J. Enzyme Inhibition, 2000, Vol. 15, pp. 517–531
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ANTIFUNGAL ACTIVITY OF AG(I) AND ZN(II) COMPLEXES OF AMINOBENZOLAMIDE (5-SULFANILYLAMIDO-1,3,4-THIADIAZOLE-2-SULFONAMIDE) DERIVATIVES

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(Received 6 January 2000)

Aminobenzolamide (5-sulfanilylamido-1,3,4-thiadiazole-2-sulfonamide) is a potent inhibitor of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1), being at the same time structurally similar to the antimicrobial sulfonamides. Here we report that the reaction of aminobenzolamide with arylsulfonyl isocyanates affords a series of new arylsulfonylureido derivatives which were subsequently used as ligands (in the form of conjugate bases, as sulfonamide anions) for the preparation of metal complexes containing Ag(I) and Zn(II). All the new compounds proved to be very potent inhibitors of CA (isozymes I, II and IV). The newly synthesized complexes, unlike the free ligands, also act as effective antifungal agents against several *Aspergillus* and *Candida spp.*, some of them showing activities comparable to ketoconazole, with minimum inhibitory concentrations in the range of 1.8–5 μg/mL. The mechanism of antifungal action of these complexes seem to be unconnected with inhibition of lanosterol-14-α-demethylase, since the levels of sterols assessed in the fungi cultures were equal in the absence or in the presence of the tested compounds. Probably the new complexes act as inhibitors of phosphomannose isomerase, a key enzyme in the biosynthesis of yeast cell walls.

Keywords: Silver complex; Zinc complex; Sulfonamide; Carbonic anhydrase; Antifungals

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INTRODUCTION

Several important classes of antifungal compounds are presently available,[1–5] such as the azoles inhibiting lanosterol-14-α-demethylase,[1–5] sterol-Δ^{14}-reductase, [1] or Δ^7-Δ^8 isomerase[2] as well as the inhibitors of the zinc enzymes phosphomannose isomerase[6a,b] or topoisomerase.[6c] All these enzymes are involved in the biosynthesis of fungi/yeast cell walls, and their inhibition leads to impaired function of the membrane and as a consequence death of the pathogenic species. Recently, some metal complexes, such as silver sulfadiazine, were shown to possess effective antifungal properties against the pathogenic yeast *Candida albicans*.[6,7,8] The mechanism of action of this complex seems to be connected with the inhibition of phosphomannose isomerase, a key enzyme in the biosynthesis of yeast cell walls.[6,7,8]

Since resistance to the different antifungal agents constantly emerges,[2–5,9–11] it is important to investigate new types of compounds, able to prevent this serious medical problem. Metal complexes, containing mainly Ag(I) and Zn(II) seem to be such a valuable alternative.[7,8,12–15] Indeed, silver sulfadiazine **1** is extensively used clinically for the prophylaxis and treatment of bacterial and fungal burn infections, alone or in combination with mafenide acetate **2**, as well as cerium(IV) nitrate.[8,12–15]

Considering compound **1** as lead molecule, we have prepared some new derivatives of aminobenzolamide **3**, a strong sulfonamide inhibitor of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1),[16] by its reaction with arylsulfonyl isocyanates of type **4**.[17a] The conjugate bases of the new derivatives **5–9** were then used as ligands for the preparation of the Ag(I) and Zn(II) complexes **10–19**. The new compounds and their complexes were assayed as CA inhibitors as well as for their antifungal properties against two *Aspergillus* and one *Candida albicans* strains.

MATERIALS AND METHODS

Elemental analyses were done by combustion with a Carlo Erba Instrument or gravimetrically for the metal ions. NMR spectra were recorded at 200 MHz, with a Varian Gemini 200 spectrophotometer; chemical shifts are reported as δ values, relative to Me_4Si as external standard, in solvents specified in each case. IR spectra were recorded with a Perkin-Elmer spectrophotometer using KBr pellets as reference. Conductimetric measurements were done at room temperature on a Radelkis KFT conductimeter.

Aminobenzolamide was prepared as described previously.[17b] Sulfadiazine, mafenide hydrochloride, arylsulfonyl isocyanates, metal salts and solvents were commercially available (from Sigma-Aldrich or Janssen) and were used without further purification.

General Procedure for Preparation of Arylsulfonylureido Aminobenzolamides 5–9

An amount of 3.5 g (10 mM) of aminobenzolamide **3** was suspended in 100 mL of highly anhydrous (kept on molecular sieves) acetonitrile and magnetically stirred at 4°C for 10 min. The stoichiometric amount of arylsulfonyl isocyanate **4** (eventually dissolved in the same solvent for the solid compounds, or in pure state for the liquid ones) was then added dropwise, maintaining the temperature under 10°C. The reaction mixture was stirred at room temperature for 2–4 h (TLC control), then the solvent was evaporated in vacuo and the residue crystallized from ethanol-water. Yields were practically quantitative.

4-(Benzenesulfonylaminocarbonyl)-aminobenzolamide, **5**: as colorless crystals, mp > 300°C. IR(KBr), cm^{-1}: 1124, 1132 and 1171 (SO_2^{sym}), 1290 (amide III), 1328, 1356 and 1380 (SO_2^{as}), 1540 (amide II), 1680 (amide I), 3065 and 3190 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.05–7.60 (m, 5H, ArH, Ph), 7.51–8.06 (m, AA′BB′, $J_{AB} = 7.1$ Hz, 4H, ArH from -NHC$_6H_4$SO$_2$NH-thiadiazole), 7.79 (br s, 2H, SO$_2$NH_2), 8.41 (br s, 2H, N*H*CON*H*-), 10.98 (s, 1H, SO$_2$N*H*-thiadiazole). Found: C, 34.53; H, 2.91; N, 16.08. $C_{15}H_{14}N_6O_7S_4$ requires: C, 34.74; H, 2.72; N, 16.21%.

4-(4-Fluorobenzenesulfonylaminocarbonyl)-aminobenzolamide, **6**: as colorless crystals, mp 285–6°C (dec.). IR(KBr), cm^{-1}: 1119, 1130 and 1175 (SO_2^{sym}), 1285 (amide III), 1321, 1355 and 1380 (SO_2^{as}), 1540 (amide II), 1680 (amide I), 3065 and 3190 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.00–7.53 (m, AA′BB′, $J_{AB} = 7.1$ Hz, 4H, ArH, *p*-F-phenylene), 7.55–8.00 (m, AA′BB′, $J_{AB} = 7.2$ Hz, 4H, ArH from -NHC$_6H_4$SO$_2$NH-thiadiazole), 7.64 (br s, 1H, SO$_2$NH_2), 8.44 (br s, 2H, N*H*CON*H*-), 10.98 (s, 1H, SO$_2$N*H*-thiadiazole). Found: C, 33.78; H, 2.51; N, 15.58. $C_{15}H_{13}FN_6O_7S_4$ requires: C, 33.58; H, 2.44; N, 15.66%.

4-(4-Chlorobenzenesulfonylaminocarbonyl)-aminobenzolamide, **7**: as colorless crystals, mp 291–4°C (dec.). IR(KBr), cm^{-1}: 1120, 1133 and 1176 (SO_2^{sym}), 1290 (amide III), 1319, 1354 and 1380 (SO_2^{as}), 1540 (amide II), 1680 (amide I), 3065 and 3190 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.02–7.53 (m, AA′BB′, $J_{AB} = 7.2$ Hz, 4H, ArH, *p*-Cl-phenylene), 7.58–8.00 (m, AA′BB′, $J_{AB} = 7.1$ Hz, 4H, ArH from -NHC$_6H_4$SO$_2$NH-thiadiazole), 7.75

(br s, 1H, SO_2NH_2), 8.41 (br s, 2H, N*H*CON*H*-), 10.99 (s, 1H, SO_2NH-thiadiazole). Found: C, 32.54; H, 2.44; N, 15.05. $C_{15}H_{13}ClN_6O_7S_4$ requires: C, 32.58; H, 2.37; N, 15.20%.

4-(4-Tosylaminocarbonyl)-aminobenzolamide, **8**: as colorless crystals, mp > 300°C. IR(KBr), cm^{-1}: 1118, 1125 and 1176 (SO_2^{sym}), 1290 (amide III), 1321, 1355 and 1382 (SO_2^{as}), 1540 (amide II), 1680 (amide I), 3065 and 3195 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.50 (s, 3H, Me from *p*-tosyl), 7.05–7.62 (m, AA′BB′, $J_{AB} = 7.2$ Hz, 4H, ArH, phenylene from tosyl), 7.54–8.05 (m, AA′BB′, $J_{AB} = 7.1$ Hz, 4H, ArH from -$NHC_6H_4SO_2NH$-thiadiazole), 7.75 (br s, 1H, SO_2NH_2), 8.46 (br s, 2H, N*H*CON*H*-), 10.95 (br s, 1H, SO_2NH-thiadiazole). Found: C, 36.23; H, 3.41; N, 15.65. $C_{16}H_{16}N_6O_7S_4$ requires: C, 36.08; H, 3.03; N, 15.78%.

4-(2-Tosylaminocarbonyl)-aminobenzolamide, **9**: as colorless crystals, mp 281–2°C. IR(KBr), cm^{-1}: 1120, 1134 and 1175 (SO_2^{sym}), 1285 (amide III), 1329, 1341 and 1381 (SO_2^{as}), 1540 (amide II), 1680 (amide I), 3065 and 3190 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.64 (s, 3H, Me from *o*-tosyl), 7.00–7.72 (m, 4H, ArH, phenylene from *o*-tosyl), 7.49–8.00 (m, AA′BB′, $J_{AB} = 7.1$ Hz, 4H, ArH from -$NHC_6H_4SO_2NH$-thiadiazole), 8.51 (br s, 2H, N*H*CON*H*-), 7.62 (br s, 2H, SO_2NH_2), 10.95 (s, 1H, SO_2NH-thiadiazole). Found: C, 36.29; H, 3.00; N, 15.70. $C_{16}H_{16}N_6O_7S_4$ requires: C, 36.08; H, 3.03; N, 15.78%.

General Procedure for the Preparation of Complexes 10–19

An amount of 6 mMol of monosodium salt of sulfonamides **5–9** was prepared by reacting the corresponding sulfonamide with the calculated amount of an alcoholic 1N NaOH solution, in ethanol as solvent. To this solution was added the aqueous metal salt solution (Zn(II), Ag(I) nitrates), working in molar ratios of aminobenzolamide derivative: M^{n+} of 2:1 for the zinc compounds, and 1:1, respectively, for the silver derivatives. The aqueous-alcoholic reaction mixture was heated on a steam bath for 1h, adjusting the pH to 7 if necessary, 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 obtained white or yellowish powders of compounds **10–19** melted with decomposition at temperatures higher than 350°C and were poorly soluble in water and alcohol, but had good solubilities in DMSO, DMF, mixtures of DMSO-water, DMF-water as well as 0.01M NaOH or KOH solutions (with the formation of the sodium/potassium salts).

Assay of Fungistatic Activity of Compounds 1–19

Fungistatic activity was determined by a modification of the growth method previously reported by us,[18–21] utilizing two *Aspergillus* and one *Candida spp.* Minimum inhibitory concentrations (MICs) were determined by the agar dilution method with Iso-Sensitest agar as described by Kinsman *et al.*[22] The fungi/moulds were cultivated in agar plates at 37°C for 5 days in the nutrient broth (NB, Diagnostic Pasteur), in the absence and in the presence of 100–0.01 μg/mL of compounds **1–19**. Stock solutions of inhibitors were obtained in DMSO (100 mg/mL) and dilutions up to 0.01 μg/mL were done with distilled deionized water. The minimum concentration at which no growth was observed was taken as the MIC value (μg/mL), and represents the mean of at least two determinations.

Assay of Sterols Present in the Fungi Cultures

A reverse-phase HPLC method adapted from the literature,[23] was used to determine the amount of sterols (ergosterol and lanosterol) present in the fungi cultures. The fungi were cultivated as mentioned above for 5 days, with or without inhibitors added in the nutrient broth. Culture media were suspended in a small volume of MOPS buffer (pH 7.4) and the cells centrifuged at 20000 × g for 30 min. Cells were weighed (wet paste) and broken by sonication. Sterols present in the homogenate were then extracted in chloroform, the solvent evaporated to a small volume and the extracts applied on a μ-Bondapak-C18 column, with acetonitrile as eluting solvent. Authentic ergosterol and lanosterol (from Sigma) were used as standards. The flow rate was of 3 mL/min. The retention times were 8.87 min for ergosterol and 7.62 min for lanosterol, respectively. Blank assays were done for cultures which were not treated with inhibitors in order to assess the normal levels of sterols present. The amount of ergosterol present in the same amount of wet cells from the culture grown in the absence of inhibitor was taken as 100%.[21,24]

Assay of Carbonic Anhydrase Inhibition

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 as described by Forsman *et al.*[25] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by Lindskog's group,[26] and enzymes were purified by affinity

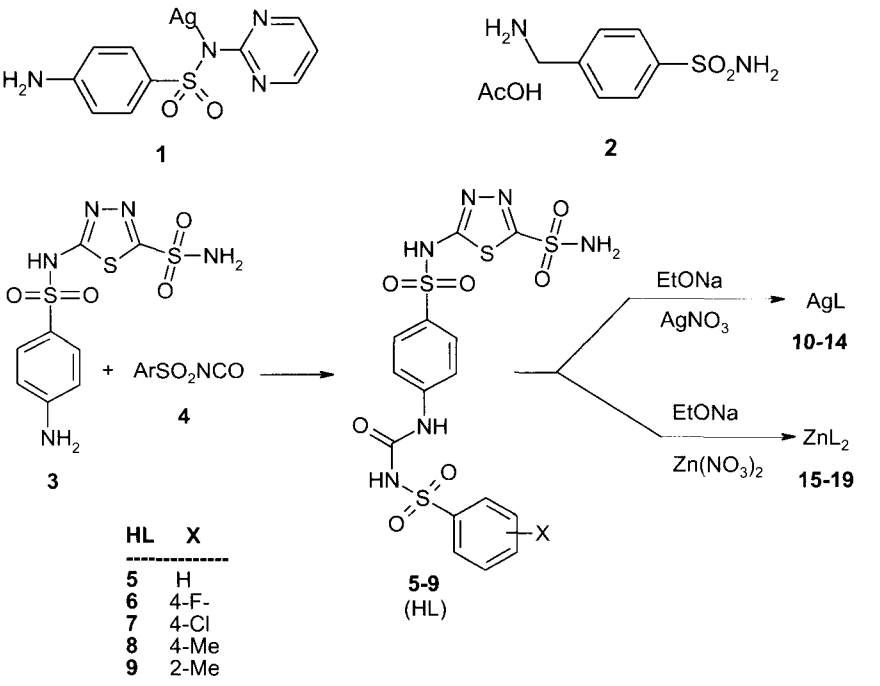
chromatography according to the method of Khalifah *et al.*[27] Enzyme concentrations were determined spectrophotometrically at 280 nm, utilizing a molar absorptivity of 49 $mM^{-1} \cdot cm^{-1}$ for CA I and 54 $mM^{-1} \cdot cm^{-1}$ for CA II, respectively, based on $M_r = 28.85$ kDa for CA I, and 29.30 kDa for CA II, respectively.[28,29] CA IV was isolated from bovine lung microsomes as described by Maren *et al.*, and its concentration was determined by titration with ethoxzolamide.[30]

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. [31] Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied between $2 \cdot 10^{-2}$ and $1 \cdot 10^{-6}$ 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 conditions of the experiments (pH 7.40), as reported in the literature.[31] 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.[31] Enzyme concentrations were 3.8 nM for hCA II, 13 nM for hCA I and 34 nM for bCA IV (this isozyme has a decreased esterase activity[32] and higher concentrations had to be used for the measurements).

RESULTS AND DISCUSSION

Reaction of aminobenzolamide **3** with arylsulfonyl isocyanates **4**, afforded the ureido derivatives **5–9**, by the procedure already reported previously by this group.[17] The new compounds obtained as outlined in (Scheme 1), were characterized by spectroscopic and analytic data that confirmed their structures (elemental analysis data for C, H and N were $\pm$ 0.4% of the theoretical values, calculated for the proposed formulas – data not shown).

Metal complexes containing the conjugate base of sulfonamides **5–9** (LH) and Ag(I) or Zn(II) ions, of types **10–19**, were also obtained (Scheme 1), and their elemental analysis data are shown in Table I. It should be noted that



SCHEME 1 Preparation of ligands **5–9** and their metal complexes **10–19**.

the new compounds **5–9** possess at least three acidic protons in their molecule, at the Ar-SO_2NHCO-, the *p*-phenylene-SO_2NH- as well as the SO_2NH_2 moieties, respectively. Of these three acidic groups, the lowest pK_a

TABLE I Prepared complexes **10–19**, containing the conjugate base of sulfonamides **5–9** (LH) as ligand and their elemental analysis data

No.	*Complex*	*Ligand*	*Analysis (calculated/found)*			
		(LH)	*%M* [a]	*%C* [a]	*%H* [b]	*%N*
10	[AgL]	**5**	17.25/17.50	28.81/28.65	2.10/2.43	13.44/13.30
11	[AgL]	**6**	16.76/16.87	28.00/27.75	1.88/1.69	13.06/12.98
12	[AgL]	**7**	16.35/16.30	27.30/27.25	1.83/2.10	12.74/12.55
13	[AgL]	**8**	16.87/16.69	30.05/29.98	2.36/2.19	13.14/13.06
14	[AgL]	**9**	16.87/17.12	30.05/30.18	2.36/2.05	13.14/12.90
15	[ZnL_2]	**5**	5.85/5.45	33.32/33.45	2.80/3.00	15.04/14.96
16	[ZnL_2]	**6**	5.67/5.39	32.28/32.37	2.53/2.39	14.57/14.40
17	[ZnL_2]	**7**	5.51/5.79	31.38/30.99	2.46/2.38	14.17/14.13
18	[ZnL_2]	**8**	5.71/5.46	34.60/34.95	3.08/2.77	14.67/14.67
19	[ZnL_2]	**9**	5.71/5.88	34.60/34.52	3.08/2.95	14.67/14.39

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

(of around 3.5)[17b] is that of the *p*-phenylene-SO_2NH- moiety, due to the electronic effects of both the phenylene-SO_2 as well as the thiadiazole ring system. Thus, this will be the group that will first ionize by treatment with one equivalent of base, leading to the mono-anionic derivatives, used then for the preparation of coordination compounds.

The new complexes have been characterized by spectroscopic, conductimetric and thermogravimetric measurements (Table I). By comparing the IR spectra of the complexes and the free ligand, the following differences were seen: (i) the shift of the two sulfonamido vibrations (both the symmetric as well as the asymmetric one) belonging to the SO_2NH-thiadiazole moiety (at 1171–1176 cm^{-1}, and 1381–1382 cm^{-1}) towards lower wavenumbers in the spectra of the complexes, as compared to the spectra of the corresponding ligand (Table II), as already documented previously for similar derivatives.[33–37] It should be noted that only one pair of such vibrations underwent the above-mentioned shift, presumably those of the SO_2NH-thiadiazole moiety, whereas the other sulfonamides (X-$C_6H_4SO_2NHCONH$ and SO_2NH_2) moiety appeared at the same wavenumbers both in ligands as well as in their metal complexes (data not shown). This is a direct indication that only the deprotonated SO_2NH-thiadiazole moiety of the ligand (which is the most acidic group of these derivatives, with pK_a values of around 3.5)[17b] interacts with the metal ions in the newly prepared coordination

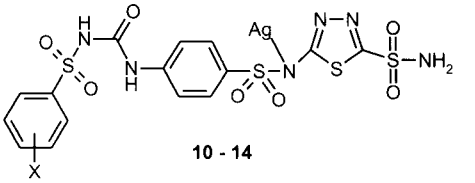
TABLE II Spectroscopic, ^{13}C-NMR and conductimetric data for the metal complexes **10–19**

Comp.	*IR Spectra*[a], cm^{-1}			*^{13}C-NMR spectra*[b]	*Conductometry*[c]
	$\nu(SO_2)^s$;	$\nu(SO_2)^{as}$	$\nu(C=O)^d$	δ, ppm *(C-5 of TDA)*	Λ_M $(\Omega^{-1} \times cm^2 \times mol^{-1})$
5	1171	1380	1680	173.8	4
6	1175	1380	1680	173.9	3
7	1176	1380	1680	173.7	3
8	1176	1382	1680	173.9	6
9	1175	1381	1680	174.0	5
10	1156	1362	1680	171.2	4
11	1159	1360	1680	170.9	3
12	1153	1362	1680	171.5	3
13	1156	1361	1680	170.8	6
14	1155	1365	1680	171.2	5
15	1151	1363	1680	170.5	6
16	1154	1360	1680	170.7	4
17	1153	1359	1680	170.8	7
18	1155	1358	1680	170.4	4
19	1154	1361	1680	170.9	5

[a] In KBr; [b] In DMSO, at 25°C; TDA = 1,3,4-thiadiazole-2-sulfonamide; [c] 1 mM solution, in DMF, at 25°C; [d] The ureido NHCONH vibration.

compounds, (ii) the thiadiazole C=N stretching vibration in the spectra of the prepared complexes are shifted by 15–20 cm^{-1} towards lower wavenumbers, as compared to the same vibration in the spectrum of sulfonamides **5–9**, indicating again that the thiadiazole moiety is probably in the vicinity of the metal ions (data not shown), (iii) the ureido vibration in the spectra of complexes **10–19**, assigned as the intense band at 1680 cm^{-1} appears at the same wavelength as that of the corresponding free ligands (Table II), suggesting that these moiety do not participate in the coordination of the metal ions.

^{13}C-NMR spectra of the ligands **5–9** and their complexes **10–19** were very similar (data not shown), except for the chemical shifts of the thiadiazolic C-5 atoms (Table II). Thus, in the spectra of the free ligands, this carbon resonates at 173.7– 174.0 ppm,[17b] whereas for the metal complexes the same signal appears 2–3 ppm at higher fields. Probably this effect (observed previously for other sulfonamide metal complexes)[33,34] is due to the proximity of the metal center to the carbon atom considered above. Conductometric data (Table II) also indicate that the new complexes **10–19** are non-electrolytes, being undissociated in DMF (or DMSO) as solvents. Thermogravimetric analysis data of complexes **10–19** showed no weight loss in the temperature interval of 100–220°C, proving thus that lattice or coordination water molecules are not present in their molecules.



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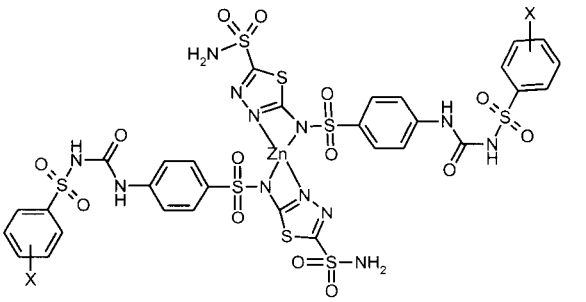


15 - 19

Based on such data, we propose that ligands **5–9** act monodentately in the new Ag(I) complexes **10–14**, the donor system being constituted by the sulfonamidic nitrogen of the *p*-phenylene-SO_2NH-thiadiazole moiety. On the other hand, the same ligands probably act bidentately in the Zn(II)

derivatives, with participation of the thiadiazolic N-4 atom too in the interaction with the metal ions in addition to the above mentioned sulfonamide nitrogen. It is very probable that it is just the N-4 atom of the heterocyclic ring interacting with the Zn(II) ion, since this behaviour has previously been documented (by means of X-ray crystallography) for related 1,3,4-thiadiazole-2-sulfonamide ligands in their complexes with Zn(II) and Cu(II) ions[31–35] (it should be noted that since so many heteroatoms are present in these new ligands of type **5–9**, their coordination chemistry is probably quite complicated, and for the moment little investigated).

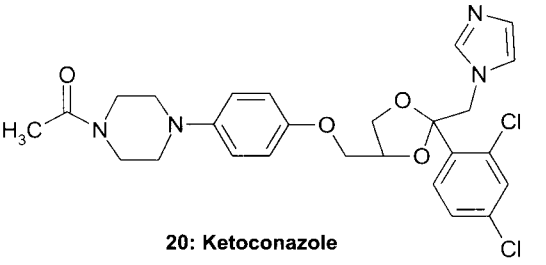
Antifungal activity data with the new derivatives **1–19** and the standard azole drug ketoconazole **20** are shown in Table III.

From the data of Table III, it should be noted that the new complexes **10–19** reported here represent a new class of antifungals with MIC-s (minor inhibitory concentration) in the (μg/mL range, which might induce strong antifungal effects. Furthermore, the ligands from which the complexes were prepared, as well as the related sulfonamide **2**, show very weak antifungal properties against the three strains investigated here. The most active derivative were the Ag(I) complexes **10–14**, followed by the Zn(II) ones containing the same type of ligand. From this point of view, the halogeno-substituted ligands led to more active antifungal complexes as compared to

TABLE III Antifungal activity of compounds **1–20** against several organisms.

Compound	*MIC* (μg/mL)		
	A. flavus C1150	*A. niger* C418	*Candida albicans* C316
1	20	23	8
2	> 100	> 100	> 100
3	> 100	> 100	> 100
5	> 100	> 100	> 100
6	94	95	> 100
7	89	81	86
8	95	83	84
9	> 100	90	82
10	10	10	2.1
11	3	4	1.3
12	5	5	1.4
13	4	6	2.0
14	6	7	2.3
15	13	9	7
16	10	8	3
17	9	11	2.5
18	14	12	6
19	13	11	8
Ketoconazole 20	1.2	1.8	0.06

the phenyl- and tosyl-ureido derivatives. *Candida* was most susceptible to inhibition, followed by *A. flavus*, whereas *A. niger* was the most resistant to this type of antifungals. In this respect, the complex derivative parallel the biological activity of ketoconazole, although they are less active. One should anyhow note that some of our new complexes are more active antifungals than silver sulfadiazine **1**, a clinically widely used derivative (Table III).



20: Ketoconazole

Ketoconazole **20** is known to act as an inhibitor of lanosterol 14-α-demethylase (CYP51A1), a microsomal cytochrome P-450 dependent enzyme system belonging to a gene superfamily involved in sterol biosynthesis in fungi, plants and animals.[32] CYP51A1 has been shown to catalyze the conversion of lanosterol to the 14-desmethylated derivative, ergosterol, through a complicated oxidative sequence. Its inhibition in fungi causes the depletion of ergosterol and accumulation of 14-methylsterols in the membrane of the cells, disturbing their membrane function and causing the death of these organisms.[32]

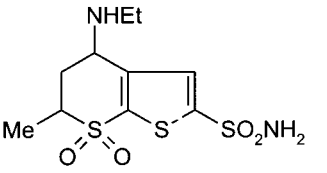
Thus, in order to investigate whether the complexes reported here act as ergosterol biosynthesis inhibitors, similarly to the azole antifungals, the amounts of ergosterol present in *C. albicans* cultures after treatment with different concentrations of the new inhibitor **11** and ketoconazole **20**, a potent CYP51A1 inhibitor,[20] were determined by means of a HPLC method (Table IV).[23]

The data of Table IV show that in contrast to ketoconazole, the silver complex investigated here, **11**, does not act as an inhibitor of CYP51A1, possessing thus a different mechanism of antifungal action. Probably, as for many other Ag(I) derivatives, the new antifungals might exert their effects through poisoning some components of the respiratory enzymes in the fungal/microbial electron transport system, or even through interaction with the DNA of the pathogenic species.[38,39]

TABLE IV Levels of ergosterol in *C. albicans* cultures after treatment with different concentrations of the azole CYP51A1 inhibitor ketoconazole **20** and the silver complex **11**

Inhibitor	*Concentration* (μg/mL)	*% Ergosterol**
Ketoconazole	0.01	89 ± 5
Ketoconazole	0.02	66 ± 7
Ketoconazole	0.05	8 ± 4
11	0.25	99 ± 1
11	0.50	99 ± 1
11	1.00	98 ± 2
11	1.50	97 ± 2

* Mean ± standard deviation ($n = 3$); The amount of ergosterol present in the same amount of wet cells from the culture grown in the absence of inhibitor is taken as 100%.



21: Dorzolamide

The new compounds **5–19** have also been tested for their carbonic anhydrase (CA) inhibitory activity, against isozymes hCA I, hCA II and bCA IV (h = human, b = bovine source) known to play important physiological functions[16,17,35,40] (Table V). Data for the standard sulfonamide inhibitors dorzolamide **21** (a clinically used topical antiglaucoma drug),[40] mafenide **2** and aminobenzolamide **3** are also included in Table V.

From the above data it is obvious that the new compounds reported here and their Ag(I) and Zn(II) complexes act as very efficient inhibitors (affinities in the low nanomolar range) against all three investigated CA isozymes, in contrast to dorzolamide (a weak CA I, but effective CA II and IV inhibitor) or mafenide (a relatively weak inhibitor against all three investigated isozymes). The new sulfonamides **5–9** are already stronger inhibitors than aminobenzolamide **3**, and these effects increase when metal ions are present into the molecules of the complex compounds. The Ag(I) derivatives were generally more effective than the corresponding Zn(II) derivatives, which in turn were more inhibitory than the free ligands **5–9**. From the point of view of the substitution pattern of the ligand, best activity was correlated with the presence of halogen atoms (fluorine and chlorine), but differences between the unsubstituted compound or the methyl-substituted ones are quite insignificant. It should also be noted that due to

TABLE V CA inhibition data with standard inhibitors dorzolamide **21** and **2–3**, the parent sulfonamides **5–9** and the metal complexes **10–19** reported in the present study, against isozymes I, II and IV

Inhibitor	K_I (nM)*		
	hCA I[a]	*hCA II*[a]	*bCA IV*[b]
Dorzolamide **21**	50000	9	45
2	25000	170	2800
3	6.2	2.1	5.2
5	2.8	1.7	3.0
6	2.1	1.2	3.1
7	2.3	1.5	3.2
8	2.5	1.6	4.5
9	2.5	1.5	4.3
10	1.2	0.4	1.7
11	1.4	0.2	1.1
12	1.5	0.2	1.2
13	1.6	0.5	1.5
14	1.5	0.4	1.6
15	1.8	0.7	2.1
16	1.6	0.5	2.0
17	1.5	0.6	2.5
18	1.7	0.9	1.9
19	1.9	0.8	2.1

* Standard error for the determination of K_I values was 5–10% (from 3 different assays); [a] Human (cloned) isozyme; [b] Isolated from bovine lung microsomes.

their putative diuretic effects, these compounds are not suitable for systemic administration, since they would produce acid-base imbalances. Their topical use instead (purpose for which they have been designed) would avoid such undesired side effects.

In conclusion we report here the preparation, antifungal and CA inhibitory activity of some Ag(I) and Zn(II) complexes of aminobenzolamide new derivatives, possessing interesting biological activity against two *Aspergillus* and *Candida* strains. Furthermore, these compounds have very high affinities for three investigated CA isozymes – in the nanomolar range – making them some of the more effective such inhibitors reported up to now.

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