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RESEARCH ARTICLE

## Synthesis and carbonic anhydrase inhibitory properties of 1,3-dicarbonyl derivatives of methylaminobenzene-sulfonamide

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### Abstract

1,3-Dicarbonyl derivatives of methylaminobenzene-sulfonamide were synthesized and their inhibitory effects on the activity of purified human carbonic anhydrase (hCA) I and hCA II were evaluated. hCA I and hCA II from human erythrocytes were purified by a simple one-step procedure by using Sepharose 4B-L-tyrosine-sulfanilamide affinity column. Our results show that the synthesized compounds inhibited the activity of carbonic anhydrase (CA) I and CA II. Among them, **2b** and **2e** were found to be the most active ( $IC_{50} = 2.12$  and  $2.52 \mu\text{M}$ ) for hCA I and hCA II, respectively.

### Keywords

Carbonic anhydrase, 1,3-dicarbonyl, enzyme inhibitor, sulfonamide

### History

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### Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are zinc metalloenzymes that assist a very simple physiological reaction, rapid interconversion of carbon dioxide and water into protons and bicarbonate ions<sup>1</sup>. The zinc ion that is essential for catalysis is found within the active site of the most CAs. The reaction is involved in many pathological and physiological processes that include respiration and transport of CO<sub>2</sub> and bicarbonate between metabolizing tissues and lungs, biosynthetic reactions, electrolyte secretion in various tissues and organs, pH and CO<sub>2</sub> homeostasis<sup>2–4</sup>.

Up to now, 16 human CA (hCA) isoforms have been identified exhibiting significant differences in catalytic activity, subcellular localization and tissues expression. They play important roles in many of physiological processes such as several biochemical pathways, cell proliferation, intra- and extracellular pH homeostasis and differentiation, and modulation of neuronal transmission<sup>5,6</sup>. In human, CAs are found in a variety of tissues such as kidneys, lungs, eyes, skins, nerves systems and gastrointestinal tract<sup>7</sup>. The different isozymes are found in different part of cell, and hCA I and hCA II are localized in the cytosol<sup>2</sup>. Biological activities of this metalloenzyme family have several medicinal applications such as treatment of glaucoma, diuretics and management of several neurological disorders, whereas several agents are in clinical evaluations as antiobesity or antidrugs<sup>8</sup>.

There are abundant number of molecules containing sulfonamide moiety<sup>9</sup>. The compounds have several applications such as modified oligonucleotides<sup>10</sup>, amines synthons<sup>11</sup>, peptidomimetics<sup>12</sup>, chiral auxiliaries<sup>13</sup> and therapeutics<sup>14</sup>. Sulfonamide

derivatives are well known pharmaceutical agents and have gained much attention due to their diverse biological activities in pharmaceutical as well as in agricultural areas<sup>15</sup>. Some of 1,3-diketone compounds and their barbituric and thiobarbituric derivatives were prepared and urease inhibition studies were reported<sup>16,17</sup>. The sulfonamide compounds have a number of biological activities such as antibacterial<sup>18</sup>, insulin releasing<sup>19</sup>, anti-inflammatory<sup>20</sup>, antitumor<sup>21</sup> and CA inhibitory<sup>22</sup>.

Sulfonamides are the best known inhibitors of CA enzymes and used for the treatment of glaucoma in medicinal chemistry<sup>23</sup>. Acetazolamide (AAZ), dorzolamide (DZA) and brinzolamide (BRZ) are sulfonamide derivatives and used in the treatment of glaucoma. However, the drugs have side effects such as numbness and tingling in the fingers and toes, blurred vision, kidney stones, an increase in urination, upset stomach, dry eye and headache or dizziness<sup>24,25</sup>.

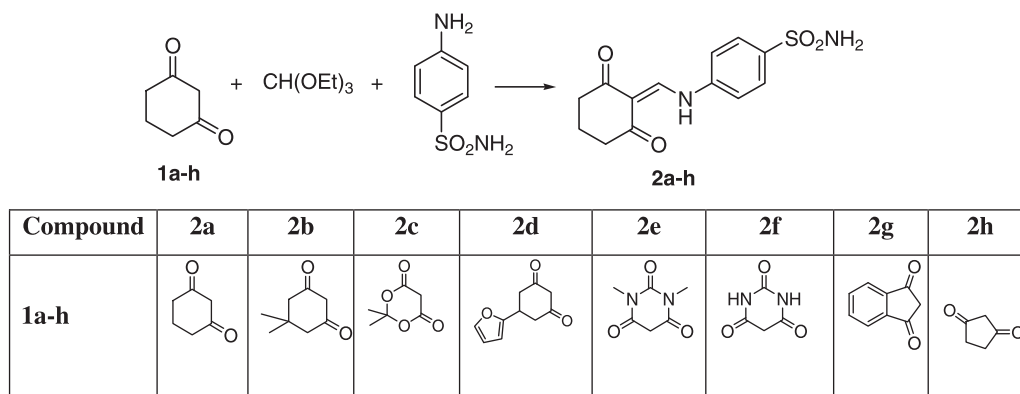
In this study, 1,3-dicarbonyl derivatives of methylaminobenzene-sulfonamide were synthesized and their inhibitory effects on the activity of purified human hCA I and hCA II were evaluated.

### Materials and methods

The compounds were prepared from a mixture of sulfanilamide, 1,3-dicarbonyl compounds and triethyl [orthoformate] in ethanol by refluxing for 3 h and shown in Scheme 1. The prepared compounds were characterized by <sup>1</sup>H NMR, <sup>13</sup>C NMR, IR and elemental analysis.

### General procedure

Melting points were taken on a Yanagimoto Barnstead Electrothermal (Surrey, UK) micro-melting point apparatus and were uncorrected. IR spectra were measured on a Shimadzu Prestige-21 (200 VCE) spectrometer (Kyoto, Japan). <sup>1</sup>H and <sup>13</sup>C NMR spectra were measured on spectrometer at Varian Infinity



Scheme 1. Synthesis of 1,3-dicarbonyl derivatives of methylaminobenzene-sulfonamide derivatives.

Plus 300 and at 75 Hz (California), respectively.  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts were referenced to the internal deuterated solvent. The elemental analysis was carried out with a Leco CHNS-932 instrument (St. Joseph, MI). Flash column chromatography was performed using Merck silica gel 60 (230–400 mesh ASTM) (Darmstadt, Germany). All chemicals were purchased from Merck (Darmstadt, Germany), Alfa Easer (Ward Hill, MA) and Sigma-Aldrich (Taufkirchen, Germany).

Synthesis of 1,3-dicarbonyl substituted methylaminobenzene-sulfonamide derivatives were prepared according to Scheme 1.

#### General procedure for the synthesis of 1,3-dicarbonyl substituted methylaminobenzene-sulfonamide derivatives

A mixture of sulfanilamide (18.25 g, 0.106 mol), 1,3-dicarbonyl compound (0.127 mol) and triethyl [orthoformate] (16 mL) in ethanol (100 mL) was refluxed for 3 h. After cooling in room temperature, the product was filtered, washed with cold ethanol and air dried.

#### 4-((2,6-Dioxocyclohexylidene)methylamino)benzenesulfonamide (2a)

Yield 85.8%, m.p. 270.2 °C; IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3296 ( $\text{NH}_2$ ), 3203 (NH), 3072 ( $=\text{C}-\text{H}$ , aromatic), 1653 ( $\text{C}=\text{O}$ );  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ , ppm): 12.62 (1H, d,  $-\text{NH}$ ,  $j = 13.5$ ), 8.56 (1H, d,  $=\text{CH}$ ,  $j = 13.5$ ), 7.84 (2H, d,  $=\text{CH}$ ,  $j = 8.7$ ), 7.71 (2H, d,  $=\text{CH}$ ,  $j = 8.7$ ), 7.41 ( $-\text{NH}_2$ ), 2.42 (2H, t,  $-\text{CH}_2$ ), 2.39 (2H, t,  $-\text{CH}_2$ ), 1.96 (2H, m,  $-\text{CH}_2$ ),  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ , ppm): 200.7, 196.4, 150.6, 142.0, 141.6, 128.0, 119.3, 111.0, 38.3, 37.9, 19.7. Anal. Calcd for  $\text{C}_{13}\text{H}_{14}\text{N}_2\text{O}_4\text{S}$  (%): C, 53.05; H, 4.79; N, 9.52; O, 21.74; S, 10.89. Found (%): C, 53.18; H, 4.82; N, 9.59; O, 21.80; S, 10.96.

#### 4-((4,4-Dimethyl-2,6-dioxocyclohexylidene)methylamino)benzenesulfonamide (2b)

Yield 87.2%, m.p. 305.4 °C; IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3277 ( $\text{NH}_2$ ), 3203 (NH), 3065 ( $=\text{C}-\text{H}$ , aromatic), 1666 ( $\text{C}=\text{O}$ );  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ , ppm): 12.65 (1H, d,  $-\text{NH}$ ,  $j = 13.5$ ), 8.50 (1H, d,  $=\text{CH}$ ,  $j = 13.5$ ), 7.80 (2H, d,  $=\text{CH}$ ,  $j = 7.9$ ), 7.58 (2H, d,  $=\text{CH}$ ,  $j = 7.9$ ), 7.28 ( $\text{NH}_2$ ), 2.38 (2H, s,  $-\text{CH}_2$ ), 2.36 (2H, s,  $-\text{CH}_2$ ), 1.02 (6H, s,  $-\text{CH}_3$ ),  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ , ppm): 199.9, 195.9, 150.0, 141.8, 141.5, 128.1, 118.9, 109.7, 51.7, 51.4, 39.2, 31.3, 28.5. Anal. Calcd for  $\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_4\text{S}$  (%): C, 55.88; H, 5.63; N, 8.69; O, 19.85; S, 9.95. Found (%): C, 55.99; H, 5.7; N, 8.71; O, 19.93; S, 9.98.

#### 4-((2,2-Dimethyl-4,6-dioxo-1,3-dioxan-5-ylidene)methylamino)benzenesulfonamide (2c)

Yield 80.6%, m.p. 263.2 °C; IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3365 ( $\text{NH}_2$ ), 3269 (NH), 3075 ( $=\text{C}-\text{H}$ , aromatic), 1631 ( $\text{C}=\text{O}$ );  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ , ppm): 11.25 (1H, d,  $-\text{NH}$ ,  $j = 13.9$ ), 8.64 (1H, d,  $=\text{CH}$ ,

$j = 13.9$ ), 7.88 (2H, d,  $=\text{CH}$ ,  $j = 8.7$ ), 7.45 (2H, d,  $=\text{CH}$ ,  $j = 8.7$ ), 7.22 ( $\text{NH}_2$ ), 1.86 (6H, s,  $-\text{CH}_3$ ),  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ , ppm): 164.4, 163.3, 153.9, 141.9, 127.8, 119.9, 105.0, 88.5, 39.3, 27.2. Anal. Calcd for  $\text{C}_{13}\text{H}_{14}\text{N}_2\text{O}_6\text{S}$  (%): C, 47.85; H, 4.32; N, 8.58; O, 29.42; S, 9.83. Found (%): C, 47.99; H, 4.39; N, 8.64; O, 29.47; S, 9.89.

#### 4-((4-(Furan-2-yl)-2,6-dioxocyclohexylidene)methylamino)benzenesulfonamide (2d)

Yield 75.3%, m.p. 278.3 °C; IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3321 ( $\text{NH}_2$ ), 3180 (NH), 3082 ( $=\text{C}-\text{H}$ , aromatic), 1596 ( $\text{C}=\text{O}$ );  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ , ppm): 12.63 (1H,  $-\text{NH}$ ,  $j = 13.6$ ), 8.53 (1H, d,  $=\text{CH}$ ,  $j = 13.6$ ), 7.83 (2H, d,  $=\text{CH}$ ,  $j = 8.7$ ), 7.70 (2H, d,  $=\text{CH}$ ,  $j = 8.7$ ), 7.52 ( $\text{NH}_2$ ), 6.41 (1H, s,  $=\text{CH}$ ), 6.38 (1H, d,  $=\text{CH}$ ,  $j = 3.1$ ), 6.16 (1H, d,  $=\text{CH}$ ,  $j = 3.1$ ), 3.51–3.59 (1H, m,  $-\text{CH}$ ), 2.68–2.95 (4H, m,  $-\text{CH}_2$ ),  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ , ppm): 198.7, 194.6, 156.8, 150.6, 142.7, 141.9, 141.7, 128.0, 119.4, 115.1, 110.6, 105.7, 42.3, 42.0, 30.7. Anal. Calcd for  $\text{C}_{17}\text{H}_{16}\text{N}_2\text{O}_5\text{S}$  (%): C, 56.66; H, 4.47; N, 7.77; O, 22.20; S, 8.90. Found (%): C, 56.73; H, 4.52; N, 7.79; O, 22.26; S, 8.92.

#### 4-((1,3-Dimethyl-2,4,6-trioxo-tetrahydropyrimidin-5(6H)-ylidene)methylamino)benzenesulfonamide (2e)

Yield 87.6%, m.p. 294.5 °C; IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3307 ( $\text{NH}_2$ ), 3190 (NH), 3062 ( $=\text{C}-\text{H}$ , aromatic), 1633 ( $\text{C}=\text{O}$ );  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ , ppm): 11.98 (1H, d,  $-\text{NH}$ ,  $j = 13.9$ ), 8.61 (1H, d,  $=\text{CH}$ ,  $j = 8.7$ ), 7.86 (2H, d,  $=\text{CH}$ ,  $j = 8.7$ ), 7.65 (2H, d,  $=\text{CH}$ ,  $j = 13.9$ ), 7.43 ( $\text{NH}_2$ ), 3.21 (6H, s,  $\text{N}-\text{CH}_3$ ),  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ , ppm): 164.5, 162.4, 152.2, 151.95, 141.7, 141.6, 128.0, 119.3, 94.2, 28.3, 27.6. Anal. Calcd for  $\text{C}_{13}\text{H}_{14}\text{N}_4\text{O}_5\text{S}$  (%): C, 46.15; H, 4.17; N, 16.56; O, 23.64; S, 9.48. Found (%): C, 46.21; H, 4.24; N, 16.59; O, 23.68; S, 9.52.

#### 4-((2,4,6-Trioxo-tetrahydropyrimidin-5(6H)-ylidene)methylamino)benzenesulfonamide (2f)

Yield 89.3%, m.p. >350 °C; IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3346 (NH), 3209 ( $\text{NH}_2$ ), 3105 (NH), 3042 ( $=\text{C}-\text{H}$ , aromatic), 1643 ( $\text{C}=\text{O}$ );  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ , ppm): 11.95 (1H, d,  $-\text{NH}$ ,  $j = 13.7$ ), 11.12 (1H,  $-\text{NH}$ ), 10.97 (1H,  $-\text{NH}$ ), 8.64 (1H, d,  $=\text{CH}$ ,  $j = 13.7$ ), 7.84 (2H, d,  $=\text{CH}$ ,  $j = 8.8$ ), 7.72 (2H, d,  $=\text{CH}$ ,  $j = 8.8$ ), 7.41 ( $\text{NH}_2$ ),  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ , ppm): 166.7, 164.1, 151.7, 151.3, 141.85, 141.4, 128.0, 119.2, 94.3. Anal. Calcd for  $\text{C}_{11}\text{H}_{10}\text{N}_4\text{O}_5\text{S}$  (%): C, 42.58; H, 3.25; N, 18.06; O, 25.78; S, 10.33. Found (%): C, 42.68; H, 3.29; N, 18.16; O, 25.98; S, 10.55.

#### 4-((1,3-Dioxo-1H-inden-2(3H)-ylidene)methylamino)benzenesulfonamide (2g)

Yield 90.5%, m.p. 307.8 °C; IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3367 ( $\text{NH}_2$ ), 3278 (NH), 3052 ( $=\text{C}-\text{H}$ , aromatic), 1651 ( $\text{C}=\text{O}$ );  $^1\text{H}$  NMR (300 MHz,

DMSO- $d_6$ , ppm): 11.25 (1H, d,  $-NH$ ,  $j = 13.8$ ), 8.38 (1H, d,  $=CH$ ,  $j = 13.8$ ), 7.84 (2H, d,  $=CH$ ), 7.80 (2H, d,  $=CH$ ), 7.78 (2H, t,  $=CH$ ), 7.72 (2H, t,  $=CH$ ), 7.39 ( $NH_2$ ),  $^{13}C$  NMR (75 MHz, DMSO- $d_6$ , ppm): 191.9, 189.6, 144.5, 142.4, 141.1, 140.2, 140.2, 134.9, 127.8, 122.5, 122.2, 119.0, 107.3. Anal. Calcd for  $C_{16}H_{12}N_2O_4S$  (%): C, 58.53; H, 3.68; N, 8.53; O, 19.49; S, 9.77. Found (%): C, 58.65; H, 3.75; N, 8.61; O, 19.57; S, 9.96.

#### 4-((2,5-Dioxocyclopentylidene)methylamino)benzene-sulfonamide (**2h**)

Yield 78.7%, m.p. 290.7 °C; IR (KBr,  $\nu$ ,  $cm^{-1}$ ): 3296 ( $NH_2$ ), 3203 (NH), 3076 ( $=C-H$ , aromatic), 1593 ( $C=O$ );  $^1H$  NMR (300 MHz, DMSO- $d_6$ , ppm): 11.95 (1H, d,  $-NH$ ,  $j = 13.2$ ), 8.27 (1H, d,  $=CH$ ,  $j = 13.2$ ), 7.80 (2H, d,  $=CH$ ,  $j = 6.7$ ), 7.77 (2H, d,  $=CH$ ,  $j = 6.7$ ), 7.36 ( $NH_2$ ), 2.39 (2H, d,  $-CH_2$ ), 2.36 (2H, d,  $-CH_2$ ).  $^{13}C$  NMR (75 MHz, DMSO- $d_6$ , ppm): 205.4, 201.9, 147.2, 141.8, 127.8, 119.6, 109.7, 34.5, 33.9. Anal. Calcd for  $C_{12}H_{12}N_2O_4S$  (%): C, 51.42; H, 4.32; N, 9.99; O, 22.83; S, 11.44. Found (%): C, 51.56; H, 4.36; N, 9.91; O, 22.94; S, 11.50.

### Preparation of haemolysate and purification from blood red cells

Blood samples (25 mL) were taken from healthy human volunteers. They were anticoagulated with acid-citrate-dextrose, centrifuged at 2000g for 20 min at 4 °C and the supernatant was removed. The packed erythrocytes were washed three times with 0.9% NaCl and then haemolysed in cold water. The ghosts and any intact cells were removed by centrifugation at 2000g for 25 min at 4 °C, and the pH of the haemolysate was adjusted to pH 8.5 with solid Tris-base. The 25 mL haemolysate was applied to an affinity column containing L-tyrosine-sulfonamide-Sepharose-4B<sup>26</sup> equilibrated with 25 mM Tris-HCl/0.1 M  $Na_2SO_4$  (pH 8.5). The affinity gel was washed with 50 mL of 25 mM Tris-HCl/22 mM  $Na_2SO_4$  (pH 8.5). The hCA isozymes were then eluted with 0.1 M NaCl/25 mM  $Na_2HPO_4$  (pH 6.3) and 0.1 M  $CH_3COONa$ /0.5 M  $NaClO_4$  (pH 5.6), which recovered hCA I and hCA II, respectively. Fractions of 3 mL were collected and their absorbance measured at 280 nm.

### CA enzyme assay

CA activity was measured by the Maren method based on the determination of the time required for the pH to decrease from 10.0 to 7.4 due to  $CO_2$  hydration<sup>19</sup>. The assay solution was 0.5 M  $Na_2CO_3$ /0.1 M  $NaHCO_3$  (pH 10.0) and phenol red was added as the pH indicator.  $CO_2$ -hydratase activity (enzyme units (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.

### In vitro inhibition studies

For the inhibition studies of sulfonamide, different concentrations of these compounds were added to the enzyme. Activity percentage values of CA for different concentrations of each sulfonamide were determined by regression analysis using Microsoft Office 2000 Excel (New York, NY). CA enzyme activity without a sulfonamide solution was accepted to be 100%.

### Results and discussion

For evaluating the physiologically relevant hCA isozymes hCA I and hCA II inhibitory activity, several 1,3-dicarbonyl derivatives of methylaminobenzene-sulfonamide were subjected to CA inhibition assay with  $CO_2$  as substrate.

Table 1. The  $IC_{50}$  values of the synthesized compounds.

Compounds	hCA I $IC_{50}$ ( $\mu M$ )	hCA II $IC_{50}$ ( $\mu M$ )
<b>2a</b>	4.25	4.93
<b>2b</b>	2.12	6.41
<b>2c</b>	6.56	18.64
<b>2d</b>	8.61	7.21
<b>2e</b>	5.20	2.52
<b>2f</b>	10.89	9.92
<b>2g</b>	3.19	3.36
<b>2h</b>	4.8	3.36

The prepared compounds were characterized by  $^1H$  NMR,  $^{13}C$  NMR, IR and elemental analysis. From the  $^1H$  NMR spectra, the hydrogen attached to the nitrogen resonances between 11.00 and 13.00 ppm, the  $=CH$  proton peak comes around 8.50 ppm and  $NH_2$  protons are seen between 7.00 and 7.50. From the  $^{13}C$  NMR spectra, 1,3-diketone carbonyl and barbiturate carbonyl carbons are observed at around 200 and 160 ppm, respectively. In the infrared spectra of compounds **2a–h**, it was possible to observe the absorptions between 3200 and 3400  $cm^{-1}$  relating to NH stretch, absorptions in 1590–1700  $cm^{-1}$  from carbonyl moiety stretching.

Sulfonamides are coordinated to the zinc (II) ion within the hCAs active site, whereas its organic scaffold fills the entire enzyme cavity, making an extensive series of van der Waals and polar interactions with amino acid residues both at the bottom, middle and entrance of the active site cavity<sup>27</sup>.

Inhibitors of CA play an important role in ophthalmology where they are used to reduce elevated intraocular pressure (IOC). Acetazolamide is the most widely used inhibitor and has advantages over the others. It is 20 times less active against CAI than against CAII in erythrocytes<sup>28</sup>. For evaluating the physiologically relevant hCA isozymes hCA I and hCA II inhibitory activity, several new sulfonamide compounds were subjected to CA inhibition assay with  $CO_2$  as substrate. The results showed that all the compounds inhibited the enzyme activity and the inhibition constants against CAs were given in Table 1. It is determined that the inhibition values are between 2.12 and 10.89  $\mu M$  for hCA I and between 2.52 and 18.64  $\mu M$  for hCA II. Among the compounds, **2b** and **2e** were found to be the most active for CAs.

Although sulfonamide compounds are mentioned very potent inhibitor of the cytosolic isoform hCAs<sup>29</sup>, all the synthesized substituted sulfonamides are moderate inhibitory activity for the hCAs. Substituents on the sulfonamides affect inhibition. In this way, methyl groups in the **2b** (2.12  $\mu M$  for CA I) and **2e** (2.52  $\mu M$  for CA II), and aromatic group in **2g** (3.19 and 3.36  $\mu M$  for hCA I and hCA II, respectively) make them strong inhibitory effects on hCAs compared to the other synthesized sulfonamides.

Although the structures of CA I and CA II are similar, there are several differences in the active sites of amino acids. Particularly, hydrophobic residues (Phe131, Val135 and Leu204) on the surface of CAII play an important stabilizing role when interacting with the hydrophobic groups of sulfonamide inhibitors<sup>30</sup>. The sulfonamides inhibit CAs by the coordination of the sulfonamide moiety to the metal ion ( $Zn^{+2}$ ) from the enzyme active site, and participation in hydrophobic and hydrophilic interactions of the organic scaffold of the inhibitor with amino acids residues are known to be involved in the binding process of CAs<sup>31</sup>. It is assumed that the effects are the results of hydrophobic and van der Waals interactions between aromatic/aliphatic part of the inhibitor molecule and active site of amino acid residues. The compounds have a lower affinity to



CA I and CA II compared to acetazolamide<sup>32</sup> which is used in the treatment of glaucoma.

In conclusion, we report here a series of 1,3-dicarbonyl derivatives of methylaminobenzene-sulfonamide which have been assayed for the inhibition of the physiologically relevant CA isozymes. They inhibit the CAs with the inhibition constants of 2.12–10.89  $\mu\text{M}$  for hCA I and 2.52–18.64  $\mu\text{M}$  for hCA II. Compounds **2b** and **2e** behaved as a moderate inhibitory activity on hCA I and hCA II with an activation constant of 2.12 and 2.52  $\mu\text{M}$ , respectively. Last but not the least, enzyme inhibition is more important issue for drug design and biochemical applications<sup>33–46</sup>. The results put forward that new sulfonamide derivatives inhibited the hCA I and hCA II enzyme activity. Therefore, our results suggested that sulfonamide derivatives are likely to be adopted as candidates to treat glaucoma and they may be taken for further evaluation in *in vivo* studies.

### Declaration of interest

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