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Synthesis, Characterization and Carbonic Anhydrase Inhibitor Properties of Metal Complexes of 4-sulfamoylbenzoic Acid

Tevfik Emre KAYA[1], Nagihan KAYA[1], Şüheda ÖZKUL[1], Burak YILDIZ[1], Ekrem TUNCA[1*], Halil İLKİMEN[2]

Highlights:

- Syntesis of metal complexes
- Measurement of Carbonic anhydrase esterase activity
- Calculation of IC_{50} and K_i values

Keywords:

- 4-sulfamoylbenzoic acid
- Metal complexes
- Carbonic anhydrase
- Esterase acivity
- Inhibition

ABSTRACT:

Six new complexes of 4-sulfamoylbenzoic acid (Hsba) {$[Mg(sba)_2].2H_2O$ (**1**), $[M(sba)_2(H_2O)x].yH_2O$, M = Mn^{2+}, x = 4, y = 2 (**2**); M = Fe^{2+} (**3**), Co^{2+} (**4**), x = 4, y = 0; M = Ni^{2+} (**5**), Cu^{2+} (**6**), x = 2, y = 0} have been prepared. The structures of the metal complexes (**1-6**) have been proposed based on data obtained from analytic and spectroscopic studies. The structures of the synthesized metal complexes were observed to be linear for **1**, octahedral for **2-4**, and tetrahedral for **5** and **6**. In the next stage of the study, the inhibitory effects of the $Mg(Ac)_2$, $FeSO_4.7H_2O$, $Mn(Ac)_2.4H_2O$, $Co(Ac)_2.4H_2O$, $Ni(Ac)_2.4H_2O$, $Cu(Ac)_2.2H_2O$, Hsba and synthesized metal complexes (**1-6**) on hCA I and hCA II isoenzymes were investigated *in vitro*. In general, the compounds significantly inhibited hCA I and hCA II. It was determined that complex **6** was 2200 times more selective for hCA I, while complex **4** was 8 times more selective for hCA II.

[1]Tevfik Emre KAYA (**Orcid ID:** 0009-0001-9717-5784), Nagihan KAYA (**Orcid ID:** 0009-0001-0146-4961), Şüheda ÖZKUL (**Orcid ID:** 0000-0002-2638-3922), Burak YILDIZ (**Orcid ID:** 0000-0002-0869-1091), Ekrem TUNCA (**Orcid ID:** 0000-0001-7556-8379), Kütahya Dumlupınar University, Faculty of Arts and Sciences, Department of Biochemistry, Kütahya, Türkiye
[2]Halil İLKİMEN (**Orcid ID:** 0000-0003-1747-159X), Kütahya Dumlupınar University, Faculty of Arts and Sciences, Department of Chemistry, Kütahya, Türkiye

*** Corresponding Author:** Ekrem TUNCA, e-mail: ekrem.tunca@dpu.edu.tr

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INTRODUCTION

4-Sulfamoylbenzoic acid (Hsba) and derivatives are known for their biological activities, such as antiviral (Curreli et al., 2011), antiglaucoma (Vullo et al., 2005), antibacterial (Dedeoglu et al., 2015), antifungal (Innocenti et al., 2009), antiepileptic (Vullo et al., 2005; Bertucci et al., 2009), anticancer (Krall et al., 2014; Lee et al., 2019), antituberculosis (Abou-Zeid et al., 1962), antimalarial (Barton et al., 2010), antitumor (Winum et al., 2005), anticonvulsant (Tanimukai et al., 1965; Goeres & Schwarz, 1967), antiinflammatory (Ganesh et al., 2014; Song et al., 2025), and inhibitory activities (Singh et al., 2014). Hsba possess the capacity to coordinate with metal ions that incorporate two carboxylate oxygen atoms, two sulfonylamino oxygen atoms, and a singular sulfonylamino nitrogen atom (Ilkimen, 2024). The simple metal complexes of Hsba {Cr(II) (Zollinger et al., 1956), Fe(III) (Ilkimen et al., 2020), Cu(II) (Ilkimen et al., 2021), Zn(II) (Guseinov et al., 1984), Cd(II), Pb(II), In(III) (Gaur et al., 1968), Hg(II) (Facsko & Nemes, 1973; Facsko & Nemes, 1974), Ag (I) (Ciuhaudu et al., 1968), UO_2 (Muller, 1918), Lu(III), Y(III), Tb(III), Nd(III), Sa(III) (Pirkes et al., 1984a), Ce(III), Pr(III), Sm(III), Eu(III), Gd(III), Dy(III), Ho(III), Er(III), Tm(III) and Yb(III) (Pirkes et al., 1984b)} have been synthesized and characterized in previous studies.

There are sixteen different isoforms of carbonic anhydrase (CA) enzymes in vertebrates, which catalyze the reversible hydration reaction of CO_2 to bicarbonate and proton (Gulec et al., 2024; Buza et al., 2025). Human carbonic anhydrase (hCA) enzymes play a role in physiological processes such as CO_2 and bicarbonate transport, pH balance and electrolyte secretion. In addition, bicarbonate, which is released as a product of the reaction, acts as a substrate for carboxylase enzymes and is involved in gluconeogenesis, lipogenesis and ureagenesis pathways (Supuran, 2008; Neri & Supuran, 2011). From this perspective, it is predictable that hCA isoenzymes are associated with many physiological/metabolic disorders. Indeed, hCA I is associated with retinal and cerebral edema, and hCA II with glaucoma, edema, epilepsy, and altitude sickness (Alterio et al., 2012; Angeli & Supuran, 2023). Studies in recent years have shown that hCA I may play a role in atherosclerosis (Adeva-Andany et al., 2015) and calcification and migration of cancer cells (García-Llorca et al., 2024). It has been reported that hCA II is expressed in cancer cells in the central nervous system and therefore may be associated with these cancers (Haapasalo et al., 2020). Although the hCA inhibitors currently used in the clinic (such as acetazolamide) are highly effective, they are not selective among hCA isoenzymes, and some side effects are observed during treatment. Therefore, new and isoenzyme selective inhibitors are needed.

In this study, six new complexes (**1-6**) of 4-sulfamoylbenzoic acid (Hsba) have been synthesized and characterized. The inhibitory effects of the synthesized metal complexes on hCA I and hCA II isoenzymes were investigated *in vitro*.

MATERIALS AND METHODS

General Methods and Materials

All chemicals used were commercially purchased from Aldrich. Perkin Elmer AAS PinAAcle 900T for AAS, Elementar Vario III EL for elemental analyses, Vertex 70 FT-IR spectrometer (Bruker Optics) (using KBr) for FT-IR, SHIMADZU UV-2550 spectrophotometer for UV-vis (200–900 nm and in DMSO), Sherwood Scientific Magway MSB MK1 for magnetic susceptibility (Gouy method using $Hg[Co(SCN)_4]$ as calibrant) and WTW Cond 315i/SET Model conductivity meter for molar conductance (in DMSO) were used for spectral and analytical measurements.

Synthesis of 1-6

A solution of 10.00 mmol Hsba (2.01 g) and 5.00 mmol metal(II) salt [1.42 g $Mg(CH_3COO)_2$ for **1** or 1.23 g $Mn(CH_3COO)_2.4H_2O$ for **2** or 1.39 g $FeSO_4.7H_2O$ for **3** or 1.25 g $Co(CH_3COO)_2.4H_2O$ for **4** or 1.24 g $Ni(CH_3COO)_2.4H_2O$ for **5** or 1.00 g $Cu(CH_3COO)_2.H_2O$ for **6**] dissolved in 50 mL of water:ethanol (1:2). After four days of stirring at 25 °C, the mixtures were filtered and left to crystallize. The complexes were obtained as powder solids (Figure 1). Compound **7** {trihydratetris(4-sulfamoylbenzoato)iron(III)} was previously synthesized and published (Ilkimen et al., 2020).

For 1 {bis(4-sulfamoylbenzoato)magnesium(II) dihydrate}: powder white, 1.15 g, 50% yield. Elemental analysis ($C_{14}H_{16}N_2O_{10}S_2Mg$): C, 36.50%; H, 3.50%; N, 6.08%; S, 13.92%; Mg, 5.28. Found: C, 36.52%; H, 3.55%; N, 6.10%; S, 13.95%; Mg, 5.30%. IR spectrum (ν_{max}, cm^{-1}): 3548 (O-H), 3472, 3146 (NH_2), 3068 $(C-H)_{Ar,}$ 1638, 1425 (C=O), 1618, 1496 (C=C), 1387, 1243, 1065 (C-O), 1243, 1167, 1065 (S=O), 458 (M-O). UV spectrum in DMSO {$[\lambda]_{max}$, nm (ε_0)]} 288 (26270). Magnetic moment 0 BM. Molar conductivity 1.2 $\Omega^{-1}cm^2mol^{-1}$.

For 2 {tetrahydratebis(4-sulfamoylbenzoato)manganese(II)}: powder white, 1.1518 g, 50% yield. Elemental analysis ($C_{14}H_{20}N_2O_{12}S_2Mn$): C, 31.88%; H, 3.82%; N, 5.31%; S, 12.16%; Mn, 10.42%. Found: C, 31.90%; H, 3.80%; N, 5.30%; S, 12.15%; Mn, 10.45%. IR spectrum (ν_{max}, cm^{-1}): 3496 (O-H), 3340, 3251 (NH_2), 3060 $(C-H)_{Ar,}$ 1636, 1415 (C=O), 1590, 1530, 1493 (C=C), 1344, 1291, 1092 (C-O), 1309, 1162, 1137 (S=O),466 (M-O). UV spectrum in DMSO {$[\lambda]_{max}$, nm (ε_0)]} 286(21910). Magnetic moment 5.90 BM. Molar conductivity 1.9 $\Omega^{-1}cm^2mol^{-1}$.

For 3 {tetrahydratebis(4-sulfamoylbenzoato)iron(II)}: powder cream, 3.96 g, 75% yield. Elemental analysis ($C_{14}H_{20}N_2O_{12}S_2Fe$): C, 31.83%; H, 3.82%; N, 5.30%; S, 12.14%; Fe, 10.57. Found: C, 31.85%; H, 3.80%; N, 5.30%; S, 12.15%; Fe, 10.60%. IR spectrum (ν_{max}, cm^{-1}): 3450 (O-H), 3360, 3261 (NH_2), 3053 $(C-H)_{Ar,}$ 1688, 1494 (C=O), 1603, 1576, 1561, 1431 (C=C), 1343, 1287, 1090 (C-O), 1314, 1184, 1125 (S=O),459 (M-O). UV spectrum in DMSO {$[\lambda]_{max}$, nm (ε_0)]} 286(13480), 334(2900), 803(110). Magnetic moment 4.88 BM. Molar conductivity 2.5 $\Omega^{-1}cm^2mol^{-1}$.

For 4 {tetrahydratebis(4-sulfamoylbenzoato)cobalt(II)}: powder pink, 1.58 g, 60% yield. Elemental analysis ($C_{14}H_{20}N_2O_{12}S_2Co$): C, 31.64%; H, 3.79%; N, 5.27%; S, 12.07%; Co, 11.09. Found: C, 31.65%; H, 3.75%; N, 5.25%; S, 12.05%; Co, 11.10%. IR spectrum (ν_{max}, cm^{-1}): 3449 (O-H), 3347, 3258 (NH_2), 3105 $(C-H)_{Ar,}$ 1690, 1492 (C=O), 1587, 1543, 1428, 1405 (C=C), 1342, 1289, 1092 (C-O), 1164, 1137, 1115 (S=O), 477 (M-O). UV spectrum in DMSO {$[\lambda]_{max}$, nm (ε_0)]} 288(16150), 632(170). Magnetic moment 3.88 BM. Molar conductivity 6.5 $\Omega^{-1}cm^2mol^{-1}$.

For 5 {dihydratebis(4-sulfamoylbenzoato)nickel(II)}: powder green, 1.73 g, 70% yield. Elemental analysis ($C_{14}H_2ON_2O_{12}S_2Ni$): C, C, 31.66%; H, 3.80%; N, 5.27%; S, 12.07%; Ni, 11.05. Found: C, 31.65%; H, 3.85%; N, 5.30%; S, 12.10%; Ni, 11.05%. IR spectrum (ν_{max}, cm^{-1}): 3640 (O-H), 3365, 3262 (NH_2), 3060 $(C-H)_{Ar,}$ 1651, 1447 (C=O), 1620, 1558 (C=C), 1344, 1289, 1041 (C-O), 1163, 1041(S=O), 455 (M-O). UV spectrum in DMSO {$[\lambda]_{max}$, nm (ε_0)]} 307(35390), 322(37030), 751(180). Magnetic moment 2.50 BM. Molar conductivity 4.2 $\Omega^{-1}cm^2mol^{-1}$.

For 6 {dihydratebis(4-sulfamoylbenzoato)copper(II)}: powder blue, 2.00 g, 80% yield. Elemental analysis ($C_{14}H_2ON_2O_{12}S_2Cu$): C, 31.37%; H, 3.76%; N, 5.23%; S, 11.96%; Cu, 11.86%. Found: C, 31.40%; H, 3.75%; N, 5.20%; S, 12.00%; Cu, 11.85%. IR spectrum (ν_{max}, cm^{-1}): 3585 (O-H), 3461, 3369 (NH_2), 3080 $(C-H)_{Ar,}$ 1591, 1410 (C=O), 1550, 1490, 1461 (C=C), 1324, 1304, 1089 (C-O), 1179, 1156, 1142 (S=O), 532 (M-O). UV spectrum in DMSO {$[\lambda]_{max}$, nm (ε_0)]} 285(13560), 744(280). Magnetic moment 1.68 BM. Molar conductivity 8.2 $\Omega^{-1}cm^2mol^{-1}$.

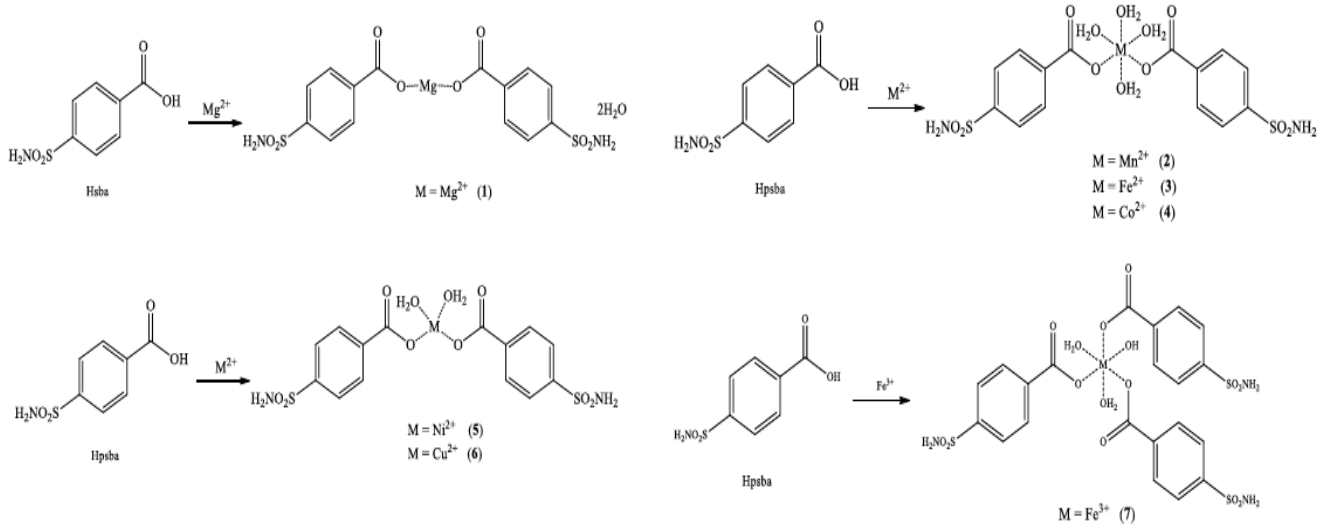

Figure 1. Syntheses of metal complexes **1-7**

Inhibition Studies

The hCA I and II enzymes required for inhibition studies were purified from human erythrocytes. The purification and characterization of hCA isoenzymes was described in detail in our previous studies (Tunca et al., 2020; Parmaksiz & Tunca, 2024).

The inhibition effects of the synthesized compounds on hCA I and II were determined by measuring the esterase activities of these enzymes. For inhibition studies, 0.1% stock solutions of the synthesized compounds in DMSO were prepared. In experimental studies, a reaction medium was prepared containing 3.0 mM 350 µL *p*-nitrophenyl acetate, 50 mM 400 µL pH 7.4 Tris-SO_4 200 µL dd. H_2O and 50 µL hCA I/II in a total volume of 1.0 mL. The absorbance change due to the produced *p*-nitrophenol was measured at 348 nm for 3 minutes by using a UV-Vis spectrophotometer. The same measurement was repeated in the presence of inhibitor solutions of different concentrations. The enzyme activity in the inhibitor-free medium was accepted as 100% and percent relative enzyme activity vs. [Inhibitor] curves were plotted and IC_{50} values were calculated (Verpoorte et al., 1967; Ilkimen et al., 2013).

K_i constants were determined by measuring esterase activity at three different inhibitor concentrations for each of five different substrate concentrations (0.3 mM, 0.4 mM, 0.5 mM, 0.6 mM and 0.7 mM) (Lineweaver & Burk, 1934; Alkan Alkaya et al., 2018).

RESULTS AND DISCUSSION

FT-IR Analysis

The FT-IR spectral data of the starting compound (Hsba) and compounds **1-6** are given in Table 1 and Figure 2. The vibration bands at 3360 and 3260 cm^{-1} of NH_2 group of Hsba are slightly shifted from those found 3472 and 3146 cm^{-1} for **1**, 3340 and 3251 cm^{-1} for **2**, 3360 and 3261 cm^{-1} for **3**, 3347 and 3258 cm^{-1} for **4**, 3365 and 3262 cm^{-1} for **5** and 3461 3369 cm^{-1} for **6** due to the weak intermolecular interactions. The carboxylate moieties present marked carbonyl absorption bands, demonstrated through the infrared (IR) spectrum, which indicates asymmetric (ν_{as}) and symmetric (ν_s) stretching vibrations at 1701 and 1456 cm^{-1} for Hsba, 1638 and 1425 cm^{-1} ($\Delta\upsilon$ = 213) for **1**, 1636 and 1415 cm^{-1} ($\Delta\upsilon$ = 221) for **2**, 1688 and 1494 cm^{-1} ($\Delta\upsilon$ = 194) for **3**, 1690 and 1492 cm^{-1} ($\Delta\upsilon$ = 198) for **4**, 1651 and 1447 cm^{-1} ($\Delta\upsilon$ = 204) for **5** and 1591 and 1410 cm^{-1} ($\Delta\upsilon$ = 188) for **6.** The observed differences ($\Delta\upsilon$) between the asymmetric and symmetric stretching frequencies of the carboxylate groups across complexes **1** to **6** imply the presence of a monodentate coordination of the carboxylate

group to the metal ion in all studied complexes (Nakamato, 1997). In all compounds, range of 3640-3449 cm^{-1} of ν(OH) vibrations, range of 3105-3053 cm^{-1} aromatic , range of 1620-1404 cm^{-1} ν(C=C) vibrations, range of 1387-1065 cm^{-1} ν(C-O) vibrations, range of 1314-1065 cm^{-1} ν(SO_2) vibrations and range of 532-455 cm^{-1} ν(M-O) have been observed.

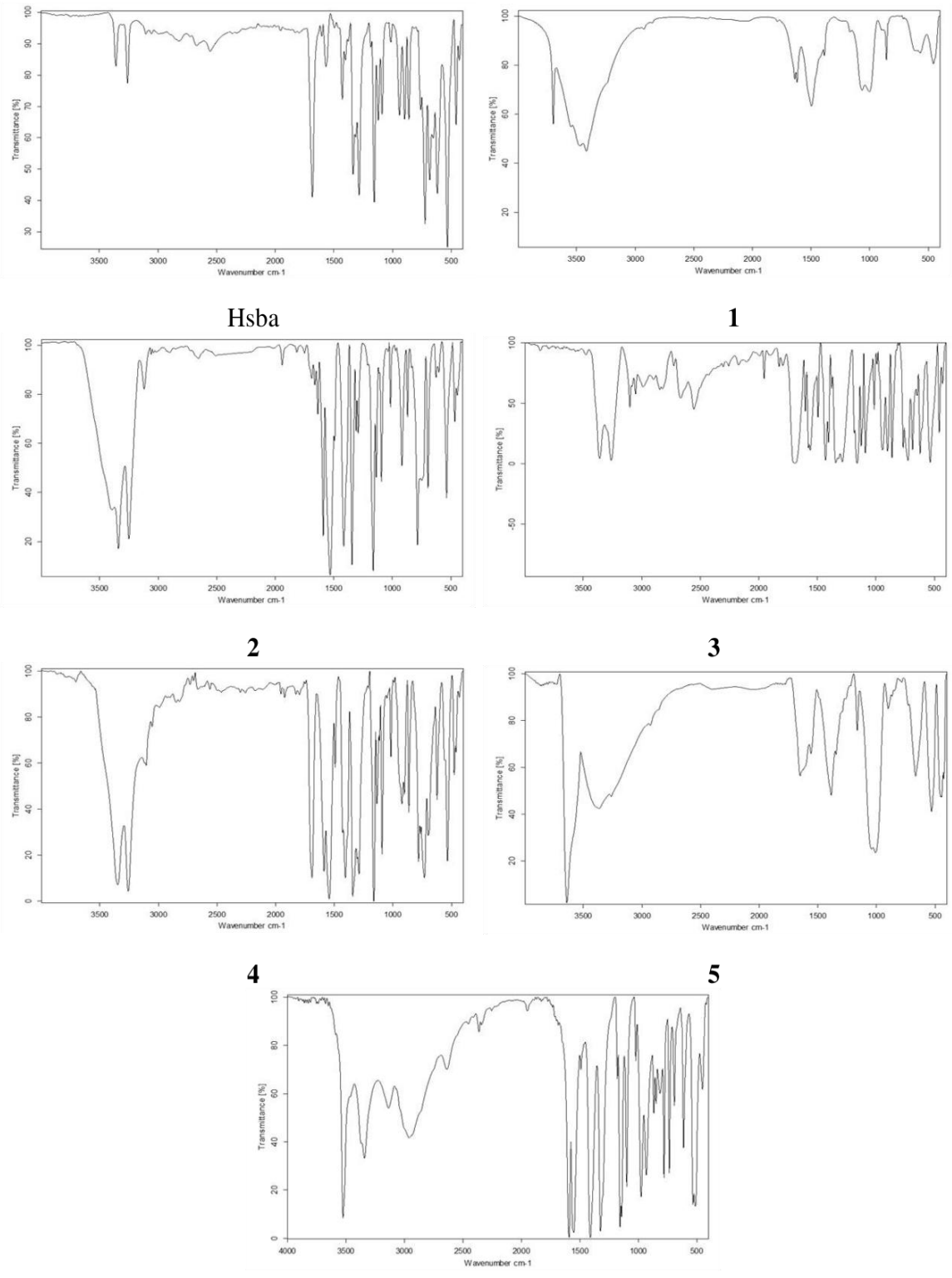

Figure 2. IR spectra of Hsba and **1-6**

Table 1. IR spectral data of Hsba and **1-6** (cm^{-1})

ν	Hsba	1	2	3	4	5	6
(O-H)	2849(br)	3548(br)	3496(br)	3450(br)	3449(br)	3640(br)	3585(br)
(NH_2)	3360(m) 3260(m)	3472(m) 3146(m)	3340(m) 3251(m)	3360(m) 3261(m)	3347(m) 3258(m)	3365(m) 3262(m)	3461(m) 3369(m)
$(C-H)_{Ar}$	3103(w)	3068(w)	3060(w)	3053(w)	3105(w)	3060(w)	3080(w)
(C=O)	1686(s) 1494(s)	1638(s) 1425(s)	1636(s) 1415(s)	1688(s) 1494(s)	1690(s) 1492(s)	1651(s) 1447(s)	1591(s) 1410(s)
(C=C)	1604(s) 1577(s) 1562(s) 1431(s)	1618(s) 1496(s)	1590(s) 1530(s) 1493(s)	1603(s) 1576(s) 1561(s) 1431(s)	1587(s) 1543(s) 1428(s) 1405(s)	1620(s) 1558(s)	1550(s) 1490(s) 1461(s)
(C-O)	1404(s) 1377(s) 1251(s) 1091(s)	1387(s) 1243(s) 1065(s)	1344(s) 1291(s) 1092(s)	1343(s) 1287(s) 1090(s)	1342(s) 1289(s) 1092(s)	1344(s) 1289(s) 1041(s)	1324(s) 1304(s) 1089(s)
(S=O)	1287(s) 1185(s) 1127(s)	1243(s) 1167(s) 1065(s)	1309(s) 1162(s) 1137(s)	1314(s) 1184(s) 1125(s)	1164(s) 1137(s) 1115(s)	1163(s) 1041(s)	1179(s) 1156(s) 1142(s)
(M-O)	-	458(w)	466(w)	459(w)	477(w)	455(w)	532(w)

UV/Vis Analysis

The UV spectra of Hsba and **1-6** were recorded in DMSO ($1x10^{-3}$ M, Table 2, Figure 3). π- π* transitions were observed at 286(11550 $Lmol^{-1}cm^{-1}$) nm for Hsba, 288(26270 $Lmol^{-1}cm^{-1}$) nm for **1**, 286(21910 $Lmol^{-1}cm^{-1}$) nm for **2**, 286(13480 $Lmol^{-1}cm^{-1}$) and 334(2900 $Lmol^{-1}cm^{-1}$) nm for **3**, 288 (16150 $Lmol^{-1}cm^{-1}$) nm for **4**, 307(35390 $Lmol^{-1}cm^{-1}$) and 322(37030 $Lmol^{-1}cm^{-1}$) nm for **5** and 285 (13560 $Lmol^{-1}cm^{-1}$) nm for **6**. The d-d transition bands were observed at 803(110 $Lmol^{-1}cm^{-1}$) nm for 3, 632(170 $Lmol^{-1}cm^{-1}$) nm for **4**, 751(180 $Lmol^{-1}cm^{-1}$) nm for **5**, and 744(280 $Lmol^{-1}cm^{-1}$) nm for **6**. d-d transition was not observed for compounds **1** and **2** due to d^0 for 1 {Mg(II)} and d^5 for **2** {Mn(II)} electronic structure of metal ion (Cetin et al., 2019).

Table 2. Optical properties of **1-6** and Hsba (nm($Lmol^{-1}cm^{-1}$)).

Hsba	1	2	3	4	5	6
286(11550)	288(26270)	286(21910)	286(13480) 334(2900) 803(110)	288(16150) 632(170)	307(35390) 322(37030) 751(180)	285(13560) 744(280)

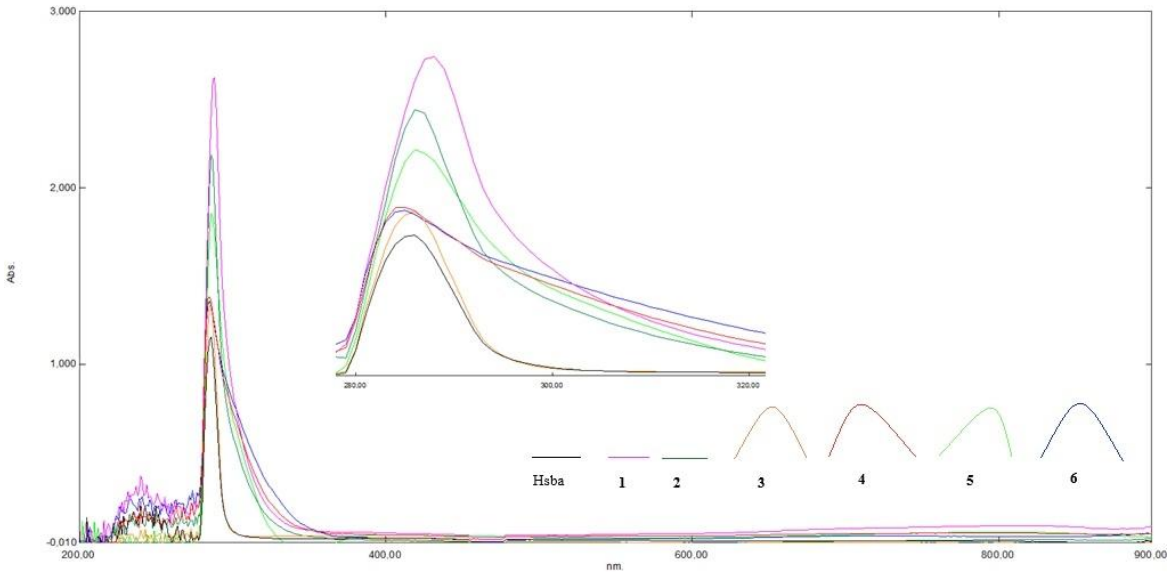

Figure 3. UV-Vis spectra of Hsba and **1-6**

Molar Conductivity Measurements

The molar conductivity in DMSO are 1.2 $\Omega^{-1}cm^2mol^{-1}$ for **1**, 1.39 $\Omega^{-1}cm^2mol^{-1}$ for **2**, 2.5 $\Omega^{-1}cm^2mol^{-1}$ for **3**, 6.5 $\Omega^{-1}cm^2mol^{-1}$ for **4**, 4.2 $\Omega^{-1}cm^2mol^{-1}$ for **5** and 8.2 $\Omega^{-1}cm^2mol^{-1}$ for **6** indicating that all of the complexes are non-ionic (Geary, 1971).

Magnetic Susceptibility Measurements

The magnetic moments of complexes **1-6** (Table 3) are 0 BM for **1**, 5.90 BM for **2**, 4.88 BM for **3**, 3.88 BM for **4**, 2.80 BM for **5** and 1.68 BM for **6** per metal ion, indicating the presence of zero (d^0), three (d^5), four (d^6), three (d^7), two (d^8) and one (d^9) unpaired electron, respectively.

Table 3. Magnetic Susceptibility Measurements

	1	2	3	4	5	6
C_{bal}	1.011	1.011	1.011	1.011	1.011	1.011
l	1.4	1.6	1.3	1.7	1.8	1.8
R	0	875	765	450	234	115
R_o	-30	-30	-30	-30	-30	-30
m	0.0529	0.0489	0.0506	0.06	0.0695	0.0529
T	295.15	295.15	295.15	295.15	295.15	295.15
M_a	460.72	527.38	528.29	531.38	495.11	499.96
μ	0	5.90	4.88	3.88	2.50	1.68
n	0	5	4	3	2	1

Cbal: Equilibrium calibration constant of the instrument, l (cm): height of the sample in the tube, R: observed value for the sample, R_0: Observed value for an empty tube, m (g): mass of the sample, T (K): ambient temperature, Ma (g/mol): molecular mass, μ (BM): magnetic moment, n: unpaired electron.

Due to the amorphous structure of the **1-6**, the single crystal X-ray diffraction technique, which is the most effective technique for structure characterization, could not be applied within the scope of our study. The structures of the complexes were suggested using elemental, atomic absorption (AAS) and spectral (FT-IR and UV-Vis) analyses and magnetic susceptibility and molar conductivity techniques.

hCA Inhibition Studies

In the present study on the *in vitro* inhibition of hCA I isoenzyme and hCA II isoenzyme, it was determined that all of the metal complexes were inhibitors of these isoenzymes, only Hsba compound among the starting compounds showed inhibitory effect, while simple metal salts did not show significant effect (Table 4).

Table 4. Esterase IC_{50} and K_i values of the compounds

Inhibitor	Esterase IC_{50} (μM)[a,b]		K_i (μM)[a,b]			
	hCA I	hCA II	hCA I	Inhibition Type	hCA II	Inhibition Type
AAZ[c]	0.420±0.004	0.310±0.008	0.260±0.003	Noncompetitive	0.140±0.005	Noncompetitive
$Mg(Ac)_2$	No effect	No effect	-	-	-	-
$FeSO_4.7H_2O$	No effect	Weak activator	-	-	-	-
$Mn(Ac)_2.4H_2O$	Weak inhibitor	No effect	-	-	-	-
$Co(Ac)_2.4H_2O$	Weak inhibitor	No effect	-	-	-	-
$Ni(Ac)_2.4H_2O$	No effect	No effect	-	-		
$Cu(Ac)_2.2H_2O$	Weak inhibitor	Weak inhibitor	-	-	-	-
Hsba	0.396±0.008	0.402±0.012	0.146±0.006	Competitive	0.690±0.021	Uncompetitive
1	1.582±0.032	0.408±0.009	0.862±0.025	Noncompetitive	0.316±0.011	Noncompetitive
2	0.216±0.007	0.100±0.001	0.045±0.001	Uncompetitive	0.288±0.006	Noncompetitive
3	0.218±0.004	0.164±0.003	0.351±0.016	Noncompetitive	0.090±0.002	Competitive

Table 4 (Continued). Esterase IC_{50} and K_i values of the compounds

4	1.487±0.029	0.510±0.009	0.545±0.019	Uncompetitive	0.070±0.002	Uncompetitive
5	77.00±1.371	138.60±2.45	-	-	-	-
6	0.164±0.002	99.00±1.861	0.014±0.001	Uncompetitive	31.21±0.750	Noncompetitive
7	0.278±0.010	0.338±0.007	0.289±0.008	Noncompetitive	0.135±0.004	Noncompetitive

[a]Mean ± standard error (three different assays).
[b]$p < 0.0001$
[c]AAZ (acetazolamide): control compound.

When esterase IC_{50} values are evaluated, it is seen that compounds Hsba, **2, 3, 6,** and **7** are more effective inhibitors than AAZ for hCA I. For hCA II, **2** and **3** are more potent inhibitors than AAZ. While **6** is the most potent inhibitor for hCA I, **2** is the most effective inhibitor for hCA II. Both compounds are approximately three times more potent inhibitors than AAZ. Moreover, **1-4** are more selective for hCA II, while **5-7** are more selective inhibitors for hCA I. In addition to **4** being three times more selective for hCA II, **6** inhibited the hCA I isoenzyme about 600 times more potently than the hCA II isoenzyme (Table 4). These differences in the inhibition potentials of the compounds can be attributed to the differences in the amino acid residues in the active sites of hCA I and hCA II isoenzymes and the differences in the three-dimensional structures of the metal complexes. Indeed, in complex **1**, the Mg(II) ion formed bonds with only two sba^- ligands. There are two sba^- ligands and four H_2O ligands in the coordination spheres of complexes **2-4**. Differences in the inhibitory effect of these complexes among themselves may also be due to the variation of the metal ion and small differences in the orientation of the ligands accordingly. In complexes **5** and **6**, the metal ion is coordinated with two sba^- ligands and two H_2O ligands. The difference between the inhibitory potentials of these two compounds may also be due to differences in metal ions. In complex **7**, the Fe(III) ion is coordinated with three sba^- ligands. This compound may have interacted more tightly with hCA I and hCA II due to its bulky structure and strengthened the inhibitory effect.

In studies conducted to determine the inhibition mechanism, and the equilibrium constant (K_i) of the inhibition reaction using different substrate concentrations, the K_i constant of **5**, one of the metal complexes, could not be calculated because this compound could not reduce the activities of hCA enzymes by more than 50%. Among the starting compounds, only the K_i value of the Hsba compound could be determined. When K_i values for hCA I are examined, it is seen that the strongest inhibitor is **6**. When the selectivity of this compound between hCA I and II was evaluated, the selectivity determined as 600-fold based on IC_{50} values was determined as 2200-fold based on K_i values. This shows that there is an increase in the affinity of the inhibitor for the enzyme at changing substrate concentrations. This compound inhibited the hCA I enzyme via an uncompetitive mechanism, whereas it inhibited the hCA II isoenzyme via a noncompetitive mechanism (Figure 4). Furthermore, **6** has about 18 times stronger inhibitory effect for hCA I than AAZ. It is followed by **2** in terms of hCA I inhibition potential. When K_i constants for hCA II are examined, it is seen that the most potential inhibitor is **4**. This compound inhibited the hCA II isoenzyme two-fold more potently than AAZ. Additionally, this compound has the highest selectivity for hCA II, approximately 8-fold. Complex **4** inhibited hCA I and hCA II via uncompetitive mechanism.

This compound is followed by complex **3** in terms of hCA II inhibition potential (Table 4). It is useful to state that the comments made on the structure-activity relationship in this section can be confirmed by preparing inhibitor-enzyme crystals and using X-ray diffraction techniques.

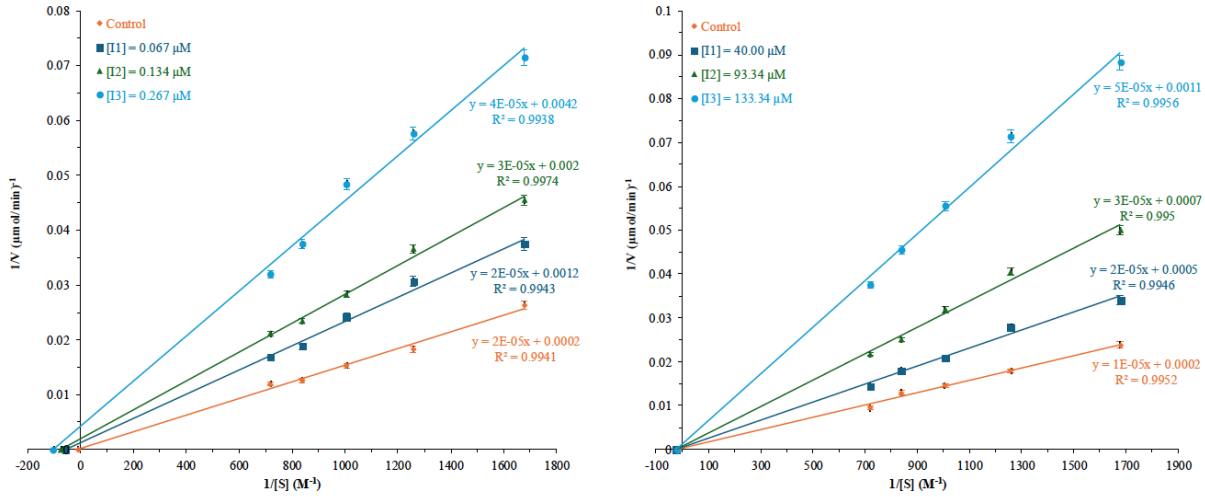

Figure 4. K_i plots of compound 6 (hCA I left, hCA II right)

CONCLUSION

The metal complexes synthesized within the scope of our study exhibited a remarkable inhibition potential on hCA isoenzymes. Particularly, complex **6** has high selectivity for hCA I. Therefore, it can be said that this compound is a candidate molecule to be subjected to further pharmacological tests such as cell culture tests and toxicity tests.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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