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Synthesis, characterization and antiglaucoma activity of a novel proton transfer compound and a mixed-ligand Zn(II) complex

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ABSTRACT

A novel proton transfer compound, pyridin-2-ylmethanaminium 2,4-dichloro-5-sulfamoylbenzoate (**1**), and a mixed-ligand Zn(II) complex, bis(2,4-dichloro-5-sulfamoylbenzoate)(2-aminomethylpyridine)aquazinc(II) monohydrate (**2**), have been synthesized from the same free ligands, which are 2,4-dichloro-5-sulfamoylbenzoic acid (Hsba) and 2-aminomethylpyridine (amp). They have been characterized by elemental, spectral (^{1}H NMR, IR and UV–vis.) and thermal analyses. Additionally, magnetic measurement and single crystal X-ray diffraction technique were applied to compound **2**. In the complex, Zn(II) ion exhibits a distorted octahedral configuration coordinated by O1 and O1^i atoms of two mono dentante sba anions and N1, N2, N2^i atoms of bidentante amp anion and a water molecule (O1w). The free ligands Hsba and amp, and the products **1** and **2,** and acetazolamide (AAZ) as the control compound, were also evaluated for their in vitro inhibitor effects on human Carbonic Anhydrase isoenzymes (hCA I and hCA II) purified from erythrocyte cell by affinity chromatography for their hydratase and esterase activities. The IC_{50} values of products **1** and **2** for hydratase activity are 0.26 and 0.13 μM for hCA I and 0.30 and 0.15 μM for hCA II, respectively. The IC_{50} values of the same inhibitors for esterase activity are 0.32 and 0.045 μM for hCA I and 0.29 and 0.23 μM for hCA II, respectively. In relation to esterase activities, the inhibition equilibrium constants (K_i) were also determined and found 0.25 and 0.058 μM on hCA I and 0.22 and 0.24 μM on hCA II for **1** and **2**, respectively. The comparison of the inhibition studies of newly synthesized compounds **1** and **2** to parent compounds Hsba and amp and to AAZ indicated that **1** and **2** have effective inhibitory activity on hCA I and II, and might be used potential inhibitors.

1. Introduction

The sulfonamides constitute an important class of drugs, with several types of pharmacological agents possessing antibacterial, antiglaucoma, diuretic, hypoglycemic, antithyroid, protease inhibitory and antitumor activity, among others.[1–4] With the sulfonamides as lead structures, different classes of pharmacological agents have been obtained, such as antibacterial sulfonamides that inhibit the zinc enzyme Carbonic Anhydrase (CA; EC 4.2.1.1), in which the zinc ion is the prosthetic group and is coordinated by histidine side chains in the active site. CA catalyzes the reversible hydration of carbon dioxide to bicarbonate and protons, a very simple but critically important physiological reaction for organisms.[5–10] This enzyme has 16 different isoenzymes presently known. Several of these isoenzymes (CA-II and CA-IV) are expressed in human eyes[11–14] causing glaucoma, which is a group of diseases characterized by the gradual loss of visual field due to an elevation in intraocular pressure (IOP), and is the second leading cause of blindness worldwide.[14,15] Since CA inhibitors have been shown to reduce intraocular pressure exclusively by lowering the aqueous humor flow, these compounds have been used for years for the treatment of glaucoma.[16,17]

Although there are limited reported crystal structure studies on Hsba derivatives,[18,19] a huge number of crystal structures of a wide range of metal complexes with aminopyridines were studied by means of X-ray diffraction. Aminopyridines and their derivatives in most cases act as monodentate ligands which coordinate the metal ions through the nitrogen of the ring,[20] though there are several works on 2-aminomethylpyridine complexes in which the amino group also participates in coordination.[21–25] 2-Aminomethylpyridines serve as useful chelating ligands in a variety of inorganic and organometallic applications.[26,27] The high incidence of pharmacological activity among heteroaromatic amines has also stimulated many research efforts to prepare compounds of this structural type.[28,29]

Zinc was chosen in this work, because zinc complexes have antimicrobial effect against bacteria, fungi, and viruses, as well as

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inhibitory effects against carbonic anhydrases, carboxy peptidases, thermolysin, and alcohol dehydrogenase.[30–32]

In this study, a novel proton transfer compound, namely pyridin-2-ylmethanaminium 2,4-dichloro-5-sulfamoylbenzoate (**1**), and a mixed-ligand Zn(II) complex, namely bis(2,4-dichloro-5-sulfamoylbenzoate)(2-aminomethylpyridine)aquazinc(II) monohydrate (**2**), formulated as $(Hamp)^+(sba)^-$ and $[Zn(sba)_2(amp)(H_2O)]\cdot H_2O$, respectively, have been synthesized and characterized by elemental, spectral (^{1}H NMR, IR and UV–vis) and thermal analyses. Magnetic measurement and single crystal X-ray analysis of the complex (**2**) were also reported. Furthermore, we have investigated the potential use of these compounds as new inhibitors of hCA isoenzymes in the treatment of glaucoma.

2. Experimental section

2.1. General methods and materials

All chemicals used were analytical reagents and were commercially purchased from Aldrich. $Zn(CH_3COO)_2\cdot 2H_2O$, 2-aminomethylpyridine and 2,4-dichloro-5-sulfamoylbenzoic acid were used as received. Elemental analyses for C, H, N and S were performed on Elementar Vario III EL and Zn was detected with Perkin Elmer Optima 4300 DV ICP-OES. ^{1}H NMR spectra were recorded with Bruker DPX FT NMR (500 MHz) spectrometer ($SiMe_4$ as internal standard and 85% H_3PO_4 as an external standard). FT-IR spectra were recorded in the 4000–400 cm^{-1} region with Bruker Optics, Vertex 70 FT-IR spectrometer using ATR techniques. Thermal analyses were performed on SII Exstar 6000 TG/DTA 6300 model using platinum crucible with 10 mg sample. Measurements were taken in the static air, within 30–700 °C temperature range. The UV–vis spectra were obtained for aqueous solutions of the compounds (10^{-3} M) with a SHIMADZU UV-2550 spectrometer in the range 900–200 nm. Magnetic susceptibility measurement at room temperature was performed using a Sherwood Scientific Magway MSB MK1 model magnetic balance by the Gouy method using $Hg[Co(SCN)_4]$ as calibrant. Molar conductance of the compound was determined in DMSO (10^{-3} M) at room temperature using a WTW Cond 315i/SET Model conductivity meter.

2.2. Synthesis of $(Hamp)^+(sba)^-$ (1) and $[Zn(sba)_2(amp)(H_2O)]\cdot H_2O$ (2)

A solution of amp (0.54 g, 5 mmol) in 10 mL ethanol was added to the solution of Hsba (1.35 g, 5 mmol) in 10 mL ethanol. The mixture was refluxed for 3 h, and then was cooled to room temperature. The reaction mixture was kept at room temperature for 3 h to give white solid of **1** (1.51 g, 80% yield).

A solution of $Zn(CH_3COO)_2\cdot 2H_2O$ (0.199 g, 1 mmol) in water (10 mL) was added dropwise to the mixed solution of free ligands (0.108 g, 1 mmol for amp and 0.54 g, 2 mmol for Hsba) in ethanol (20 mL) with stirring at room temperature for 1 h. The reaction mixture was kept at room temperature for two weeks to give white crystalline solid (0.412 g, 55% yield). On recrystallisation, colorless crystals of **2** suitable for X-ray diffraction were separated and washed with water.

Anal. Calcd for **1** ($C_{13}H_{13}Cl_2N_3O_4S$): C, 41.25; H, 3.45; N, 11.13; S, 8.48. Found: C, 41.28; H, 3.46; N, 11.11; S, 8.48; Anal. Calcd for **2** ($C_{20}H_{18}Cl_4N_4O_{10}S_2Zn$): C, 32.20; H, 2.40; N, 7.50; S, 8.58; Zn, 8.70. Found: C, 32.21; H, 2.43; N, 7.51; S, 8.60; Zn, 8.77.

2.3. Crystal structure determination of 2 ($C_{20}H_{18}Cl_4N_4O_{10}S_2Zn$)

The crystal and instrumental parameters used in the unit-cell determination and data collection are summarized in Table 1 for the compound **2**. Crystallographic data of **2** were recorded on a Stoe IPDS II CCD X-ray diffractometer employing plane graphite monochromatized with Mo Kα radiation ($\lambda = 0.71073$ Å), using $\omega - 2\theta$ scan mode. Unit cell parameters were refined from the setting angles of 25 centered reflections in the range of $2.15 \leqslant \theta \leqslant 26.50$ for the complex compound. The structures were solved by the direct methods and refined by full-matrix least-squares techniques on F^2 using the solution program SHELXS-97 and refined using SHELXL-97.[33] The empirical absorption corrections were applied by the integration method via X-RED software.[34] All non-hydrogen atoms were refined with anisotropic displacement parameters and hydrogen atoms were included in their idealized positions and refined isotropically except for the water molecules. Hydrogen atoms of water molecules were located from difference maps and refined with isotropic thermal parameters, with distance of O–H = 0.813(13)–0.822(11) Å and with $U_{iso}(H) = 1.2U_{eq}(O)$. The crystal data and structure refinement details for compound **2** are given in Table 1. ORTEP drawings[35] of the complex compound with 40% probability displacement thermal ellipsoids and atom-labelling schemes are shown in Figures 1, 2 and 3.

Table 1
Crystal data and structure refinement details for compound **2**

Empirical formula	$C_{20}H_{18}Cl_4N_4O_{10}S_2Zn$
Formula weight	745.71
Temperature (K)	296(2)
Wavelength (Å)	0.71073
Crystal system, space group	Monoclinic, $P\,2_1/m$
Unit cell dimensions: (Å, °)	
a	7.8650(4)
b	18.9429(9)
c	9.1899(5)
β	89.839(4)
V (Å^3)	11369.16(12)
Z	2
μ (mm^{-1})	1.502
D_{calcd} (Mg m^{-3})	1.809
$F(0\,0\,0)$	752
θ range (°)	2.15–26.50
Crystal size (mm)	0.580 × 0.460 × 0.340
Absorption correction	Integration
Limiting indices	$-9 \leqslant h \leqslant 9$, $-23 \leqslant k \leqslant 23$, $-11 \leqslant l \leqslant 11$
Reflections collected	17,221
Unique reflections	2920
Observed reflections	2728
Parameters refined	223
Refinement method	Full-matrix least-squares on F^2
R_{int}	0.0298
Final R indices [$I \geqslant 2\sigma(I)$]	$R_1 = 0.0267$, $wR_1 = 0.0664$
R indices (all data)	$R_2 = 0.0292$, $wR_2 = 0.0676$
Goodness of fit on F^2	1.076
Largest difference peak and hole (e Å^{-3})	−0.467 and 0.427

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

In order to purify hCA-I and 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 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).[36,37] The amount of purified protein was estimated by the Bradford method[38] and SDS–PAGE was carried out to determine whether the elute contained the enzyme.[39]

2.5. Hydratase activity assay

Carbonic anhydrase activity was assayed by following the hydration of CO_2 according to the method described by Wilbur and Anderson.[40] CO_2-hydratase activity as an enzyme unit (EU) was calculated by using the equation $((t_0 - t_c)/t_c)$ where t_0 and t_c 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 free ligands amp and Hsba, and the synthesized compounds **1** and **2,** and acetazolamide (AAZ) as the control compound.

2.6. 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.[41,42] 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 ligands amp and Hsba, and the synthesized compounds **1** and **2,** and AAZ as the control compound.

2.7. Determination of K_i values

The method for determination of K_i values is described elsewhere.[43–47] According to this study, in its first part, IC_{50} values have been obtained as in vitro. IC_{50} of the inhibitors (free ligands amp and Hsba, and the synthesized compounds **1** and **2**, 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.[43] Regression analysis graphs were drawn by plotting inhibitor concentrations versus enzyme activity by using Microsoft Excel Program.

In the second part of the study, the concentrations of inhibitors which give results 30%, 50%, and 70% inhibition on isoenzymes were determined at five different substrate concentrations (2.4 μM, 4.8 μM, and 7.3 μM). At each of these inhibitor concentrations (30%, 50%, and 70%), enzyme activity was then measured in the presence of various substrate concentrations given above and the data were linearized with Lineweaver–Burk plot in order to obtain K_i value (Fig. 4).

3. Results and discussion

3.1. Crystal structure of $[Zn(sba)_2(amp)(H_2O)]\cdot H_2O$ (2)

The molecular structure of $[Zn(sba)_2(amp)(H_2O)]\cdot H_2O$ (**2**), with the atom labeling of asymmetric unit and symmetric unit of **2**, are shown in Figures 1 and 2, respectively. The crystal packing diagram of **2** is shown in Figure 3. The details of the crystal structure solution are summarized in Table 1. The selected bond lengths and angles are listed in Table 2. The compound crystallizes in the monoclinic *P* 21/*m* space group. The asymmetric unit contains

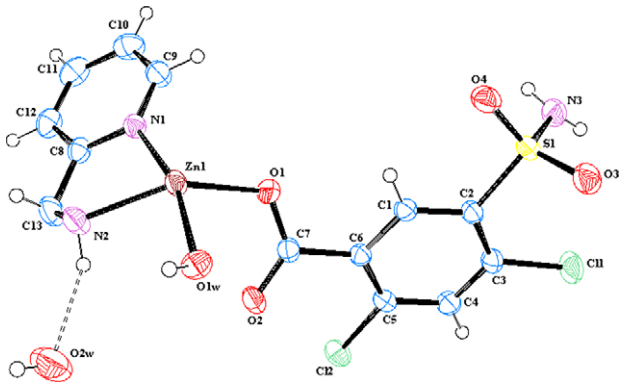


Figure 1. An ORTEP drawing of asymmetric unit of **2** with the atom-numbering scheme. Displacement ellipsoids are drawn at the 40% probability level.

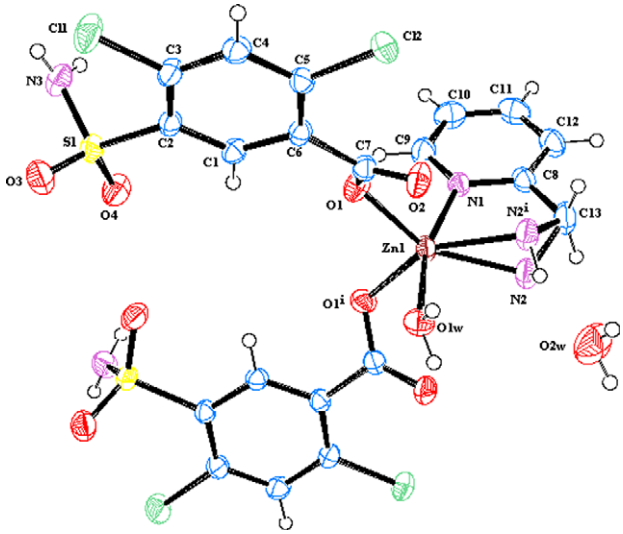


Figure 2. An ORTEP drawing of symmetric unit of **2** with the atom-numbering scheme. Displacement ellipsoids are drawn at the 40% probability level.

one sba anion, one amp anion, in which methylene carbon (C13) has one hydrogen atom and amine nitrogen (N2) has half hydrogen atom, one Zn(II) cation, half coordinated water molecule and half uncoordinated water molecule (Fig. 1).

In the symmetric unit (Fig. 2), the coordination environment of the Zn(II) ion has a distorted octahedral configuration coordinated by O1 and $O1^i$ atoms of two mono dentante sba anions and N1, N2, $N2^i$ atoms of bidentante amp anion and a water molecule (O1w). One of the H atoms on methylene carbon (C13) of amp molecule belongs to neighbor symmetric unit, while amine nitrogen (N2) in the amp molecule bears one H atom. The other H atom of $-NH_2$ group of free amp molecule was probably lost during complex formation in order to balance the charge in the complex (**2**). Two sba oxygen atoms (O1, $O1^i$) and two amp nitrogen atoms (N1, N2) comprise equatorial plane, while water oxygen atom (O1w) and amp nitrogen atoms (N1) occupy the axial positions [O1w–Zn–N1 = 164.88(9)°]. The Zn–O bond distances range from 1.9926(13) to 2.191(2) Å, and Zn–N1 and Zn–N2 bond distances are 2.072(3) and 2.161(2) Å, respectively. These values are in good agreement in the literature.[48–52] The Zn–O1w bond distance, arising from coordination of the water molecule to the central metal ion, is 2.191(2) Å, which is much longer than the Zn–O (carboxylate) bond distances.

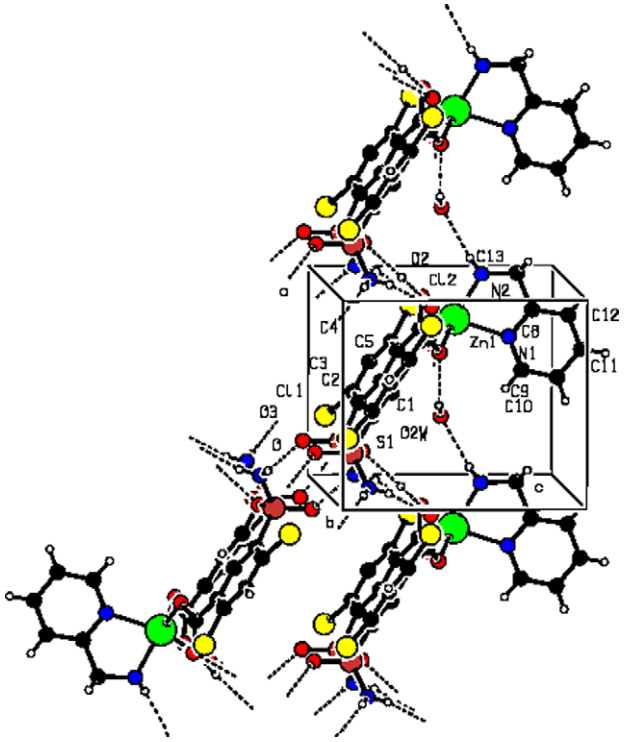


Figure 3. Crystal packing diagram of **2**.

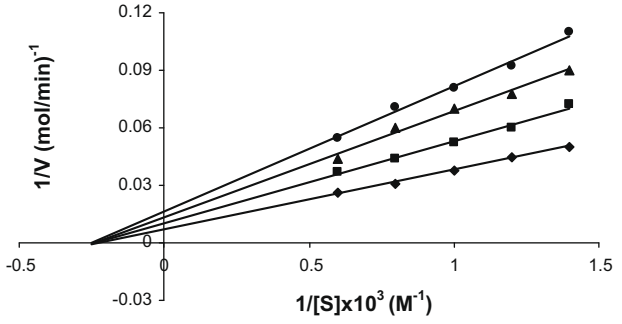


Figure 4. K_i graph obtained from in vitro studies for compound **1** on hCA-II.

Table 2
Selected bond distances (Å) and angles (°) for **2**

C2–S1	1.7793(18)	Zn1–O1	1.9926(13)
C3–Cl1	1.7239(19)	Zn1–O1^{i}	1.9926(13)
C5–Cl2	1.7288(18)	S1–O3	1.4295(16)
Zn1–N1	2.161(2)	S1–O4	1.4314(16)
Zn1–N2	2.072(3)	S1–N3	1.5836(18)
Zn1–N2^{i}	2.072(3)	Zn1–O1w	2.191(2)
N2–N2^{i}	1.011(8)		
O1–Zn1–N1	97.77(6)	N1–Zn1–N2	78.26(11)
O1–Zn1–N2	145.38(11)	N1–Zn1–O1w	164.88(9)
O1–Zn1–O1w	92.17(6)	N2–Zn1–O1w	87.08(11)
O3–S1–O4	119.26(10)	N2–Zn1–N1	78.26(11)
O3–S1–N3	107.84(10)	N2–Zn1–N2^{i}	28.3(2)
O4–S1–N3	107.28(10)	O1^{i}–Zn1–N2^{i}	145.38(11)
O1–Zn1–O1^{i}	97.49(9)	O1–Zn1–N2^{i}	117.13(11)

Symmetry codes: (i) *x*, −*y*+1/2, *z*.

Hydrogen bond between the uncoordinated water molecule and N–H group of amp plays an important role in stabilizing the crystal structure (Figs. 1 and 3). The parameters for hydrogen bonding interaction in the complex are as follows: H···O2w 2.21(2) Å, N2···O2w 2.932(4) Å, N2–H2···O2w 143.2(11)°.

3.2. FT-IR measurements

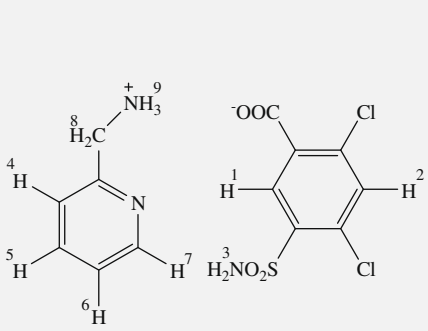
In the high frequency region, weak bands between 3100 and 3037 and between 3037 and 2850 cm^{-1} are attributed to the stretching ν(C–H) vibrations of aromatic and methylene groups, respectively. There is a broad absorption band at 2900 cm^{-1} attributed to the ν(OH) vibrations of carboxylate group of Hsba. This band is not observed in compound **1** due to proton transfer. There are also broad absorption bands at 3495 and 2623 cm^{-1} attributed to the ν(OH) vibrations of coordinated and uncoordinated water molecules, respectively, in the complex compound **2**. NH_2 vibrations at 3364 and 3289 cm^{-1} in amp are shifted to lower frequencies in compound **1** as 2821, 2740, 2707 cm^{-1} due to proton transfer to amine group.[53] An absorption band was arisen at 3321 cm^{-1}, which was assigned to NH group of amp in IR spectrum of the complex compound (**2**). NH_2 vibrations of sulfonamides in free Hsba (3425, 3278 cm^{-1}), in compounds **1** (3340, 3159 cm^{-1}) and **2** (3365, 3174 cm^{-1}) are observed with similar pattern. The absorption bands for compounds **1** and **2** have shifted 80 cm^{-1} in average to lower frequencies due to molecular interaction in solid state. The strong absorption bands for SO_2 groups in Hsba in compounds **1** and **2** are observed at the region of 1400–1070 cm^{-1} with similar profiles and almost similar vibrations.[54] The strong C=O vibration at 1684 cm^{-1} of Hsba is shifted to 1621 and 1624 cm^{-1} for compounds **1** and **2**, respectively, indicating the role of carboxylic acid group of free Hsba on the structures of the compounds prepared in this study.[55] The ν(C–O) vibration of Hsba is also shifted from 1169 cm^{-1} to 1116 and 1123 cm^{-1} in **1** and **2**, as indicated in.[56,57] The strong absorption bands at the region of 1400–1590 cm^{-1} are attributed to the ν(C=N) and ν(C=C) vibrations in free ligands, amp and Hsba, and compounds **1** and **2**.[58] The ring wagging vibrations of the pyridine groups are also observed at 751 and 625 cm^{-1} for amp, 770 and 628 cm^{-1} for compound **1**, and 799 and 687 cm^{-1} for compound **2**. The weak bands in the region of 573 and 520 cm^{-1} are responsible for the Zn–N and Zn–O vibrations, respectively, of the complex **2**.

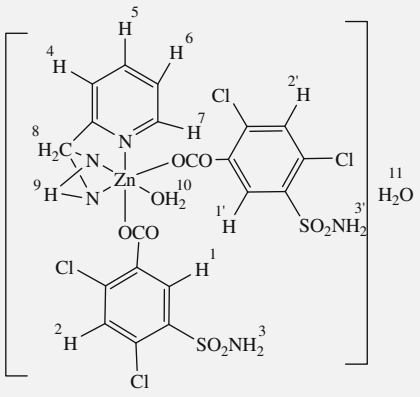
3.3. ^{1}H NMR studies of $(Hamp)^+(sba)^-$ (1) and $[Zn(sba)_2(amp)(H_2O)]\cdot H_2O$ (2)

The ^{1}H NMR spectra of the compounds (**1** and **2**) were obtained in DMSO-d_6 at room temperature using TMS as internal standard. Table 3 lists complete ^{1}H NMR assignments for the compounds **1** and **2**. The ^{1}H signals were assigned on the basis of chemical shifts, multiplicities, intensity of the signals and coupling constants. The CH_2 protons (H^8) are easily distinguishable as singlets, and they are observed at 4.19 ppm for **1** and 4.16 ppm for **2**. H^5 and H^6 protons of the amp rings are triplets and they are observed at 7.39 ppm ($^3J_{H5\text{-}H4,6}$ = 6.15 Hz) and 7.83 ppm ($^3J_{H6\text{–}H5,7}$ = 7.72 Hz) in compound **1**, respectively, and at 8.00 ppm ($^3J_{H5\text{–}H4,6}$ = 7.61 Hz) and 7.51 ppm ($^3J_{H6\text{–}H5,7}$ = 6.36 Hz, one of the bands of the triplet is thought to be under neighbor doublet due to the different coupling constants for these sets of peaks, see Figure 6) in compound **2**, respectively. In addition, H^4 and H^7 protons of the amp rings are doublets as expected and arisen at 7.43 ppm ($^3J_{H4\text{–}H5}$ = 7.82 Hz) and 8.57 ppm ($^3J_{H7\text{–}H6}$ = 4.65 Hz) in compound **1**, respectively, and at 8.61 ppm ($^3J_{H4\text{–}H5}$ = 6.36 Hz) and 7.54 ppm ($^3J_{H7\text{–}H6}$ = 8.07 Hz) in compound **2**, respectively. The ^{1}H NMR spectrum of compound **2** contains singlet peaks for H^1–$H^{1'}$ and H^2–$H^{2'}$ on aromatic ring of sba at 8.22 and 7.76 ppm with the intensity of two H atoms, respectively. However, in compound **1**, these peaks are seen at 7.91 ppm (H^1) and 7.62 ppm (H^2) with the intensity

of one H atom, which clearly indicate the formation of the proton transfer compound with 1:1 ratio of amp and Hsba. The broad singlet peaks at 4.3 ppm (H^9, with one H intensity) and 7.74 ppm (H^3–$H^{3'}$, with four H intensity) are assigned to –NH and $-SO_2NH_2$ groups in compound **2**. This result also indicates that one hydrogen atom was lost from NH_2 group of amp to give NH^- in order to bal-

Table 3
^{1}H NMR chemical shifts (ppm) with coupling constants and assignments for compounds **1** and **2**





H^8	4.19 (2H, s)	H^8	4.16 (2H, s)
H^5	7.39 (1H, t) [$^3J_{H5-H4,6}$ = 6.15 Hz]	H^9	4.30 (1H, s) (broad)
H^4	7.43 (1H, d) [$^3J_{H4-H5}$ = 7.82 Hz]	H^6	7.51 (1H, t) [$^3J_{H6-H5,7}$ = 6.36 Hz]
H^2	7.62 (1H, s)	H^7	7.54 (1H, d) [$^3J_{H7-H6}$ = 8.07 Hz]
H^6	7.83 (1H, t) [$^3J_{H6-H5,7}$ = 7.72 Hz]	H^3, $H^{3\prime}$	7.74 (4H, s) (broad)
H^1	7.91 (1H, s)	H^2, $H^{2\prime}$	7.76 (2H, s)
H^7	8.57 (1H, d) [$^3J_{H7-H6}$ = 4.65 Hz]	H^5	8.0 (1H, t) [$^3J_{H5-H4,6}$ = 7.61 Hz]
H^9, H^3	Not observed	H^1, $H^{1\prime}$	8.22 (2H, s)
		H^4	8.61 (1H, d) [$^3J_{H4-H5}$ = 6.36 Hz]
		H^{10}, H^{11}	Not observed

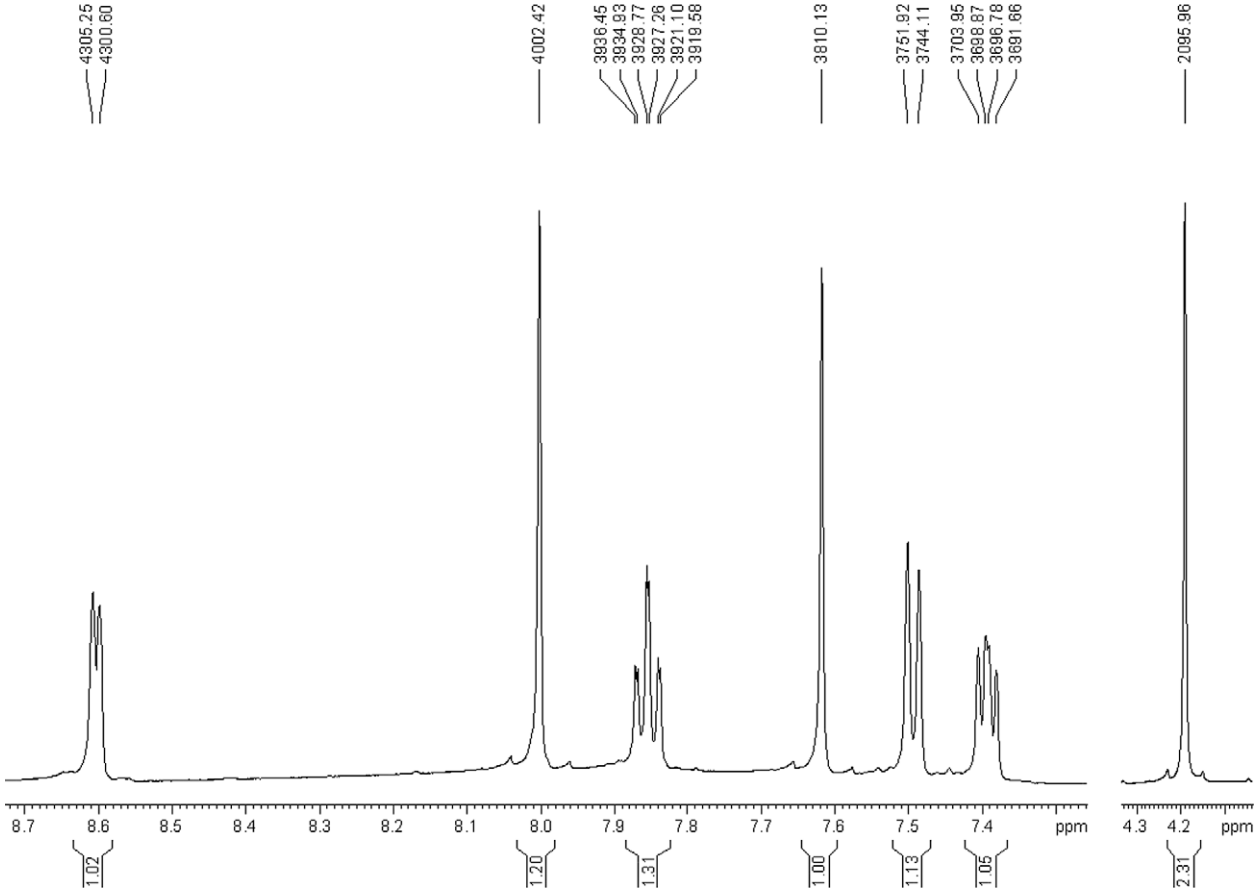


Figure 5. ^{1}H NMR spectrum of compound **1**.

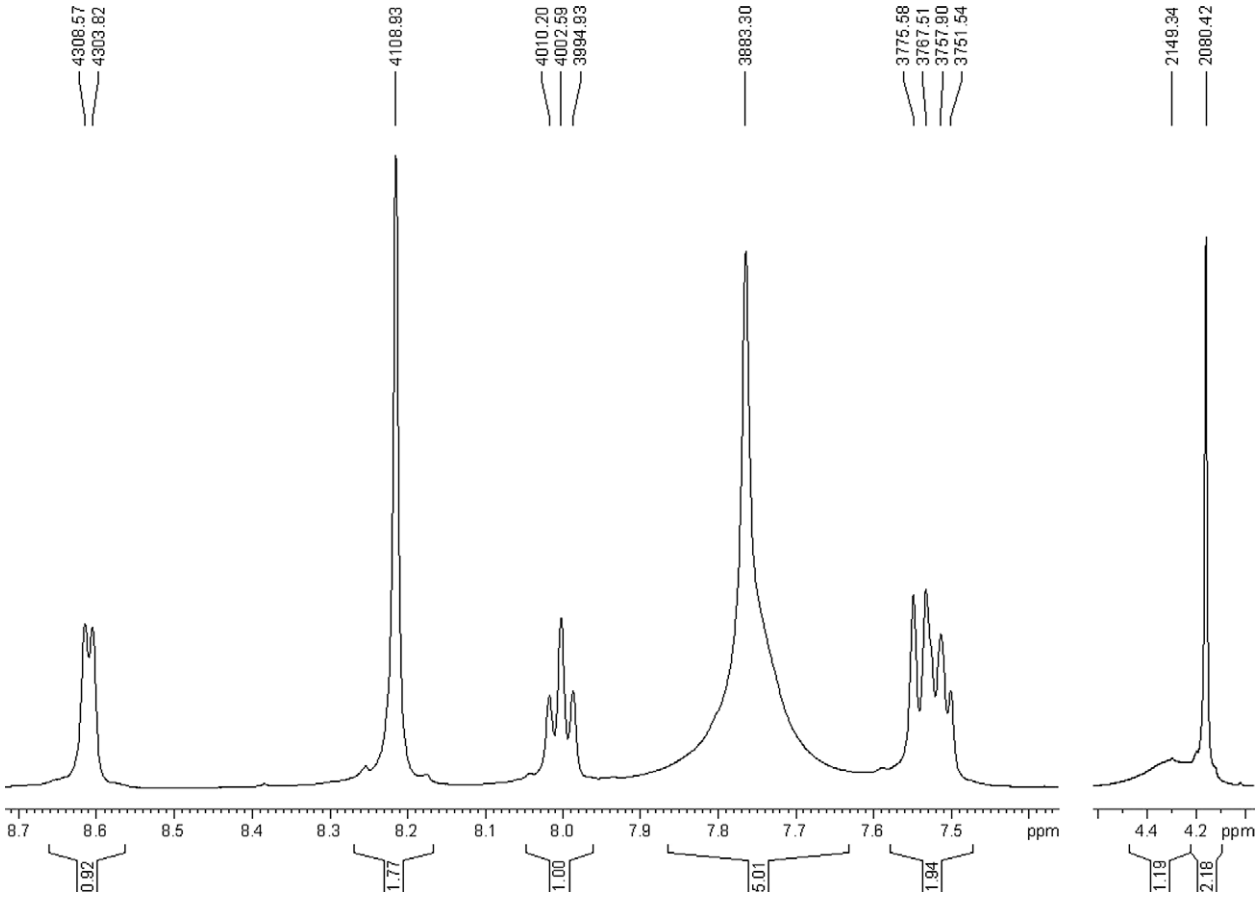


Figure 6. ^{1}H NMR spectrum of compound **2**.

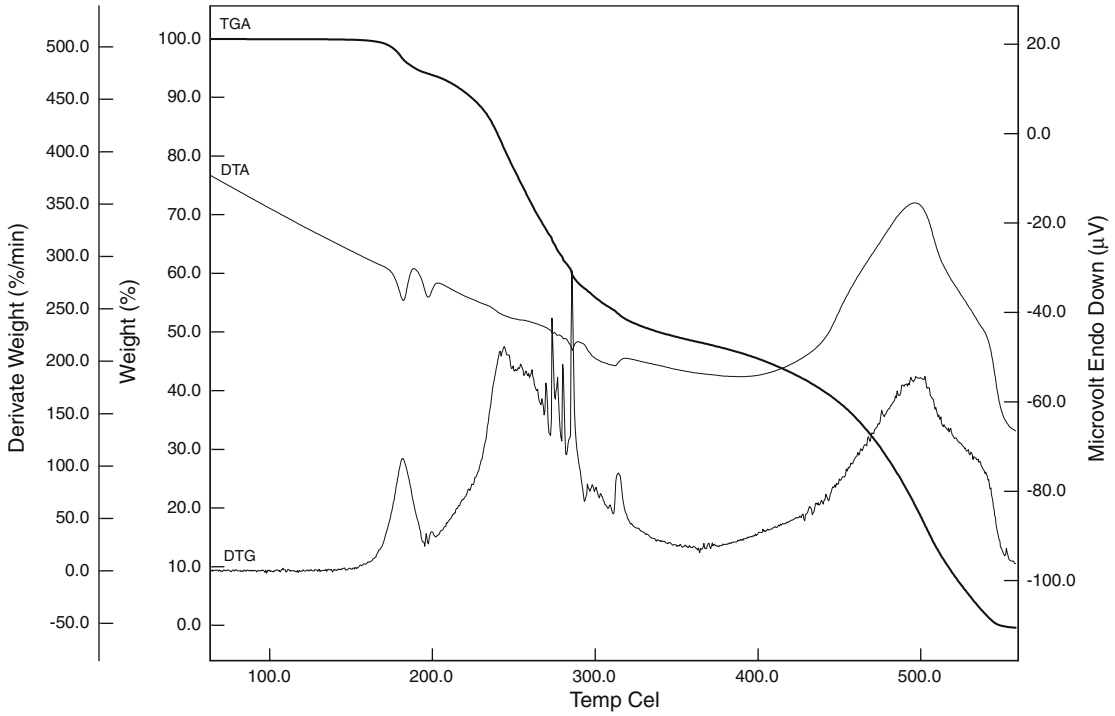


Figure 7. The TG-DTG and DTA curves of **1**.

ance the charge in complex compound (**2**). The hydrogen atoms, on $-NH_3$ (H^9), $-SO_2NH_2$ (H^3) in compound **1**, and on both water molecules (H^{10} and H^{11}) in compound **2**, have not been observed in the ^{1}H NMR spectra.

The room temperature ^{1}H NMR spectra, 500 MHz, for both compounds (Figs. 5 and 6) clearly indicate the formation of proton transfer compound and complex compound, and confirm that solid state structure of **2** is retained in solution.[48]

3.4. Thermal analyses of 1 ($C_{13}H_{13}Cl_2N_3O_4S$) and 2 ($C_{20}H_{18}Cl_4N_4O_{10}S_2Zn$)

Figures 7 and 8 show the TG-DTG and DTA curves of compounds **1** and **2**, respectively. The endothermic first stage (DTG_{max} = 182 °C), between 30 and 188 °C, corresponds to the loss of the NH_3 from amine group of **1** (found 4.50, calcd 4.50). The second stage, an endothermic peak (DTG_{max} = 241, 254 and 261 °C) between 188 and 263 °C, corresponds to the loss of C_6H_6N from amine group of **1** (found 24.5, calcd 24.4). Sulfonamide (SO_2NH_2) and carboxylate (COO) groups of Hsba are decomposed in the third stage between 236 and 450 °C with DTG_{max} at 273, 276, 280 and 314 °C (found 32.8, calcd 32.8). The final stage was the decomposition of the residue of sba, $C_6H_2Cl_2$, between 450 and 560 °C with DTG_{max} at 496 °C (found 38.2, calcd 38.3).

For the compound **2,** the first stage, an endothermic peak (DTG_{max} = 173 and 175 °C) between 30 and 184 °C, corresponds to the loss of the 2 mol of crystal water molecules (found 4.2, calcd 4.8). The endothermic second stage, (DTG_{max} = 187 °C) between 184 and 266 °C, is consistent to the loss of the C_2H_3N from amine group of **2** (found 5.2, calcd 5.5). The residue of amine group (C_4H_3N) and 2 moles sulfonamide (SO_2NH_2) are decomposed endothermically in the third stage between 266 and 371 °C with DTG_{max} at 302, 319, 334 and 345 °C (found 30.0, calcd 30.1). The fourth stage, an endothermic peak (DTG_{max} = 372 and 443 °C) between 371 and 485 °C, agrees to the loss of the 4 moles of chlorine of sba unit in compound 2. In the fifth stage, decomposition of the residue of sba is observed between 485 and 600 °C with DTG_{max} at 568 and 595 °C (found 32.0, calcd 31.8). The final decomposition product was ZnO identified by IR spectroscopy (found 9.7%, calcd 10.8).

Table 4
Optical properties of compounds **1** and **2**, and the free ligands amp and Hsba in water and DMSO

	λ_{max} (ε)			
	Amp	Hsba	(**1**)	(**2**)
Water		209(25520)	209(26150)	209(23460)
		236(12340)		236(10530)
	262(27370)		280(10200)	
DMSO	261(44340)	257(35120)	257(30100)	256(24450)
			264(41020)	
		285(11230)	280(15750)	283(16800)

3.5. UV–vis spectrum, magnetic susceptibility and conductivity

The electronic spectra of compounds **1** and **2**, and the free ligands amp and Hsba were recorded in solution at a 1×10^{-3} M concentration at room temperature (Table 4). Characteristic π–π^* transitions are in the spectra of **1** (209 and 280 nm) and of **2** (209 and 236 nm) in water solution with the same profiles as the free ligands Hsba (209 and 236 nm) and amp (262 nm). Two sets of characteristic π–π^* transitions were observed for all compounds, except amp in DMSO solutions, which were located around 257 and 283 nm. The more energetic π–π^* transition bands in water around 209 nm for all compounds, except amp, were red shifted to around 257 nm in DMSO solution spectra. The other π–π^* absorption bands at 283 nm for compound **1** in water were also observed in the same region (280 nm) in the spectra recorded in DMSO. The intensities of the absorption bands for all compounds in DMSO are, in general, higher than in water.

All the data which we obtained from the electronic spectra of the free ligands do not show any marked differences from those of either proton transfer compound or Zn(II) complex in both solutions.

Zn(II) complex does not show any magnetic properties on measurements at room temperature due to d^{10} electronic structure of

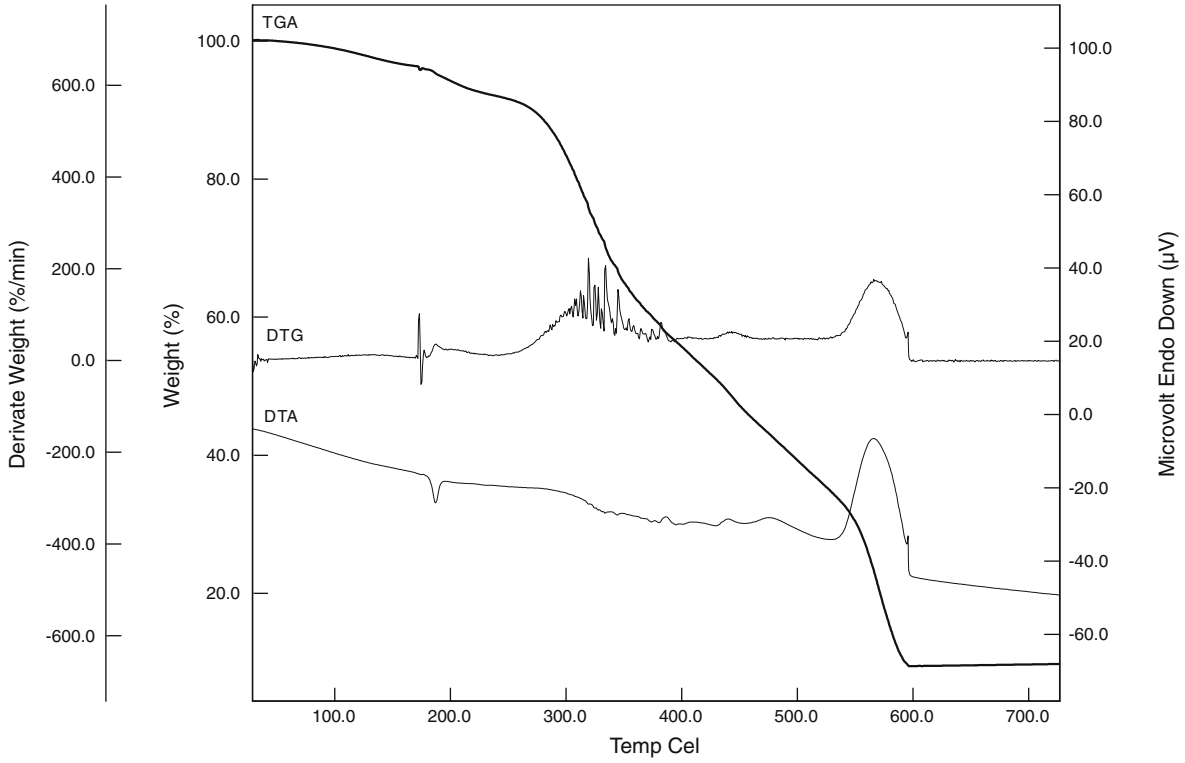


Figure 8. The TG-DTG and DTA curves of **2**.

Table 5
IC_{50} values of hydratase and esterase activities on hCA I and hCA II isoenzymes and K_i values

Inhibitor	Hydratase IC_{50} (μM)		Esterase IC_{50} (μM)		K_i values (μM)	
	hCA I	hCA II	hCA I	hCA II	hCA I	hCA II
AAZ	3.6	2.8	6.1	4.5	5.2	3.8
Amp	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition
Hsba	0.43	0.38	3.2	18.0	7.7	22
1	0.26	0.30	0.32	0.29	0.25	0.22
2	0.13	0.15	0.045	0.23	0.058	0.24

zinc(II) ion. The conductivity data in DMSO and in water are the 4.1 and 11.5 Ω^{-1} cm^2 mol^{-1}, respectively, indicating that the complex **2** is non-ionic.[59] This result agrees that one mole of sba anion and one mole of amp anion in asymmetric unit to form neutral coordination compound.

3.6. In vitro inhibition studies

Inhibition effects of parents compounds (amp and Hsba), newly synthesized proton transfer compound (**1**) and Zn(II) complex (**2**) and AAZ as the control compound on hCA I and hCA II enzymes were studied by hydratase and esterase activity methods and then K_i values were determined for each compound (Table 5).

According to in vitro studies, the IC_{50} values of hydratase activities of newly synthesized compounds **1** and **2** (0.26 and 0.13 μM for hCA I and 0.30 and 0.15 μM for hCA II, respectively) are lower than IC_{50} values of Hsba (0.43 and 0.38 μM for hCA I and II) and of AAZ (3.6 and 2.8 μM for hCA I and II). The IC_{50} values obtained from esterase activities of all compounds studied have been observed in similar trend, which are 0.32 and 0.045 μM for hCA I and 0.29 and 0.23 μM for hCA II for **1** and **2**, respectively. Hsba and AAZ have esterase activities as 3.2 and 6.1 for hCA I and 18.0 and 4.5 μM for hCA II, respectively. Other parent compound, amp, does not present any hydratase and esterase activities.

In relation to esterase activities, the inhibition equilibrium constants (K_i) were also determined. Novel compounds **1** and **2** have significant K_i values which are, respectively 0.25 and 0.058 μM for hCA I and 0.22 and 0.24 μM for hCA II compared to Hsba (7.7 and 22.0 μM for hCA I and II) and AAZ (5.2 and 3.8 μM for hCA I and II). Coordination compound (**2**) has shown remarkable inhibition on hCA I (K_i: 0.058 μM), showing higher inhibition than **1**, probably due to the effect of Zn(II) ion.[30–32] However, proton transfer compound and coordination compound of zinc(II) ion, which are prepared from the same ligands, have shown almost similar inhibition result on hCA-II isoenyzme. Products (**1** and **2**) have quite higher potential inhibitory effects than parent inhibitors (Hsba and amp) and AAZ. Hence, they can be seen as candidates for the treatment of glaucoma.

4. Conclusions

In the present work, a novel proton transfer compound (**1**) and a mixed-ligand Zn(II) complex (**2**) were prepared for the first time. The compound **2** crystallizes in $P\,21/m$ space group. The coordination environment of the Zn(II) ion has a distorted octahedral configuration coordinated by O1 and $O1^i$ atoms of two mono dentante sba anions and N1, N2, $N2^i$ atoms of bidentante amp anion and a water molecule (O1w). Hydrogen bond between the uncoordinated water molecule and N–H group of amp plays an important role in stabilizing the crystal structure. Elemental analyses and all measurements show good agreement with the structures of synthesized compounds. These two novel compounds possess significant inhibition effect on hCA-I and on hCA-II, and thus might be considered as possible drugs for glaucoma. The preliminary biological evaluation suggests that derivatives of such compounds should be subject to further inhibition in vivo tests. Proton transfer compound and coordination compound of zinc(II) ion, which are prepared from the same ligands, have shown almost similar inhibition result on hCA-II isoenyzme. These results also suggest that further studies on such compounds are worthwhile to obtain correlation of inhibitory effects on hCA-II.

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Supplementary data

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 745613 for **2**. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.11.031.

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