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RESEARCH ARTICLE

Synthesis and characterization of metal complexes of heterocyclic sulfonamide as carbonic anhydrase inhibitors

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Abstract
Three novel metal complexes of N-[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]-4-benzoyl-1-(3-nitrophenyl)-5-phenyl-1H-pyrazole-3-carboxamide which possess strong carbonic anhydrase (CA) inhibitory properties have been synthesised. The structure of these compounds has been investigated by elemental analysis, FT-IR, LC/MS, UV–vis spectrophotometric method and magnetic susceptibility. Human carbonic anhydrase isoenzymes hCA-I and hCA-II were purified from erythrocyte cells by the affinity chromatography. The inhibitory effects of newly synthesized complexes and acetazolamide (**AAZ**) as a control compound on hydratase and esterase activities of these isoenzymes have been studied *in vitro* by comparing IC_{50} and K_i values and it has been found that the newly synthesised complexes behave as very powerful inhibitors against hCA-I and hCA-II than parent ligand (**1**) and than **AAZ**.

Keywords: Pyrazole, sulfonamide, carbonic anhydrase, metal complexes, acetazolamide

Introduction

Carbonic anhydrase (CA, EC 4.2.1.1) is a zinc metalloenzyme that catalyzes the reversible reactions of CO_2 and water: $CO_2 + H_2O \rightarrow H^+ + HCO_3^-$. The treatment of glaucoma with inhibitors of the metalloenzyme carbonic anhydrase (CA II and CA IV) is very effective in reducing elevated intraocular pressure (IOP) due to CO_2, characteristic of this disease[1–5]. The 16 different isoenzymes of carbonic anhydrase (CA, EC 4.2.1.1) are known in humans[6–10]. Sulfonamides inhibiting the zinc enzyme carbonic anhydrase are widely used pharmacological agents for the treatment of glaucoma[11–16]. 1,3,4-Thiadiazole-2-sulfonamide derivatives[17–21] played a critical role in the development of the antiglaucoma drugs with carbonic anhydrase (CA) inhibitory action[22,23]. Neutral sulfonamides are expected to be poor ligands because of the withdrawal of the electron density from the nitrogen atom onto the electronegative oxygen atoms. However, if the sulfonamide N atom bears a dissociable hydrogen atom, this same electron-withdrawing effect increases its acidity and, in the deprotonated form, sulfonamide anions are effective σ-donor ligands. Metal complexes of sulfonamides containing a large number of main group or transition metal ions, were shown to possess very strong CA inhibitory properties[24–29]. Coordination metal compounds of heterocyclic sulfonamides are 10–100 times more active as CA inhibitors than the free sulfonamides[24–29].

The ligand **1** used in this study has already been developed in our laboratories as CA inhibitor[30] and we only prepared its metal complexes **2**, **3** and **4** by the reaction of **1** with Co(II), Ni(II) and Cu(II) (Scheme 1). These complexes can be seen as candidates for lowering intraocular pressure (IOP). The newly synthesised complexes **2-4** were characterised by spectroscopic methods in order to assign their structures, and were assayed as CA inhibitors against hCA-I and hCA-II isoenzymes.

Materials and methods

Metal salts ($CoCl_2.6H_2O$, $Ni(NO_3)_2.6H_2O$ and $CuCl_2.2H_2O$) and the other reagents were the highest grade commercially available and used without further purification. Compound **1** was synthesized by literature

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methods[30]. Elemental analyses for C, H, N and S were performed on a Leco CHNS-932 instrument. Mass spectra data were determined by Varian Mat III 80 eV. IR spectra were recorded on a Bruker Optics, vertex 70 FT-IR spectrometer using ATR techniques. The UV-vis spectra were carried out with a SHIMADZU UV-2550 spectrometer in the range 900–200 nm. Magnetic susceptibility measurements at room temperature were taken using a Sherwood Scientific Magway MSB MK1 model magnetic balance by the Gouy method using $Hg[Co(SCN)_4]$ as calibrant.

General procedure for syntheses of complexes 2-4

A solution of metal salt (7.54×10^{-5} mol) in 10 mL ethanol was added with continuous stirring to 10 mL of an ethanolic solution containing N-[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]-4-benzoyl-1-(3-nitrophenyl)-5-phenyl-1H-pyrazole-3-carboxamide (**1**) (1.5×10^{-4} mol). The pH of the reaction mixture was adjusted by addition of 0.1 M aqueous NaOH solution in the range between 6.0 and 7.0. The reaction mixture was stirred for 3 h at room temperature to give a solid of complexes **2-4**.

Synthesis of $[CoL_2(H_2O)_2].2H_2O$ (2)

Pink solid, 40%, m.p. decompose; IR (cm^{-1}) : 3500-3200 (-OH), 3385 and 3265 (-NH_2), 3094 (ar-CH), 1662 (benzoyl C=O), 1602 (amide C=O), 1535, 1492 (C=C and C=N), 1349 and 1173 (-SO_2), 595 (Co-O) and 515 (Co-N); UV-vis, [λ (nm), ε_{max} ($M^{-1}\,cm^{-1}$)]: 267 (8690), 282 (18530), 330 (25720) (π-π*), 587 (220), 666 (240), 787(290) (d-d); MS(CI) *m/z* : 1207.0 $[Co(C_{25}H_{17}N_7O_6S_2)_2]^+$, 1157.08 $[(C_{25}H_{17}N_7O_6S_2)2]^+$, 575.9 $[C_{25}H_{17}N_7O_6S_2]^+$; Anal. Calcd for $C_{50}H_{40}CoN_{14}O_{16}S_4$: C, 46.91; H, 3.15; N, 15.32; S, 10.02. Found: C, 46.93; H, 3.09; N, 15.07; S, 10.35.

Synthesis of $[NiL_2(H_2O)_2]$ (3)

Blue solid, 65%, m.p. decompose; IR (cm^{-1}) : 3500-3200 (-OH), 3222(-NH_2), 3068 (ar-CH), 1667 (benzoyl C=O), 1600 (amide C=O), 1530, 1501 (C=C and C=N), 1348 and 1173 (-SO_2), 593 (Ni-O) and 512 (Ni-N); UV-vis, [λ (nm), ε_{max} ($M^{-1}\,cm^{-1}$)]: 275 (10890), 282 (18370), 329 (24450) (π-π*), 414 (440), 564 (400), 797(270) (d-d); MS(CI) *m/z*: 1206.0 $[Ni(C_{25}H_{17}N_7O_6S_2)_2]^+$, 1157.08 [(C25H17N7O6S2)2]+, 1151.0 $[(C_{25}H_{17}N_7O_6S_2)_2]^+$, 576.0 $[(C_{25}H_{17}N_7O_6S_2)]^+$; Anal. Calcd for $C_{50}H_{36}NiN_{14}O_{14}S_4$:C, 48.28; H, 2.92; N,1 5.76; S, 10.31. Found: C, 47.64; H, 2.52; N, 15.43; S, 10.24.

Synthesis of $[CuL_2(H_2O)_2].H_2O$ (4)

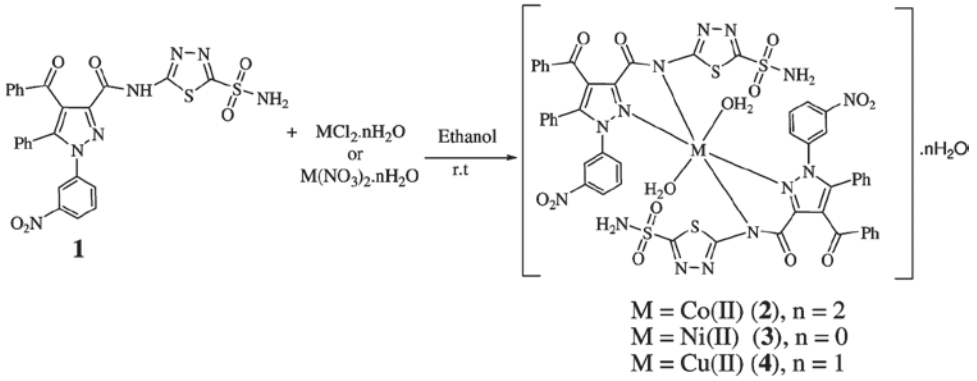
Green solid, 70%, m.p. 300°C; IR (cm^{-1}) : 3500-3200 (-OH), 3191(-NH_2), 3066 (ar-CH), 1668 (benzoyl C=O), 1616 (amide C=O), 1530, 1500 (C=C and C=N), 1348 and 1169 (-SO_2), 591 (Cu-O) and 487 (Cu-N); UV-vis, [λ (nm), ε_{max} ($M^{-1}\,cm^{-1}$)]: 273 (4530), 282 (9500), 312 (8540) (π-π*), 699 (180) (d-d); MS(CI) *m/z*: 1210.8 $[Cu(C_{25}H_{17}N_7O_6S_2)_2]^+$, 1150.9 $[(C_{25}H_{17}N_7O_6S_2)_2]^+$, 576.0 $[(C_{25}H_{17}N_7O_6S_2)]^+$; Anal. Calcd for $C_{50}H_{38}CuN_{14}O_{15}S_4$: C, 47.41; H, 3.02; N, 15.48; S, 10.13. Found: C, 47.69; H, 2.96; N, 15.05; S, 10.98.

Purification of isoenzymes hCA-I and hCA-II from human erythrocytes

In order to purify hCA-I and hCA-II isoenzymes, first, human blood was centrifuged at 1500 rpm for 20 min, and after the removal of the plasma, the erythrocytes were washed with an isotonic solution (0.9 % NaCl). After that, the erythrocytes were lysed with 1.5 volume of ice-cold water. The lysate was centrifuged at 20,000 rpm for 30 min to remove cell membranes and non-lysed cells. The pH of the supernatant was adjusted to 8.7 with Tris and was then loaded onto an affinity column containing Sepharose-4B-L-tyrosine-p-aminobenzene sulfonamide as the binding group. After extensive washing with 25 mM Tris–HCl/22 mM Na_2SO_4 (pH 8.7), the hCA-I and hCA-II isoenzymes were eluted with 1.0 M NaCl/25 mM Na_2HPO_4 (pH 6.3) and 0.1 M CH_3COONa/0.5 M $NaClO_4$ (pH 5.6)[31,32]. The amount of purified protein was estimated by the Bradford method[33] and SDS–PAGE was carried out to determine whether the elute containing the enzyme[34]. SDS polyacrylamide gel electrophoresis was performed after the purification of the enzyme (Supplementary Figure 1).

Hydratase activity assay

Carbonic anhydrase activity was assayed by following the hydration of CO_2 according to the method described by Wilbur and Anderson[35]. CO_2-hydratase activity as an enzyme unit (EU) was calculated by using the equation



Scheme 1. Syntheses of complexes **2**-**4**.

((t_0-tc)/tc) where t_0 and tc are the times for pH change of the nonenzymatic and the enzymatic reactions, respectively. IC_{50} values (the concentration of inhibitor producing a 50% inhibition of CA activity) have been obtained as *in vitro* for the newly synthesized compounds **2**-**4** and **AAZ** as the control compound.

Esterase activity assay

Carbonic anhydrase activity was assayed by following the change in absorbance at 348 nm of 4-nitrophenylacetate (NPA) to 4-nitrophenylate ion over a period of 3 min at 25°C using a spectrophotometer (CHEBIOS UV–VIS) according to the method described in the literature[36,37]. The enzymatic reaction, in a total volume of 3.0 mL, contained 1.4 mL of 0.05 M Tris–SO_4 buffer (pH 7.4), 1 mL of 3 mM 4-nitrophenylacetate, 0.5 mL H_2O and 0.1 mL enzyme solution. A reference measurement was obtained by preparing the same cuvette without enzyme solution. IC_{50} values have been obtained as *in vitro* for free the novel synthesized compounds **2**-**4** and **AAZ** as the control compound.

Determination of K_i values

The method for determination of K_i values is described elsewhere[38–42]. In the first part of this study, IC_{50} values have been obtained as *in vitro*. IC_{50} of the inhibitors (the synthesized complexes (**2**-**4**) and **AAZ** as the control compound) were assayed by the hydrolysis of *p*-nitrophenylacetate on esterase activities of CA isoenzymes in the presence of various inhibitor concentrations. The absorbance was determined at 348 nm after 3 min[38]. Regression analysis graphs were drawn by plotting inhibitor concentrations versus enzyme activity by using Microsoft Excel Program.

In the second part of the study, enzyme activity was measured in the presence of five different substrate concentrations at each of these inhibitor concentrations (30%, 50%, and 70%), and the data were linearized with Lineweaver-Burk plot in order to obtain K_i values.

Results and discussion

We herein report the syntheses of the complexes **2**-**4** by treatment of **1**, with metal salts in ethanol (Scheme 1).

FT-IR measurments

The modifications of the characteristic IR bands of **1**[30] are indicative of the deprotonation and/or coordination of the ligand to the metal ions.

The bands in the region 3500–3200 cm^{-1} are associated with the stretching vibrations of the O-H bond in the complexes **2**-**4**. Two stretching frequencies were observed for the asymmetric (3385 cm^{-1}) and symmetric (3265 cm^{-1}) vibrations of the sulfonamido -NH_2 moiety for compound **2**. This band was observed at 3222 cm^{-1} for **3** and 3191 cm^{-1} for **4**. The bands corresponding to the amide ν(C=O) vibration of the pyrazole carboxamide (1651 cm^{-1}) of **1**[30] is observed as a single band shifted to lower frequencies (about 1616–1600 cm^{-1}) in the complexes (**2**-**4**). This fact could be attributed to the modifications that take place on the deprotonation of the nitrogen atom of carboxamido group[43]. The changes found in the bands assigned to the ν(C=C) and ν(C=N) vibrations are at 1535–1530 and 1501–1492 cm^{-1} for complexes **2**-**4** and as these vibrations appear at 1588 and 1494 cm^{-1} for **1**[30]. These data are also attributed to the coordination occurring from N atom of pyrazole ring of **1**[30]. M-O and M-N vibrations are observed in the range 595–591 and 515–487 cm^{-1} for complexes **2**-**4**.

UV/vis spectrum and magnetic susceptibility

The electronic spectra of compounds **2**-**4** were recorded in DMSO solutions at a 1×10^{-3} M concentration at room temperature. The electronic spectra of the complexes confirmed their geometry. The electronic spectrum of **2** exhibits three intense absorption bands at 267, 282 and 330 nm attributed to π-π* transitions. These bands are observed at 275, 282 and 329 nm for compound **3** and 273, 282 and 312 nm for compound **4** similarly to other sulfonamide derivatives containing 1,3,4-thiadiazole-2-sulfonamide ring[44]. In addition, the electronic spectra of complexes (**2** and **3**) show three weak intensity bands at 587, 666 and 787 nm for complex **2** and the bands at 414, 564 and 797 nm for complex **3** and a band at 699 nm for compound **4** which may tentatively be assigned to octahedral geometry of metal(II) ions with d^7, d^8, d^9, respectively[45].

Magnetic susceptibility measurements were carried out on powdered samples at room temperature. The effective magnetic moments, 4.91 B.M. for **2**, 3.64 B.M. for **3** and 1.89 B.M. for **4** are consistent with d^7, d^8, d^9 octahedral metal (II) complexes, respectively[46,47].

Mass spectra of 2-4

The mass spectra give additional structural information about the chemical structure of the studied complexes **2**-**4**. None of the mass spectra of complexes shows a molecular ion (M^+) peak. The peaks at *m/z* 1207.0, 1157.08 and 575.9 for **2**, *m/z* 1206.0, 1151.0 and 576.0 for **3** and *m/z* 1210.8, 1150.9 and 576.0 for **4** are due to the fragments, $[ML_2]^+$ and $[L_2]^+$ and $[L]^+$, respectively which suggest the monomeric nature of the complexes. These data confirm the proposed formulas of the complexes (Scheme 1).

In vitro inhibition studies

Inhibition effects on hCA-I and hCA-II isoenzymes of the newly synthesized compounds (**2**-**4**) and acetazolamide (**AAZ**) as a control compound were studied by hydratase and esterase activity methods and K_i values were determined for each compound and compared to inhibition effect of free ligand (**1**)[30] (Table 1).

According to the *in vitro* studies, the IC_{50} values of hydratase activities of newly synthesized compounds **2**, **3** and **4** (0.49, 0.075 and 0.19 μM for hCA-I and 0.013, 0.0625 and 2.2 μM for hCA-II, respectively) are generally lower than the IC_{50} values of **1** (1.2 and 0.4 μM for hCA-I

Table 1. IC_{50} and K_i values of **AAZ** and **1** and newly synthesized compounds **(2-4)** for hCA I and hCA II isoenzymes.

	Hydratase (IC_{50} μM)		Esterase (IC_{50} μM)		K_i (μM)	
Compound	hCA-I	hCA-II	hCA-I	hCA-II	hCA-I	hCA-II
AAZ	3.3	2.4	4.6	3.9	2.8	2.1
1	1.2	0.4	1.4	3.0	1.40	3.0
2	0.49	0.013	0.25	0.1	0.280	0.145
3	0.075	0.0625	0.125	0.170	0.081	0.175
4	0.19	2.2	0.280	0.260	0.316	0.325

and hCA-II, respectively)[30] and of **AAZ** (3.3 and 2.4 μM for hCA-I and II, respectively). The IC_{50} values of esterase activities of compounds **2**-**4** are in the same trend with hydratase activities of **2**-**4** (0.25, 0.125 and 0.280 μM for hCA-I and 0.1, 0.170 and 0.260 for hCA-II, respectively). The IC_{50} values of esterase activities for free ligand (**1**)[30] and **AAZ**, have been obtained as 1.4 and 4.6 μM for hCA-I and 3.0 and 3.9 μM for hCA-II, respectively.

In relation to the esterase activities, the inhibition equilibrium constants (K_i) were also determined. The pyrazole carboxamide group of **1** might form a hydrogen bond with the histidine residue in the active site of CA to inhibit the isozymes (K_i = 1.40 and 3.0 μM for hCA-I and II), resulting in less movement of the carbonic acid toward CO_2 production as suggested in a study[48]. The coordination compounds (**2**-**4**) show remarkable inhibition on hCA-I and II (K_i: 0.28, 0.081 and 0.316 μM and 0.145, 0.175 and 0.325 μM, respectively), having a higher inhibition as compared to **1**, as well as to the control compound AAZ (2.8 and 2.1 μM for hCA I and II), which is probably due to the further effect of the metal(II) ions on the histidine residues in the active site of carbonic anhydrase[49,50]. Especially compound **2** has shown remarkable inhibition against hCA-II and compound **3** has shown remarkable inhibition against hCA-I isoenzymes. The order of the metal ions for the inhibition of hCA-I for hydratase activity is Ni>Cu>Co, and of hCA-I for esterase activity is Ni>Co>Cu, and of hCA-II for hydratase activity is Co>Ni>Cu, and of hCA-II for esterase activity is also Co>Ni>Cu.

Conclusions

We propose here three novel metal complexes **2**-**4** of heterocyclic sulfonamide **1**; we found that these compounds proved to be stronger CA inhibitors than ligand **1** and **AAZ** against hCA-I and hCA-II isoforms. Electronic spectra, magnetic measurements and the other analyses show good agreement with the proposed structures of newly synthesized complexes **2**-**4**.

Declaration of interest

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