we have calculated the complete molecular orbital correlation diagram for the [Zn(5-amino-1,3,4-thiadiazole-2-sulfonamidate)$_2$(NH_3)].H_2O complex. Although the extent of mixing between ligand orbitals interacting with metal orbitals is sometimes so great that a clear individuality is difficult, Figure 7 exhibits only the more important contributions to the interesting energy levels derived from our analysis.

Molecular orbital calculations have been carried out on the studies of Carbonic Anhydrase Inhibitors.[29,32] An aim of these investigations is to determine which orbitals, both from the sulfonamide ligand and from the metal ion, have relevance in the bonding between the metal and the inhibitor with the purpose of a better understanding of the interaction CA-Inhibitor. For our compound these studies can be of special interest due to its crystal structure has shown an unusual ligand coordination to the Zn(II) ion, not only through the sulfonamido nitrogen atom but also through the thiadiazole nitrogen one.

Thus for this complex it is noteworthy that the metal orbitals are not involved in the HOMO and LUMO (Figure 8), which consist of nonbonding orbitals formed by combinations of p atomic orbitals of the thiadiazole rings. The energy gap between both frontier levels is very large (4.228 eV).

In the formation of the molecular orbitals involved in the metal coordination, mainly four fragments molecular orbitals (FMO) of the ligands participate (Figure 9). The FMOs are composed by different atomic orbitals combinations of the ammonia and sulfonamido and thiadiazole nitrogen atoms.

The 4s orbital of the metal interacts with a FMO constituted by the p of the ammonia nitrogen, the p of the sulfonamido nitrogens and to a less extent of the p of the thiadiazole nitrogen to give the bonding MO 92 and the corresponding antibonding MO 42. The $4p_y$ orbital of the metal interacts with a FMO composed by the orbitals of the nitrogens of two sulfonamido group. The $4p_x$ and $4p_z$ orbitals are involved only slightly in the bonding MO orbitals as we can appreciate in the

Figure 8. In spite of the coordination of the N thiadiazole the orbitals of this N atom do not participate significantly in the FMOs, although some contribution can be inferred (see FMOs 92 and 63).

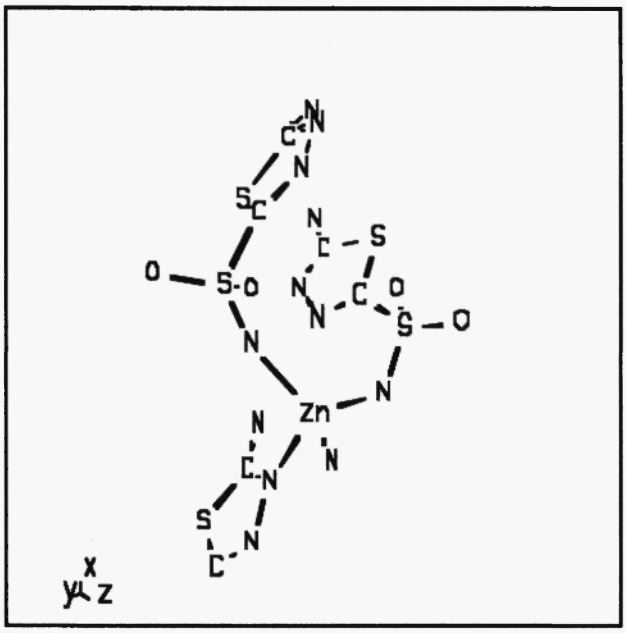


Figure 6. Idealised structure of the coordination polyhedron of the metal in the $[Zn(ats)_2(NH_3)](H_2O)$ complex.

A comparison of the results of these EHMO calculations with those of the one previously reported $[Zn(macm)_2(NH_3)_2]$ complex[22] shows that the two complexes have the HOMO and LUMO orbitals without contribution of the metal orbitals, in agreement with the crystal data of the CA-acetazolamide compound reported by Liljas et al[26] that have pointed out the importance of the thiadiazole ring interactions in the CA-inhibitor interaction. For the [Zn(5-amino-1,3,4-thiadiazole-2-sulfonamidate)$_2$(NH$_3$)].H$_2$O another important aspect to be note is the participation of the thiadiazole N orbitals in the principal FMOs due to the interaction of the nitrogen atom of the thiadiazole ring with the Zn(II) ion. This fact is not observed in the $[Zn(macm)_2(NH_3)_2]$ complex, where only the nitrogen atoms from the sulfonamido groups were involved.

Inhibition data with the newly synthesized complex inhibitors, the ligands from which they derive, as well as the standard inhibitor acetazolamide, are shown in Table VI.

Table VI. Inhibition data (IC_{50} values) against human isozymes CAI and CAII with the complex inhibitors, the ligands and acetazolamide.

Compound	IC_{50} CAI (μM)	IC_{50} CAII (x 10^{-9} M)
H_2aaz (acetazolamide)	24 + 2	56 ± 4
H_2uacm	21 + 2	51 ± 4
Hmuacm	13 + 1	50 ± 5
H_2pyacm	17 ± 2	29 ± 2
Hats (aminothiadiazolesulfonamide)	155 + 8	230 ± 12
$Zn_3(uacm)_2(NH_3)_2Cl_2$	20 ± 2	14 + 1.
$Zn_3(uacm)_2(EtNH_2)_2Cl_2$	36 ± 2	25 ± 2
Zn(muacm)(NH$_3$)Cl	4 ± 0	19 ± 1
Zn(pyacm)(MeOH)$_2$	8 ± 1	12 + 1
$Zn(ats)_2(NH_3)(H_2O)$	18 ± 1	68 ± 4

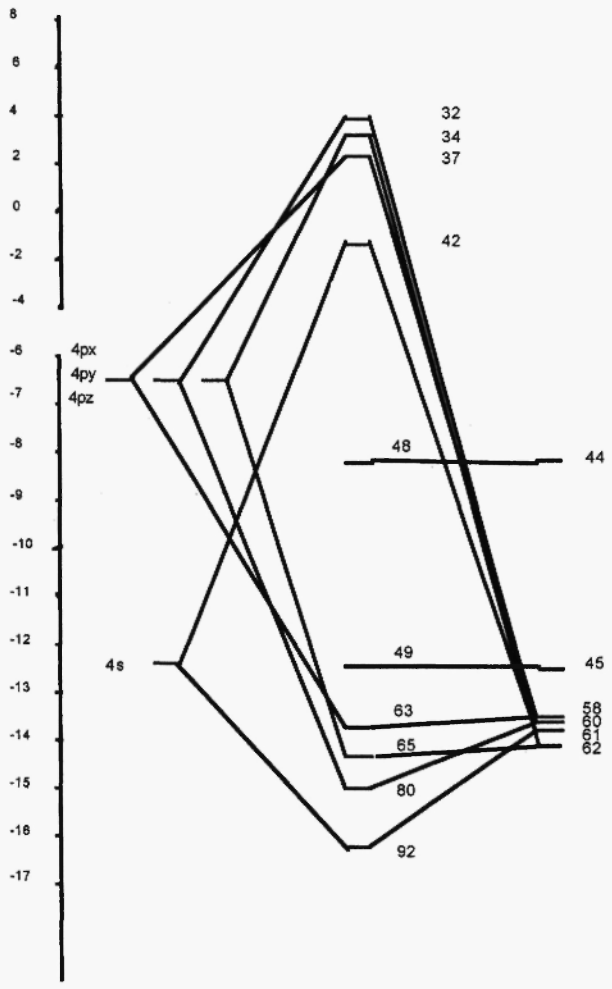


Figure 7. Molecular Orbital energy level of the $[Zn(ats)_2(NH_3)](H_2O)$ complex.

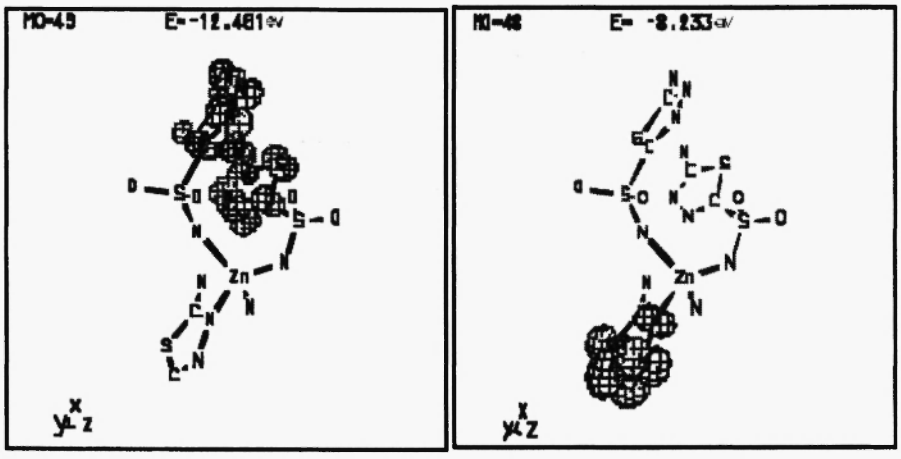


Figure 8. HOMO and LUMO of the $[Zn(ats)_2(NH_3)](H_2O)$ complex.

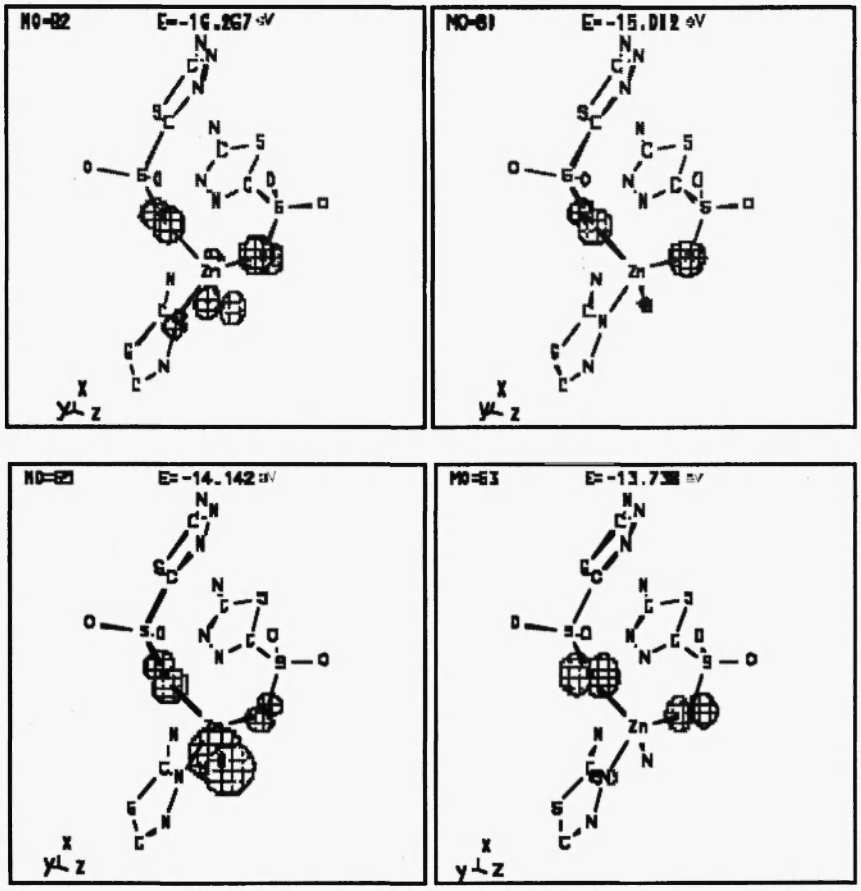


Figure 9. Molecular orbitals of the ligands interacting with Zn(II) atomic orbitals.

The first observation which can be done taking into account the above data, is that the complexes reported here, behave as very strong CAI and CAII inhibitors, with potencies superior to the ligands from which they derive, as has been explained previously for other analogous.[5,33] Only the $Zn_3(uacm)_2(NH_3)_2Cl_2$ compound shows an inhibition of the CAI in the range found for the free ligand. A careful inspection of the data in the table indicates that the inhibition of CA by the compounds is not only due to the free ligand coming from partial dissociation of the complexes because the IC_{50}-s for the compounds do not correspond those of the free ligand. In general, ligands used in the synthesis of the coordination compounds behave as stronger CAI and CAII inhibitors as compared to acetazolamide, the classical and widely used drug.[34,35] 5-amino-1,3,4-thiadiazole-2-sulfonamide (Hats) on the other hand is a much weaker inhibitor, as already shown in the classical study of Anderson's group.[36] Still its complexes behave as very strong inhibitors. It should be mentioned that a large difference exists between the two isozymes CAI and CAII, in their susceptibility to be inhibited by sulfonamides and their metal complexes.[35] Thus, CAII is much more sensitive to these inhibitors, with IC_{50} values around 10^{-8} M, whereas the isozyme I is more resistant to them, with IC_{50}-s in the micromolar range. The presence of a larger ammine, such as ethylamine in one complex, led to a weaker inhibitor towards both isozymes which may be due to a more bulky complex than those containing ammonia. The very diverse behaviour of the two isozymes towards this type of inhibitors constitutes a good prerequisite for an eventual development of isozyme-specific CA inhibitors for the nine isozymes presently isolated in higher vertebrates.

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Supplementary material

A list of structure amplitudes, anisotropic thermal parameters, H-atom parameters, distances and angles involving H atoms and principal torsion angles is available upon request from Dr. Borrás.

References

1. I. Bertini, H. B. Gray, S. J. Lippard and J. S. Valentine, "Bioinorganic Chemistry" University Science Books, Mill Valley, California **1994**.
2. V. E. Kinsey, *Arch. Oftalmol.*, **1957**, *50*, 401.
3. T. H. Maren, *Mol. Pharm.*, **1992**, *41*, 419.
4. T. H. Maren, *Ann. N. Y. Acad. Sci.*, **1984**, *429*, 568.
5. G. Alzuet, S. Ferrer, J. Borrás and C. T. Supuran, *Roum. Quaterly Rev.*, **1994**, *2*, 283.
6. C. T. Supuran, *Roum. Quaterly Rev.*, **1993**, *1*, 77.
7. C. T. Supuran, A. T. Balaban, M. D. Georghiu, A. Dinculescu and I. Puscas, *Rev. Roum. Chim.*, **1990**. *35*. 399.
8. C. T. Supuran, *Rev. Roum. Chim.*, **1995**, *40*, 643.
9. C. T. Supuran, A. Popescu and M. D. Banciu, *Rev. Roum. Chim.*, **1992**, *37*, 289.
10. A. Altomare, M. C. Burla, M. Camalli, G. Cascarano, G. Giacovazzo, A. Guagliardi and G. Polidori, SIR92, *J. Appl. Crystallogr.*, **1994**, *27*, 435.
11. J. M. Stewart, P. A. Machi, C. W. Dickinson, H. L. Ammon, H. Heck and H. Flack, **1976**. The X-ray 76 System, Computer Science Center, Univ. Of Maryland, College Park, Maryland (USA).
12. M. Martinez-Ripoll and J. Fayos, *Zeit. Für Kristallog.*, **1980**, *152*, 189.
13. D. Rogers, *Acta Cryst.*, **1981**, *A37*, 734.
14. C. Mealli and D. Proserpio, *J. Chem. Educ.*, **1990**, ***67***, 399.
15. C. Forsman, G. Behravan, A. Osterman and B.H. Jonsson, *Acta Chem. Scand.*, **1988**, B42, 314.
16. G. Behravan, P. Jonasson, B. H. Jonsson and S. Lindskog, *Eur. J. Biochem.*, **1991**, 198, 589.
17. R. G. Khalifah, D. J. Strader, S. H. Bryant and S. M. Gibson, *Biochemistry*, **1977**, 16, 2241.
18. P. O. Nyman and S. Lindskog, *Biochem. Biophys. Acta*, **1964**, *85*, 141.
19. L. E. Henderson, D. Henriksson and P. O. Nyman, *J. Biol. Chem.*, **1976**, *251*, 5457.
20. Y. Pocker and J. S. Stone, *Biochemistry*, **1967**, *6*, 668.
21. S. Ferrer, J. Borrás and E. García-Espana, *J. Inorg. Biochem.*, **1990**, *39*, 297.
22. G. Alzuet, J. Casanova, J. A. Ramirez, J. Borrás and O. Carugo, *J. Inorg. Biochem.*, **1995**, 57, 219.
23. U. Hartman and H. Vahrenkamp, *Inorg. Chem.*, **1991**, *30*, 4676.
24. S. L. Sumalan, J. Casanova, G. Alzuet, J. Borrás, A. Castineiras and C. T. Supuran, *J. Inorg. Biochem.*, **1996**, *62*, 31.
25. T. Koike, E. Kimura, I. Nakamura, Y. Hashimoto and M. Shiro, *J. Am. Chem. Soc.*, **1992**, *114*, 7338.
26. J. Vidgren, A. Liljas and N.P.C. Walker, *Int. J. Biol. Macromol.*, **1990**, *12*, 342.
27. S. Ferrer, J. Borrás, C. Miratvilles and A. Fuertes, *Inorg. Chem.*, **1989**, *28*, 160.
28. S. Ferrer, J. Borrás, C. Miratvilles and A. Fuertes, *Inorg. Chem.*,**1990**, *29*, 206.
29. J. Liang and W. Lipscomb, *Biochemistry*, **1989**, ***28***, 9724.
30. J. C. Pedregosa, G. Alzuet, J. Borrás, S. Fustero, S. García-Granda and M.R. Diaz, *Acta Cryst.*, **1993**, *C49*, 630.
31. E. Pretsch, T. Clerc, J. Seibl and W. Simon, "Tabellen zur Strukturaufklärung Organischer Verbindungen mit Spektroskopischen Metoden", Springer Verlag, Berlin **1976.**
32. A. Vedani and D. W. Huhta, *J. Am. Chem. Soc.*, **1990**, *112*, 4759.
33. C. T. Supuran, G. L. Almajan, *Main Group Met. Chem.*, **1995**, *2*, 347.
34. T. H. Maren, *Pharmacol. Rev.*, **1967**, *47*, 595.
35. C. T. Supuran, "Carbonic Anhydrase Inhibitors" in "Carbonic Anhydrase and Modulation of Physiologic and Pathologic Processes in the Organism", I. Puscas De., Helicon, Timisoara **1994**.
36. a) J.R. Vaughan, J.A. Eichler, and G.W. Anderson,*J. Org. Chem.*, **1956**, *21*, 700. b) R.W. Yourn, K.H. Wood, J.A. Eichler, J.R. Vaughan and G.W. Anderson, *J. Am. Chem. Soc.*, **1956**, *78*, 4649.

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