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Carbonic Anhydrase Inhibitors: Schiff's Bases of Aromatic and Heterocyclic Sulfonamides and their Metal Complexes

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Schiff's bases were obtained from aromatic/heterocyclic sulfonamides and amino-sulfonamide derivatives, such as sulfanilamide, homosulfanilamide, 4-aminoethyl-benzenesulfonamide and 5-amino-1,3,4-thiadiazole-2-sulfonamide. Metal complexes of some of these Schiff's bases, incorporating Zn(II), Co(II), Ni(II) and Cu(II) ions, were also prepared and tested as inhibitors of the zinc enzyme carbonic anhydrase (CA), and more specifically the red blood cell isozymes I and II. The Schiff's bases behaved as medium potency CA I and CA II inhibitors, whereas their metal complexes showed a highly enhanced potency, with several low nanomolar CA II inhibitors detected.

Keywords: Carbonic anhydrase; Isozymes I, II; Aromatic/Heterocyclic sulfonamides; Schiff base; Metal complexes

INTRODUCTION

The importance of sulfonamides as pharmacological agents appeared when Domagk[1] showed that sulfanilamide was the metabolite of the antibacterial drug Prontosil. Later, a great number of sulfanilamide derivatives were synthesised, characterised and tested as antibacterial agents, with many such derivatives currently used for the treatment of bacterial infections.[2] Such sulfonamide derivatives widely used in clinical medicine as pharmacological agents with a wide variety of biological actions, were designed from the simple sulfanilamide lead molecule.[3,4] In addition to the antibacterials mentioned above, the unsubstituted aromatic/heterocyclic sulfonamides act as carbonic anhydrase inhibitors (CAIs),[5,6] whereas other types of derivatives show diuretic activity (high-ceiling diuretics or thiadiazine diuretics), hypoglycemic activity, anticancer properties,[7] or may act as inhibitors of the aspartic HIV protease, being used for the treatment of AIDS and HIV infection among others. [3]Historically, the first sulfonamide metal complex reported was the silver(I) derivative of sulfanilamide, prepared from the sulfonamide sodium salt and silver nitrate by Braun and Towle.[8] The metal complexes of substituted sulfanilamides were then investigated in detail by Bult;[9] however few crystal structures of such complexes were reported, whereas metal complexes of other type of sulfonamides have not been investigated. Several heterocyclic/aromatic sulfonamides acting as inhibitors of the metallo-enzyme carbonic anhydrase (CA, EC 4.2.1.1), are clinically used: acetazolamide **1**, methazolamide **2**, ethoxzolamide **3**, dichlorophenamide **4**, dorzolamide **5**, or brinzolamide **6**.[5,6] These compounds are also attractive ligands for complexation by metal ions. Indeed, many metal complexes of these and structurally related heterocyclic sulfonamides have been reported,[10–26] characterized by spectroscopic and X-ray crystallographic methods,[10–26] and investigated for inhibition of different CA isozymes as well as for potential applications as diagnostic tools/pharmacological agents.[5,6] The most interesting fact regarding these metal complexes of sulfonamide CA inhibitors is that they generally are 10–100 times more potent inhibitors of isozymes CA I, CA II and CA IV, as compared to the parent sulfonamide from which they were obtained, and this has been rationalized from the mechanistic point of view.[5,6] Thus, it is believed that this powerful inhibition is due

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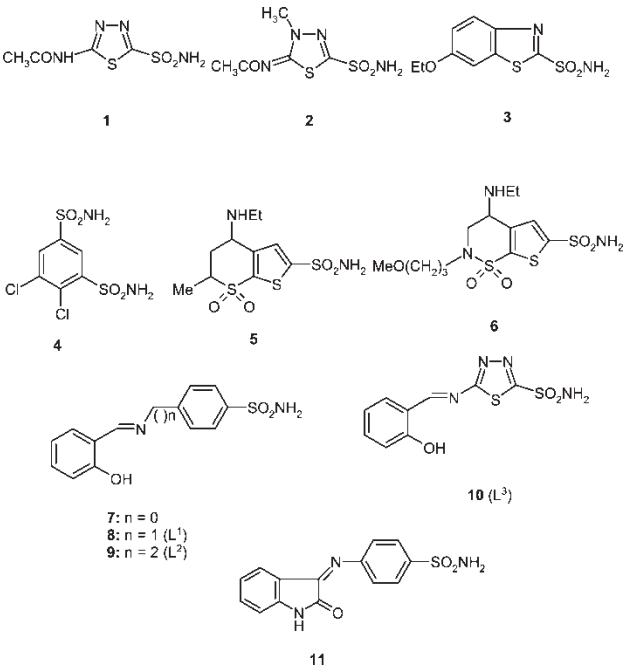
to a dual mechanism of action, through sulfonamide anions, and metal ions, obtained in dilute solution by dissociation of the coordination compounds. Sulfonamide anions formed in this way then bind to the Zn(II) ion within the enzyme active site, whereas the metal ions block the proton shuttle residues of CA, i.e., His 64 for isozyme II, His 64 and 67 for isozyme I, and probably the entire histidine cluster in the case of isozyme II as well.[5,6]



Several interesting applications have been recently reported for some metal complexes of heterocyclic sulfonamides possessing powerful CA inhibitory properties. Thus, zinc or copper complexes of highly lipophilic thiadiazole sulfonamides were shown to act as very efficient intraocular pressure (IOP) lowering agents when administered topically in normotensive or glaucomatous rabbits, although the parent sulfonamides from which the complexes were prepared did not possess topical antiglaucoma activity.[20] It was then shown that many other such metal complexes possess topical antiglaucoma action,[20–22] and this has been explained by a modulation due to the presence of the metal ion of the physico-chemical properties of the complex, which in some cases becomes more polar and thus penetrates better through the cornea for inhibition of the ciliary process CAs (isozymes CA II and CA IV).[20–22] Some aluminum sulfonamide complexes were then shown to act as efficient antisecretory agents in dogs.[23] Gastric acid secretion parameters three days after treatment with such CA inhibitors were drastically reduced, as compared to the same parameters in animals that did not receive these enzyme inhibitors.[23]

Taking into account such findings, here we extend our previous studies in which we investigated metal complexes of compound **7**, a Schiff's base obtained from sulfanilamide and 2-hydroxybenzaldehyde.[27] We report the synthesis and evaluation as CA inhibitors of Schiff's base transition metal complexes, obtained from sulfanilamide analogs (homosulfanilamide, 4-aminoethyl-benzenesulfonamide and 1,3,4-thiadiazole-2-sulfonamide) and salicylaldehyde, of types **8**–**10**. An isatin-derived sulfanilamide Schiff's base **11** has also been obtained. The new complexes of ligands **8**–**10**, containing Zn(II), Co(II), Ni(II) and Cu(II) are also reported here, and were characterized by standard procedures and assayed as inhibitors of the physiologically relevant CA isozymes: hCA I, and hCA II.

MATERIALS AND METHODS

Melting points were determined with a heating plate microscope and are not corrected. IR spectra were obtained in KBr pellets with a Perkin-Elmer 16PC FTIR spectrometer. Electronic spectra of the metal complexes were obtained by the diffuse reflectance technique in MgO as reference, with a Perkin Elmer Lambda 15 apparatus, in the range 200–900 cm^{-1}. Elemental analyses were done by combustion for C, H, N, with an automated Carlo Erba analyzer, or gravimetrically for the metal ions, and were $\pm 0.4\%$ of the theoretical values. The Schiff base **8**–**10** have been prepared as reported previously.[26] Compound **11** has been obtained as described below from isatin and sulfanilamide. Sulfonamides used as standards were commercially available from Sigma, Acros or Aldrich. Solvents used in the synthesis were doubly distilled and kept on molecular sieves in order to maintain them in anhydrous conditions. Metal salts used for the preparation of the coordination compounds were analytical grade from E. Merck.

Human CA I and CA II cDNAs were expressed in *Escherichia coli* strain BL21 (DE3) from the plasmids pACA/HCA I and pACA/HCA II described by Forsman *et al.*[27] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by Lindskog's group,[28] and enzymes were purified by affinity chromatography according to the method of Khalifah *et al.*[29] Enzyme concentrations were determined spectrophotometrically at 280 nm, utilizing a molar absorptivity of 49 $mM^{-1}\,cm^{-1}$ for CA I and 54 $mM^{-1}\,cm^{-1}$ for CA II, respectively, based on $M_r = 28.85$ kDa for CA I, and 29.30 kDa for CA II, respectively.[30–32]

Initial rates of 4-nitrophenyl acetate hydrolysis catalyzed by different CA isozymes were monitored spectrophotometrically, at 400 nm, with a Cary 3

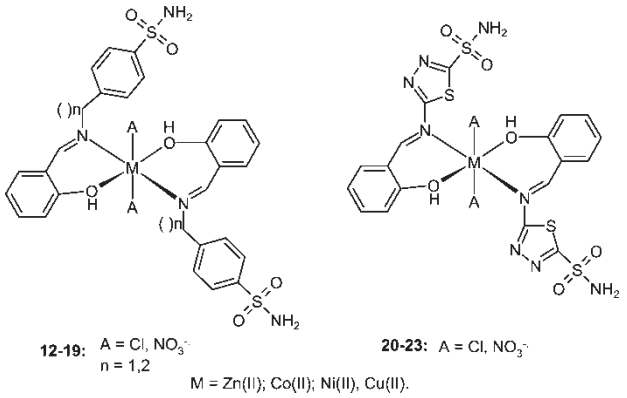
instrument interfaced with an IBM compatible PC.[33] Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied between 2×10^{-2} and 1×10^{-6} M, working at 25°C. A molar absorption coefficient ε of 18,400 $M^{-1} cm^{-1}$ was used for the 4-nitrophenolate formed by hydrolysis, under the conditions of the experiments (pH 7.40), as reported in the literature.[33] Non-enzymic 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.[33]

Preparation of Ligand 11

To an ethanolic (25 mL) solution of sulfanilamide (1.2 g, 0.007 moles) an ethanolic solution of isatin (1 g, 0.007 moles) was added with stirring. Then 1–2 drops of conc H_2SO_4 were added and the mixture refluxed for 2 h. After cooling to room temperature, the solution obtained was filtered and left overnight at room temperature, which led to the formation of a crystalline product. This crystallized product was filtered and recrystallized from ethanol: chloroform (50%) to give the orange–red desired products (68%). Purification was checked by TLC which indicated a single spot.

Preparation of Coordination Compounds 12–23

To a hot ethanolic (25 mL) solution of sulfonamides **8–10** (0.02 moles) an aqueous solution of the corresponding metal(II) salt (0.01 M) was added. The mixture was refluxed for 3 h, the solution filtered and reduced to half of its volume, by evaporation *in vacuo*. The concentrated solution was left overnight at room temperature, which led to the formation of a solid product. This solution was filtered and the solid washed with ethanol (2 × 15 mL) and dried. Recrystallization from 50% aqueous ethanol gave the desired products. Unfortunately only microcrystalline powders could be obtained, which could not be used for X-ray structural determinations. In fact this is the usual technical problem related to the thorough characterization of this type of metal complex.[19]



RESULTS AND DISCUSSION

The Schiff's bases **8–10**, prepared from aromatic/heterocyclic sulfonamides and salicylaldehyde,[22] were shown to behave as moderate CAIs against isozyme I, and as much more effective CA II inhibitors.[26] Since metal complexes of heterocyclic/aromatic sulfonamides have recently been shown[10–25] to possess even stronger inhibitory properties, it appeared of interest to synthesize some metal complexes of these Schiff bases, mainly due to their interesting donor system, comprising the nitrogen incorporated in the Schiff's base moiety, as well as the phenolic OH group. Thus, the Zn(II), Cu(II); Co(II) and Ni(II) complexes (Table I) of ligands **8–10** and different counter-anions were obtained. It should be noted that similar to other ligands investigated in previous studies as metal complexing sulfonamides with CA inhibitory properties,[19] the compounds investigated here acts as neutral ligands, and not in the deprotonated state (see later in the text). Practically the new derivatives reported here (**12–23**) are non-chelate metal complexes of sulfonamide CAIs. These complexes have been purposely prepared in the absence of deprotonating agents (NaOH, NH_3 or organic amines) so as to favor the participation of the Schiff base as neutral ligand in the interaction with the metal ions. Working in the presence of bases, generally leads to deprotonation of the sulfonamido moiety of the ligand, which thereafter interacts with the metal ion(s) present in the coordination compound.[10–26]

The new complexes **12–23** were characterized by elemental analysis (Table I) and spectral data that allowed us to assign the coordination mode of ligand L (Table II) in these complexes. As seen from data of Table I, the elemental analysis data of the new complexes were within ± 0.4% of the theoretical data calculated for the proposed formulas.

The main differences in the IR spectra of the new complexes **12–23** as compared to the corresponding spectra of the parent ligands **8–10** are: (i) the two

TABLE I Metal complexes **12**–**23** containing sulfonamide **8**–**10** (L^1–L^3) as ligands, their formulae, melting points and elemental analysis data

No.	Complex	Formula	Analysis (calculated/found) %M[a]	%C[b]	%H[b]	%N[b]	M.p. (°C)
12	$[ZnL^2_1Cl_2]$	$C_{28}H_{28}ZnN_4O_6S_2Cl_2$	9.1/8.5	46.9/45.3	3.9/3.3	7.8/8.1	211–2
13	$[CoL^1_2(NO_3)_2]$	$C_{28}H_{28}CoN_6O_{12}S_2$	7.7/8.4	44.0/42.6	3.7/3.1	11.0/11.2	208–11
14	$[NiL^1_2(NO_3)_2]$	$C_{28}H_{28}NiN_6O_{12}S_2$	7.7/7.8	44.0/49.5	3.7/4.1	11.0/7.4	208–10
15	$[CuL^1_2(NO_3)_2]$	$C_{28}H_{28}CuN_6O_{12}S_2$	8.3/7.8	43.7/39.0	3.6/3.1	10.9/7.0	208–9
16	$[ZnL^2_2Cl_2]$	$C_{30}H_{32}ZnN_4O_6S_2Cl_2$	8.7/8.5	48.4/45.3	4.3/3.3	7.5/8.1	211–2
17	$[CoL^2_2(NO_3)_2]$	$C_{30}H_{32}CoN_6O_{12}S_2$	7.4/8.4	45.5/42.6	4.0/3.1	10.6/1.2	208–11
18	$[NiL^2_2(NO_3)_2]$	$C_{30}H_{32}NiN_6O_{12}S_2$	7.4/7.8	45.5/49.5	4.0/4.1	10.6/7.4	208–10
19	$[CuL^2_2(NO_3)_2]$	$C_{30}H_{32}CuN_6O_{12}S_2$	8.0/7.8	45.2/39.0	4.0/3.1	10.6/7.0	208–10
20	$[ZnL^3_2Cl_2]$	$C_{18}H_{16}ZnN_8O_6S_4Cl_2$	9.3/8.5	30.7/45.3	2.3/3.3	15.9/8.1	211–2
21	$[CoL^3_2(NO_3)_2]$	$C_{18}H_{16}CoN_{10}O_{12}S_4$	7.8/8.4	28.8/42.6	2.1/3.1	18.6/11.2	208–11
22	$[NiL^3_2(NO_3)_2]$	$C_{18}H_{16}NiN_{10}O_{12}S_4$	7.8/7.8	28.8/49.5	2.1/4.1	18.6/7.4	208–10
23	$[CuL^3_2(NO_3)_2]$	$C_{18}H_{16}CuN_{10}O_{12}S_4$	8.4/7.8	28.6/39.0	2.1/3.1	18.5/7.0	208–9

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

sulfonamide vibrations, and (ii) the Schiff base vibration ν(C=N). Thus, in metal complexes reported here, the two sulfonamide vibrations (appearing at 1140–1145, and 1325–1330 cm^{-1}, respectively, in ligands **8**–**10**) are very slightly shifted to higher wavenumbers (1140–1150, and 1335–1340 cm^{-1}) in complexes **12**–**23**, a situation previously seen for similar metal derivatives.[14–20,27] The C=N vibration on the other hand is subjected to a more significant shift (of around 15 cm^{-1}), mainly due to the involvement of this moiety in coordinating the metal ion.[14–20,27]

The electronic spectroscopic data for the Zn(II), Cu(II), Co(II) and Ni(II) derivatives (Table II), showed that the Zn(II) derivative contains only the band due to the ligand, whereas for the paramagnetic metal ion derivatives, the corresponding maxima due to d–d transitions characteristic for each specific ion in octahedral geometry were seen, as previously reported for similar complexes of sulfonamide CA inhibitors.[14–20,27]

The following observations can be made about inhibition of isozymes CA I and II with the obtained Schiff's bases **8**–**11** and complexes **12**–**23** (Table III). Thus, Schiff's bases derived from aromatic sulfonamides of type **7**–**9** behave as weak inhibitors against isozymes hCA I, being slightly better hCA II inhibitors. On the other hand, the heterocyclic derivatives **10** and **11** are much better inhibitors against both isozymes, with K_I-s in the range of 65–325 nM against hCA I, and 33–74 nM against hCA II, respectively. Metal complexes **12**–**23** on the other hand, are much more potent inhibitors against both isozymes, their potency being enhanced as compared to that of the parent sulfonamides **8**–**10** as well as sulfanilamide (the standard inhibitor), a feature shared with many metal complexes of sulfonamide CA inhibitors reported up to now.[10–25] Thus, all the prepared complexes showed

TABLE II IR and electronic spectroscopic data for the ligands **8**–**11** and the metal complexes **12**–**23**

Compound	IR Spectra[a], cm^{-1} $\nu(SO_2)^s$;	$\nu(SO_2)^{as}$	ν(C=N)	Electronic Spectra[b] Wavenumber [ν, (cm^{-1})]
8	1140	1330	1625	28,255
9	1140	1325	1625	28,145
10	1145	1330	1630	28,315
11	1140	1330	1630	28,350
12	1150	1335	1645	28,255
13	1150	1340	1640	7,540, 17,245, 20,525
14	1150	1335	1645	10,215, 15,565, 26,240
15	1145	1335	1645	15,185, 19,580, 30,355
16	1145	1340	1640	28,145
17	1150	1335	1640	7,760, 17,145, 20,520
18	1150	1335	1645	10,315, 15,610, 26,175
19	1145	1335	1645	15,215, 19,485, 29,885
20	1150	1340	1640	28,315
21	1150	1335	1645	7,610, 17,355, 20,615
22	1150	1335	1645	10,315, 15,560, 26,345
23	1145	1335	1645	15,255, 19,565, 30,240

[a] In KBr. [b] In MgO as standard, by the diffuse reflectance technique.

TABLE III CA inhibition data with sulfanilamide, the Schiff's bases **7**–**11** and its metal complexes **12**–**23**, against isozymes I, and II, by the esterase method[33]

Compound	K_I (nM)*	
	hCA I#	hCA II#
Sulfanilamide	28000	300
7	35000	410
8	30000	240
9	12000	105
10	325	74
11	65	33
12	330	24
13	320	25
14	300	23
15	325	22
16	440	30
17	450	25
18	350	17
19	250	18
20	210	13
21	240	10
22	230	9
23	250	7

Human recombinant isozyme. * Errors in the range of ±10% of the reported value.

affinities in the range of 7–30 nM against hCA II, and 210–440 nM against hCA I, respectively (Table III). Against hCA II, the most effective inhibitors were generally those containing Cu(II), followed by those containing Co(II) ions, which in turn were more effective than those containing Ni(II) ions and Zn(II), but the differences in activity between these compounds are rather small. The nature of the counterion present in the coordination compound seems also to be of little importance for the biological activity of these derivatives (Table III).

In conclusion, in the present paper we report novel Schiff's bases metal complexes which possess a highly increased affinity in binding to the physiologically relevant isozymes hCA I and hCA II, as compared to the parent sulfonamides from which they were derived.

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