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CARBONIC ANHYDRASE INHIBITORY ACTIVITIES OF NOVEL PROTON TRANSFER SALTS AND THEIR Cu(II) COMPLEXES

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In this study, two new proton transfer salts of sulfonamide derivatives of maleic acid, namely (ClHabt)$^+$(*m*absmal)$^-$ (**1**) and (ClHabt)$^+$(*p*absmal)$^-$ (**2**), were obtained from 2-amino-6-chlorobenzothiazole (Clabt) and *N*-(3-sulfamoylphenyl)maleamide acid (H*m*absmal) and *N*-(4-sulfamoylphenyl)maleamide acid (H*p*absmal), respectively. Also, the Cu(II) complexes (**3** and **4**) of salts (**1** and **2**) and of H*m*absmal (**5**) were prepared. Compounds **1–5** were characterized by elemental, NMR (^{1}H and ^{13}C), FTIR, and thermal analyses, as well as UV-Vis, magnetic moment, and molar conductivity measurements. Carbonic anhydrase isoenzymes (hCA I and II) were purified from human erythrocyte cells by affinity chromatography. The effects of the synthesized compounds on the hydratase and esterase activities of CA isoenzymes were studied *in vitro.* The results reveal that the synthesized compounds inhibit both esterase and hydratase activities of hCA I and hCA II. The inhibition constants of the compounds (K_i) were determined according to the esterase activity measurements. K_i values of **1–5** are in the range of 0.06 ± 0.003 μM and 4.25 ± 0.100 μM for hCA I, and of 0.02 ± 0.001 μM and 3.21 ± 0.200 μM for hCA II.

Keywords: 2-amino-6-chlorobenzothiazole; *N*-(3/4-sulfamoylphenyl)maleamide acids; proton transfer salt; Cu(II) complexes; carbonic anhydrase inhibition

ИНХИБИТОРНА АКТИВНОСТ ВРЗ КАРБОНСКАТА АНХИДРАЗА НА НОВИ СОЛИ СО ТРАНСФЕР НА ПРОТОНИ И НИВНИТЕ Cu(II)-КОМПЛЕКСИ

Во оваа студија, од 2-амино-6-хлоробензотиазол (Clabt) и соодветно на *N*-(3-сулфамоилфенил)малеамидна киселина (H*m*absmal) и *N*-(4-сулфамоилфенил)малеамидна киселина (H*p*absmal), се добиени две нови протон трансфер соли на сулфонамидни деривати на малеинска киселина, имено (ClHabt)$^+$(*m*absmal)$^-$ (**1**) и (ClHabt)$^+$(*p*absmal)$^-$ (**2**). Исто така беа приготвени Cu(II) комплекси (**3** и **4**) на солите (**1** и **2**) и на H*m*absmal (**5**). Соединенијата **1–5** беа карактеризирани со елементна, NMR (^{1}H и ^{13}C), FTIR и термичка анализа, како и со UV-Vis и мерења на магнетниот момент и на моларната спроводливост. Изоензимите на карбонска анхидраза (hCA I и II) беа пречистени од човечки еритроцитни клетки со афинитетна хроматографија. Ефектите на синтетизираните соединенија на активноста на хидратазата и на естераза на CA-изоензимите беа проучени *in vitro.* Резултатите откриваат дека синтетизираните соединенија ја инхибираат активноста и на естеразата и на хидратазата на hCA I и на hCA II. Инхибиторните константи на соединенијата (K_i) беа определени со мерења на активноста на естеразата. Вредностите на K_i на **1–5** се во опсегот од 0,06 ± 0,003 μM и 4,25 ± 0,100 μM за hCA I и од 0,02 ± 0,001 μM и 3,21 ± 0,200 μM за hCA II.

Клучни зборови: 2-амино-6-хлоробензотиазол; *N*-(3/4-сулфамоилфенил)малеамидни киселини; соли со трансфер на протони; Cu(II) комплекси; инхибиција на карбонска анхидраза

1. INTRODUCTION

Sulfonamides and 2-aminobenzothiazoles perform a wide range of various biological activities, such as anti-HIV protease[1] as well as anti-inflammatory,[2] antiglaucoma,[3] antibacterial, antifungal,[4] antiviral, and antitumor actions.[5] Carbonic anhydrases (CAs, EC 4.2.1.1) are present in almost all living organisms as metalloenzymes catalyzing the hydration of carbon dioxide to bicarbonate and a proton.[6,7] CAs are encoded by eight different independent gene families (α-, β-, γ-, δ-, ζ-, η-, θ-, and ι-).[8] α-CAs contain Zn^{2+} in their active site; they are contained in mammals and vertebrates, whereas sixteen different isozymes of them are present in humans (hCAs).[9] The hCA I is a major cytosolic carbonic anhydrase present in erythrocytes' gastrointestinal tract and eyes.[10] It is physiologically relevant to retinal edema and cerebral edema.[11] The hCA II is the most active isoform among the α-CAs, present in erythrocytes' eye, gastrointestinal tract, bone osteoclasts, kidney, lung, testes, and brain, and it is physiologically relevant to glaucoma, edema, epilepsy, and altitude sickness.[12] Just these two isoforms are associated with various physiological disorders. This shows how important these enzymes are from a pharmacological point of view. Primary sulfonamides are the most well-known and clinically used inhibitors of CA isoenzymes. However, since the isoform selectivity of these compounds is weak, their side effects are considerably higher. In order to overcome the isoform selectivity problem, researchers are currently trying to develop inhibitors that would bind to different moieties in the active site or derivatize the primary sulfonamides that bind to Zn^{2+} in such a way that they ought to interact with different amino acids in the active site. A primary sulfonamide derivative coded SLC-0111,[13] which has been in clinical trials in recent years, is an effective example of an isoform-selective sulfonamide derivative strategy.

Also, the Cu(II) complexes of carboxylic acids with 2-aminobenzothiazole have been extensively studied in recent years.[14–16] The metal complexes of proton transfer salts have shown better biological activities than free ligands and salt.[2,3] The simple transition metal complexes of H*m*absmal have not been synthesized; nevertheless, there are a few reports on the synthesis of proton transfer salts and mixed ligand metal complexes {Co(II) and Cu(II)} of H*m*absmal with other ligands, such as 2-aminopyridine derivatives.[17,18] Fe(II), Co(II), and Cu(II) complexes of H*p*absmal, proton transfer salts of H*p*absmal with 2-aminopyridine or 1*H*-benzimidazole, and the Co(II), Ni(II), Cu(II) and Zn(II) complexes have been synthesized earlier and reported in our laboratories.[17–19]

In this study, relying on the importance of the sulfonamide compounds in the inhibition of CA, two new proton transfer salts $(ClHabt)^{+}(\textit{m}absmal)^{-}$ (**1**) and $(ClHabt)^{+}(\textit{p}absmal)^{-}$ (**2**) were obtained from the reaction between Clabt (the base) and H*m*absmal or H*p*absmal (the acid), respectively. The Cu(II) complexes (**3** and **4**) of salts (**1** and **2**) and of H*m*absmal (**5**) were characterized by spectroscopic methods. The potential use of these compounds as new inhibitors of hCA I and hCA II isoenzymes was also investigated.

2. EXPERIMENTAL SECTION

2.1. *Synthesis of **1–5***

The materials and equipment used in our experimental study are provided in a supplementary file. The starting compounds are the acids, H*m*absmal[20] and H*p*absmal,[21] which were synthesized according to the literature. The spectroscopic data of H*m*absmal and H*p*absmal are given in Tables S1 and S2 for ^{1}H and ^{13}C NMR, in Table S3 for FT-IR, and in Table S5 for UV-Vis, whereas Figures S1-S4 render the NMR spectra.

A solution of 0.920 g Clabt in 35 ml absolute ethanol was added to the solution of 1.35 g maleic acid (H*m*absmal for **1** or H*p*absmal for **2**) in 35 ml of absolute ethanol. The mixture was stirred at room temperature for two days in order to obtain white solids of **1** and **2** (1.934 g, 85 % yield for **1**; 1.817 g, 80 % yield for **2**).

1 mmol of copper(II) acetate monohydrate and 1 mmol proton transfer salt {**1** (0.420 g) for **3** and **2** (0.455 g) for **4**} and 1 mmol H*m*absmal (0.270 g) were dissolved in technical ethanol (50%) (50 ml) with stirring for two days. The mixture was induced to give a solid powder 0.358 g (blue, 65 % yield) for **3**, 0.258 g for **4** (green, 70 % yield), and 0.246 g for **5** (blue, 75% yield) (Fig. 1).

Anal. Calcd. for **1** ($C_{17}H_{15}N_4O_5S_2$): C, 44.88%; H, 3.32%; N, 12.32%; S, 14.10%. Found: C, 44.90%; H, 3.55%; N, 12.30%; S, 14.05%; for **2** ($C_{17}H_{15}N_4O_5S_2$): C, 44.88%; H, 3.32%; N, 12.32%; S, 14.10%. Found: C, 44.85%; H, 3.52%; N, 12.35%; S, 14.08%; for **3** ($C_{34}H_{34}N_8O_{13}S_4Cl_2Cu$): C, 39.83%; H, 3.34%; N, 10.93%; S, 12.51%; Cu, 6.20%. Found: C, 39.85%; H, 3.35%; N, 10.90%; S, 12.50%; Cu, 6.50%; for **4** ($C_{24}H_{22}N_6O_7S_3Cl_2Cu$): C, 39.11%; H, 3.01%; N, 11.40%; S, 13.05%; Cu, 8.62%. Found: C, 39.10%; H, 3.05%; N, 11.38%; S, 13.10%; Cu, 8.63%; for **5** ($C_{20}H_{24}N_4O_{13}S_2Cu$):

C, 36.61%; H, 3.69%; N, 8.54%; S, 9.77%; Cu, 8.54%. Found: C, 39.65%; H, 3.65%; N, 8.55%; S, 9.80%; Cu, 8.60%.

In addition, the Cu(II) metal complexes of H*p*absmal (CuH*p*absmal)[18] and Clabt (CuClabt)[22] were prepared according to the literature.

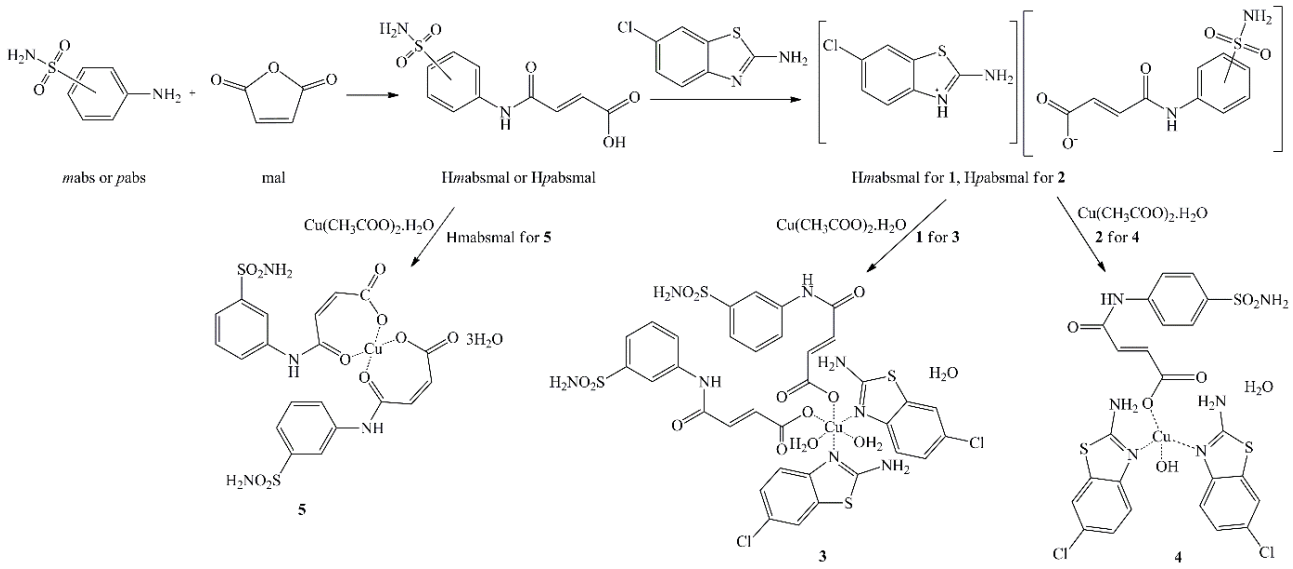


Fig. 1. Syntheses of compounds **1**–**5**

2.2. *Purification of hCA I and hCA II*

The purification of the enzymes was described in detail in our previous studies.[15–19] To review, blood samples collected in anticoagulated tubes were centrifuged, while erythrocytes were separated and hemolyzed. Following centrifugation, the supernatant, whose pH had been adjusted to 8.7, was loaded onto a Sepharose®-4B-L-tyrosine-*p*-aminobenzenesulfonamide column. After extensive washing, the hCA I and II isoenzymes were eluted with 1.0 M NaCl/25.0 mM Na_2HPO_4 (pH 6.3) and 0.1 M CH_3COONa/0.5 M $NaClO_4$ (pH 5.6).[23] Protein quantity was determined in the eluates.[24] The purified enzymes were characterized by SDS-PAGE analysis in the presence of standard hCA I and hCA II.[25] (Table S6, Fig. S5).

2.3. *Hydratase and esterase activity assay and the determination of the IC_{50} values and inhibition constants (K_i)*

Hydatase and esterase activities of purified hCA I and hCA II were determined according to the literature.[26,27] The activity measurement was described in detail in our previous studies.[15–19]

The IC_{50} values were determined by measuring hydratase and esterase activity in the presence of the following compounds: free ligands, simple Cu(II) complexes, and **1**-**5**. The regression analysis graphs were drawn by plotting inhibitor concentrations against percent enzyme activity using the Microsoft Excel Package Program (Microsoft Office 2016).

The method used to determine the K_i values was described in detail in our previous studies.[15–19] In short, in order to determine the K_i values, esterase activity measurements were made at five different substrate concentrations for each of the three different inhibitor concentrations (30 %, 50 %, and 70 % inhibition effect). The data were linearized with a Lineweaver-Burk plot in order to obtain K_i values.[28]

The IC_{50} and K_i values were determined by averaging the results from triplicate experiments.

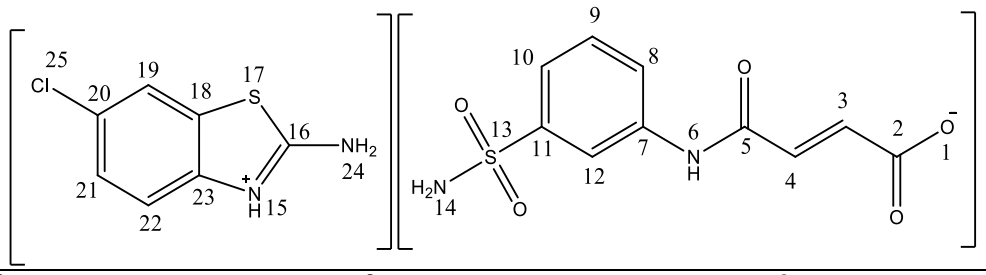
3. RESULTS AND DISCUSSION

3.1. *NMR studies of **1** and **2***

The NMR spectra (^1H and ^{13}C) of **1** and **2** are given in d_6-DMSO with and without D_2O in Figures S6-S9, whereas the chemical shift values can be seen in Tables 1 and 2.

Table 1

*^{1}H NMR and ^{13}C NMR results for compound **1***



H^3	6.50 (1H, d) [$^3J_{H3-H4}$ = 11.82 Hz]	C^2	167.37
H^4	6.35 (1H, d) [$^3J_{H4-H3}$ = 11.78 Hz]	C^3	131.39
H^6	11.60 (1H, s)	C^4	131.93
H^8	7.55 (1H, d) [$^3J_{H8-H9}$ = 7.34 Hz]	C^5	164.00
H^9	7.65 (1H, t) [$^3J_{H9-H8,10}$ = 7.17 Hz]	C^7	139.52
H^{10}	7.35 (1H, d) [$^3J_{H10-H9}$ = 7.54 Hz]	C^8	121.32
H^{12}	8.35 (1H, s)	C^9	130.84
H^{19}	7.75 (1H, s)	C^{10}	129.99
H^{14}, H^{21}, H^{22}, H^{24}	7.40-7.60 (6H, m)	C^{11}	145.20
		C^{12}	116.88
		C^{16}	166.89
		C^{18}	130.84
		C^{19}	121.27
		C^{20}	122.67
		C^{21}	125.88
		C^{22}	118.19
		C^{23}	153.25

In the ^{1}H NMR spectrum of **1** at room temperature **(**Fig. S6a, Table 1), the H^3 and H^4 protons of **1** are observed at 6.50 ppm (H^3, doublet, $^3J_{H3-H4}$ = 11.82 Hz) and 6.33 ppm (H^4, doublet, $^3J_{H4-H3}$ = 11.78 Hz) with 1H intensities, indicating the transposition of H^3 and H^4. The H^8, H^9, H^{10} and H^{12} protons of **1** are detected at 7.55 ppm (H^8, doublet, $^3J_{H8-H9}$ = 7.34 Hz), 7.35 ppm (H^{10}, triplet, $^3J_{H10-H9}$ = 7.54 Hz), 7.65 ppm (H^9, doublet, $^3J_{H9-H8,10}$ = 7.17 Hz), and 8.35 ppm (H^{12}, singlet) with 1H intensities, respectively. H^{14}, H^{21}, $H^{22,}$ and H^{24} protons of the **1** are observed in the range of 7.40–7.60 ppm (multiplet) with 6H intensities. The H^6 and H^{19} protons of the **1** are observed as singlets at 11.60 ppm with 1H intensity and 7.75 ppm with 1H intensity, respectively.

In the ^{1}H NMR spectrum of **2** at room temperature (Fig. S8a, Table 2), the H^3 and H^4 protons of **2** are detected at 6.35 ppm (H^3, doublet, $^3J_{H3-H4}$ = 11.74 Hz) and 6.50 ppm (H^4, doublet, $^3J_{H4-H3}$ = 11.74 Hz) with 1H intensities, indicating the transposition of H^3 and H^4. The H^8, H^9, H^{11}, H^{12}, and H^{19} protons of the **2** are observed at 7.80 ppm (singlet) with 5H intensities. The H^{21} and H^{22} protons of **2** are observed at 7.23 ppm (H^{21}, doublet, $^3J_{H21-H22}$ = 7.74 Hz) and 7.32 ppm (H^{22}, doublet, $^3J_{H22-H21}$ = 8.20 Hz) with 1H intensity. The H^6, H^{14}, and H^{24} protons of **2** are observed as singlets at 11.60 ppm with 1H intensity, 7.60 ppm with 2H intensity, and 7.27 ppm with 2H intensity, respectively.

The H^{15} protons of **1** and **2** were not detected in the ^{1}H NMR spectrum. This can be due to the mobility of the proton between the position O1 and N15. H^6, H^{14} and H^{24} protons of **1** and **2** were not detected in the spectra obtained in d_6-DMSO after D_2O addition due to the deuterium exchange (Figs. S6b and S8b). For both compounds (**1** and **2**), the –COOH hydrogen (H^1) was not observed due to the salt formation and proton transfer, although it was distinguishable in the H*m*absmal and H*p*absmal ^{1}H NMR spectra (12.95 and 12.90 ppm) (Figs. S1 and S3).

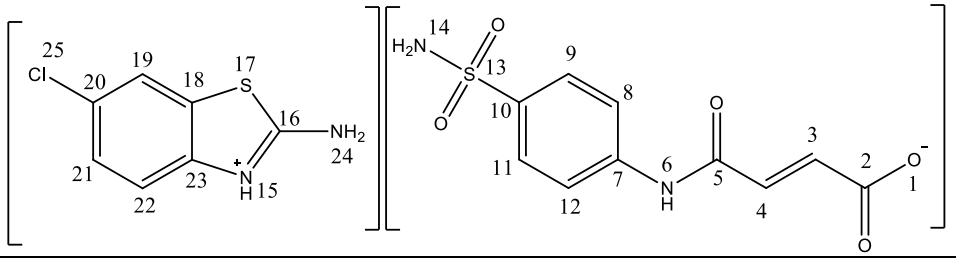
The ^{13}C NMR spectra of **1** and **2** exhibit seventeen and fifteen resonances (Tables 1 and 2, Figs. S7 and S9) as expected. Seven peaks out of these resonances for **1** and **2**, particularly 166.89 and 167.37 ppm (C^{16}), 130.84 and 130.81 ppm (C^{18}), 121.27 and 121.01 ppm (C^{19}), 122.67 and 124.98 ppm (C^{20}), 125.88 and 126.02 ppm (C^{21}), 118.19 and 119.05 (C^{22}), and 153.25 and 152.21 ppm (C^{23}), can be designated to the carbons of

HClabt$^+$ groups. The other ten peaks for **1** at 167.37 ppm (C^2), 131.39 ppm (C^3), 131.93 ppm (C^4), 164.00 ppm (C^5), 139.52 ppm (C^7), 121.32 ppm (C^8), 130.84 ppm (C^9), 129.99 ppm (C^{10}), 145.20 ppm (C^{11}) and 116.88 ppm (C^{12}) can be designated to the carbons of *m*absmal$^-$ ring moiety. The rest of the peaks for **2** at 167.61 ppm (C^2), 131.98 ppm (C^3), 133.06 ppm (C^4), 164.13 ppm (C^5), 139.25 ppm (C^7), 127.20 ppm (C^8, C^{12}), 119.46 ppm (C^9, C^{11}), and 142.08 ppm (C^{10}) can be designated to the carbons of *p*absmal$^-$ ring moiety.

The NMR spectra clearly indicate the formation of the salts between Clabt and H*m*absmal or H*p*absmal with 1:1 ratio for **1** and **2** (Fig. 1).

T a b l e 2

*^{1}H NMR and ^{13}C NMR results for compound **2***



H^3	6.35 (1H, d) [$^3J_{H3-H4}$ = 11.74 Hz]	C^2	168
H^4	6.50 (1H, d) [$^3J_{H4-H3}$ = 11.74 Hz]	C^3	139
H^6	11.60 (1H, s)	C^4	130
H^8, H^9, H^{11}, H^{12}, H^{19}	7.80 (5H, s)	C^5	164
H^{14}	7.60 (2H, s)	C^7	132
H^{21}	7.23 (1H, d) [$^3J_{H21-H22}$ = 7.74 Hz]	C^8, C^{12}	127
H^{22}	7.32 (1H, d) [$^3J_{H22-H21}$ = 8.20 Hz]	C^9, C^{11}	119
H^{24}	7.27 (2H, s)	C^{10}	142
		C^{16}	167
		C^{18}	133
		C^{19}	119
		C^{20}	124
		C^{21}	126
		C^{22}	121
		C^{23}	152

3.2. *FT-IR results*

The infrared spectral data of the free ligands (H*m*absmal, H*p*absmal and Clabt) and **1**–**5** are given in Table S3. The ν(OH) vibrations of H*m*absmal and H*p*absmal were observed as a broad band at 2900 cm^{-1}, but these bands are not observed in **1** and **2** due to the proton transfer to Clabt and in **3** and **4** due to complex formation. The ν(OH) vibration bands, uncoordinated for **3** and **5** and coordinated water for **3–4** and hydroxide moieties **4,** are in the range of 3544–3453 cm^{-1}. The band of the ν(N^+–H) vibration indicating the salt formation for **1** and for **2** (HClabt)$^+$ is observed in the range of 2759–2462 cm^{-1}. These bands were not observed for **3** and **4** due to the deprotonation of the salt after the complex formation (Fig. 1). The N-H vibrations of –SO_2NH_2, –CONH–, and –NH_2 in Clabt (3453 and 3265), in H*m*absmal (3315, 3229 and 3205 cm^{-1}), in H*p*absmal (3352, 3263, and 3212 cm^{-1}), in **1** (3392, 3339, 3313, 3282, and 3187 cm^{-1}), in **2** (3388, 3304, 3261, 3239, and 3207 cm^{-1}), in **3** (3385, 3223, 3304, 3271, and 3244 cm^{-1}), in **4** (3391, 3256, 3240, and 3174 cm^{-1}) and in **5** (3331, 3261, 3294, 3234, and 3211 cm^{-1}) are survey with similar texture. The strong C=O vibrations at 1688 and 1620 cm^{-1} of H*m*absmal and at 1695 and 1630 cm^{-1} of H*p*absmal change for the salts to 1665 and 1620 cm^{-1} for **1**, and 1658 and 1612 cm^{-1} for **2**, whereas for the complexes, to 1641 and 1617 cm^{-1} for compound **3**, 1691 and 1641 cm^{-1} for **4**, and 1666 and 1600 cm^{-1} for **5**. This indicates the formation of salts and complexes. The strong absorption bands of all compounds were detected in the range of 1401–1627 cm^{-1} for *ν*(C=N) and *ν*(C=C), 1264–1142 cm^{-1} for SO_2 groups, except Clabt, and 1397–1054 cm^{-1} for C–O, except Clabt. The weak bands at 3095–3040 cm^{-1} are from the aromatic C–H

and conjugated H–C=C–H vibrations of all compounds, 550 cm^{-1} and 585 cm^{-1} are from the M-N vibrations of **3** and **4**, and 454 cm^{-1}, 498 cm^{-1}, and 462 cm^{-1} are from the M–O vibrations of **3**–**5**, respectively.

3.3. *Thermal analyses result of **3**–**5***

The TG, DTA, and DTG curves of the compounds **3–5 are** shown in Figures S10–S12 and the results of the thermal analyses are given in Table S4.

For **3–5**, in the first stage, the endothermic peaks correspond to the loss of three moles water for **3**, one mole water and one mole OH for **4**, and three moles water for **5**. In the second stage, the endothermic peaks for **3** and **5** and the exothermic peak for **4** are consistent with the loss of $C_{18}H_{18}N_4O_6S_2$ for **3**, $C_{10}H_{12}N_2O_4S_2$ for **4**, and $C_5H_6NO_2S$ unit for **5**. In the third stage, the exothermic peaks are the decomposition of the residue of $C_{16}H_{10}N_4O_4S_2Cl_2$ for **3**, $C_4H_2N_2O_3$ for **4**, and a $C_{19}H_{14}N_5O_3S_2Cl_2$ unit for **5**. The final decomposition product is CuO for **3–5**.

3.4. *UV spectra results*

The UV spectra values of all compounds are shown in DMSO solution at $1 \cdot 10^{-3}$ $moll^{-1}$ concentrations (Table S5). π-π* transitions in the range of 297–288 nm (34550–19990 l mol^{-1} cm^{-1}) are observed for **1-5**. The π-π* transitions are observed for the free ligands Clabt, H*m*absmal, and H*p*absmal, and there are no significant differences from those of **1–5**. The bands for d-d transitions of compounds (**3-5**) are observed at 767, 782, and 763 nm (13, 40, and 36 l mol^{-1} cm^{-1}), respectively.

3.5. *Magnetic susceptibility*

The results of the magnetic moments of compounds **3–5** are 1.64, 1.63, and 1.65 BM, respectively, which reveals the presence of one unpaired (Cu(II), d^9) electron.

3.6. *Molar conductivity*

The molar conductivity data of the compounds **3–5** in DMSO are 2.5, 6.7, and 11.2 Ω^{-1} cm^2 mol^{-1}, which indicates that all complexes are non-ionic.[29]

3.7. *CA inhibition assay*

In this part of the study, the effects of all compounds on human cytosolic carbonic anhydrase isoenzymes, hCA I and hCA II, were examined *in vitro*. The inhibitory effects on carbonic anhydrase isoenzymes were observed as expected due to the sulfonamide group present in the compounds. Both hydratase and esterase activities of hCA I and hCA II are inhibited by the synthesized compounds (Fig. 2).

The inhibition of the hydratase activity shows that the compounds interact (directly or indirectly) with the zinc ion in the active site of the isoenzymes.[30] Sulfonamide derivative H*p*absmal is a less potent inhibitor than *p*abs, but the Cu(II) complex of H*p*absmal proves to be a more effective inhibitor than *p*abs and H*p*absmal for the hydratase activities of the isoenzymes. The proton transfer salt of the H*p*absmal compound (**2**, $HClabt^{+}$*p*$absmal^{-}$) is a more effective inhibitor than *p*absmal, H*p*absmal, and CuH*p*absmal compounds. It is estimated that the inhibition effect of compound **2** emerges from the presence of the $HClabt^{+}$ ion in its structure. The Cu(II) complex of **2** (**4**) shows nearly four times better inhibition effects on the hydratase activities of the isoenzymes as compared to **2**. The fact that *m*abs and its derivatives, H*m*absmal and **1**, did not have any inhibitory effects on hydratase activity indicates that the enzyme-inhibitor interaction was significantly weakened in the presence of $–SO_2NH_2$ at the *meta* position. The inhibitory property of compounds **3** and **5** shows that these compounds, which are bulkier than the starting compounds (H*m*absmal and **1**), interact more robustly with the active site. Among the synthesized compounds, the most potent hydratase inhibitor is **3** (Table 3). This compound, which is the most voluminous compound in our study, can easily interact with both the hydrophilic and hydrophobic parts of the active site. This is due to its hydrophilic and hydrophobic groups.

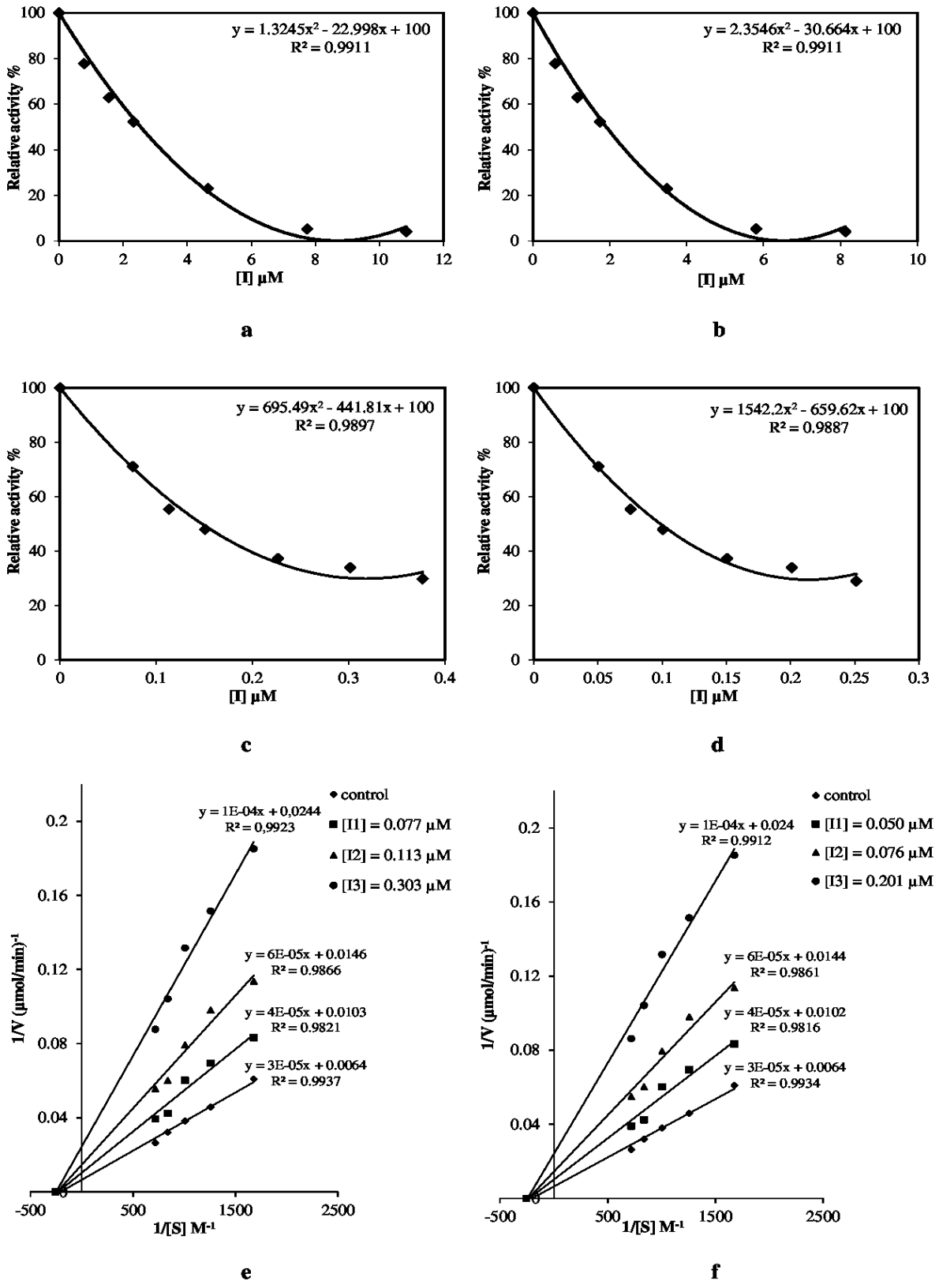


Fig. 2. Some IC_{50} and K_i plots from our study. hCA I hydratase IC_{50} plot of **3** (**a**), hCA II IC_{50} plot of **3** (**b**), hCA I esterase IC_{50} plot of **4** (**c**), hCA II esterase IC_{50} plot of **4** (**d**), hCA I Lineweaver-Burk plot of **4** (**e**), and hCA II Lineweaver-Burk plot of **4** (**f**)

Table 3

The inhibition data and K_i values of hCA I and hCA II isozymes for hydratase and esterase activity

Compound	Hydratase IC_{50}[a,b] (μM)		EsteraseIC_{50}[a,b] (μM)		K_i [a,b] (μM)	
	hCA I	hCA II	hCA I	hCA II	hCA I	hCA II
AAZ	0.39 ± 0.01	0.20 ± 0.01	0.42 ± 0.01	0.31 ± 0.01	0.26 ± 0.01	0.14 ± 0.01
*m*abs	–	–	3.14 ± 0.02	2.52 ± 0.09	0.89 ± 0.01	0.65 ± 0.01
*p*abs	30.44 ± 0.01	5.67 ± 0.01	28.14 ± 0.01	5.36 ± 0.01	26.32 ± 0.01	4.14 ± 0.01
mal	–	–	–	–	–	–
Clabt	–	–	850.00 ± 0.02	820.02 ± 0.01	–	–
CuClabt	–	–	290.01 ± 0.01	270.04 ± 0.01	–	–
H*m*absmal	–	–	7.11 ± 0.17	6.24 ± 0.25	3.64 ± 0.14	3.02 ± 0.17
H*p*absmal	84.07 ± 0.02	56.48 ± 0.01	1.64 ± 0.01	1.51 ± 0.01	1.02 ± 0.01	0.93 ± 0.01
CuH*p*absmal	13.34 ± 0.01	10.58 ± 0.01	1.43 ± 0.01	1.03 ± 0.01	0.98 ± 0.01	0.91 ± 0.01
1	–	–	8.71 ± 0.19	5.42 ± 0.14	4.25 ± 0.10	3.21 ± 0.20
2	5.04 ± 0.05	4.12 ± 0.10	0.36 ± 0.04	0.24 ± 0.02	0.15 ± 0.01	0.11 ± 0.02
3	1.09 ± 0.11	0.86 ± 0.06	1.43 ± 0.09	0.98 ± 0.07	0.86 ± 0.05	0.71 ± 0.05
4	1.68 ± 0.09	1.19 ± 0.03	0.15 ± 0.03	0.08 ± 0.01	0.06 ± 0.01	0.02 ± 0.01
5	2.33 ± 0.08	1.37 ± 0.02	4.11 ± 0.12	3.74 ± 0.16	1.92 ± 0.16	0.97 ± 0.04

[a]Mean ± standard error from three different assays.
[b]$p < 0.0001$ for all analyses.

The number of compounds inhibiting esterase activities of hCA I and II is higher than the number of compounds inhibiting hydratase activities. The fact that the *p*-nitrophenyl acetate, which is the esterase substrate, is a molecule larger than the hydratase substrate, CO_2, and it is more affected by the presence of inhibitory agents, may help explain the difference in the inhibition status. The esterase inhibition effects of the Clabt and CuClabt compounds are negligible in such a way that the K_i values could not be calculated since these compounds do not show an inhibition effect of more than 50 % required for K_i calculation. The order of esterase inhibition potential of many compounds is similar to the order of hydratase inhibition potential. However, contrary to expectations, the esterase inhibition potential of the *m*abs compound is considerably stronger than the esterase inhibition potential of the *p*abs compound. This is true only for the starting compounds, whereas for other derivatives, as expected, the inhibitory potential of the *para* derivatives is greater. The strongest inhibitor compound among the *m*abs derivatives is **3**. This can be explained by the bulky structure of the compound and the presence of various groups in its structure, as in the inhibition of hydratase activity. The inhibition potentials of the *para* derivatives increased steadily from the free ligand to the proton transfer salt complex (*p*abs < H*p*absmal < CuH*p*absmal < **2** < **4**). The addition of new groups to the structure increased the inhibition effect, especially in compounds **2** and **4**. Compound **4**, the most potent esterase inhibitor in the present research, fits the "dual-tail approach" strategy already studied by some research groups.[31,32] In this way, compounds can interact more strongly with active site amino acids (Fig. 3).

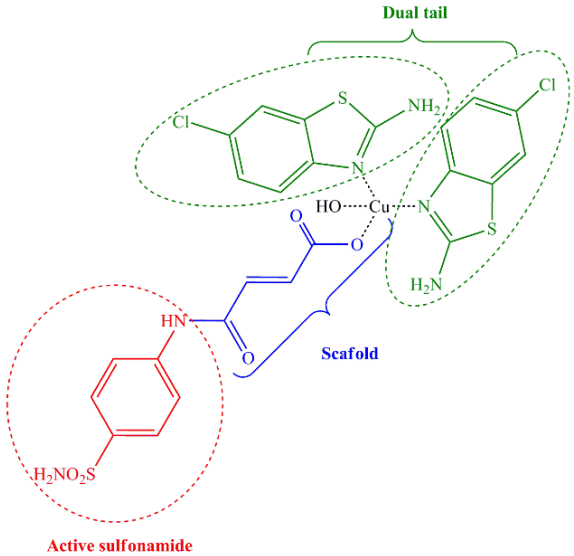


Fig. 3. Representation of compound **4** according to the dual-tail approach strategy

In addition, compound **4** shows a nearly three times stronger inhibitory effect than the control compound, **AAZ** (Table 3). The inhibition constants of the compounds (K_i values) have been determined with esterase activity method. Since

the inhibition potentials of the compounds are in the same order as the esterase inhibition potential, the structure-activity relationships in the inhibition of esterase activity are also valid in this section. The fact that the K_i values of the compounds can be determined indicates that they interact with the enzyme with sufficient strength.

In case the isoenzyme selectivity strategy mentioned in the introduction of the study is to be evaluated, it can be said that compound **4** is relatively successful in this respect. This compound inhibited the hCA II isoenzyme approximately three times stronger than the hCA I isoenzyme.

4. CONCLUSIONS

In the present work, two new salts (**1** and **2**) of sulfamoylphenylmaleamide acid and three Cu(II) complexes (**3**–**5**) were prepared for the first time. The structures of **1**–**5** are explained by the results of the spectroscopic methods.

All compounds have potential inhibition of the esterase activities of hCA I and hCA II isoenzymes. As seen from the K_i values, especially compounds **2**–**4** along with H*p*absmal and CuH*p*absmal show remarkable inhibition effects. In addition, compound **4** reveals more potential than other compounds in terms of isoenzyme selectivity. From the results of this study, it can be seen that the isoenzyme selectivity can be increased by using the dual-tail approach strategy and making some modifications to the existing compounds. The compounds synthesized in our research should help and assist future studies in this respect.

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Conflict of interest. The authors declare that they have no conflict of interest.

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