

## Some *N*-(5-methyl-1,3,4-thiadiazol-2-yl)-4-[(3-substituted)ureido/thioureido]benzenesulfonamides as carbonic anhydrase I and II Inhibitors

Sevda TÜRK, Fatih TOK, Hülya ÇELİK, Sevgi KARAKUŞ, Hayrunnisa NADAROĞLU, Bedia KOÇYİĞİT-KAYMAKÇIOĞLU, Kaan KÜÇÜKOĞLU

### ABSTRACT

In the present study, *N*-(5-methyl-1,3,4-thiadiazol-2-yl)-4-[(3-substituted)ureido]benzenesulfonamide (**1-9**) and *N*-(5-methyl-1,3,4-thiadiazol-2-yl)-4-[(3-substituted)thioureido]benzenesulfonamide (**10-14**) derivatives were synthesized from 4-amino-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (sulfamethizole). All new compounds were characterized by elemental analysis and various spectroscopic methods (FTIR, <sup>1</sup>H-NMR and MS). These new sulfonamide derivatives were investigated as inhibitors of carbonic anhydrase especially human carbonic anhydrase I and II. The new

compounds showed higher activity against the human cytosolic CA I (IC<sub>50</sub> values 0.144-15.65 nM) and CA II (IC<sub>50</sub> values 0.109-17.95 nM) in comparison with the clinically used CA inhibitor acetazolamide.

**Keywords:** Carbonic anhydrase inhibitors, sulfonamide, sulfamethizole, urea and thiourea.

*This study had partly been presented on The 3<sup>rd</sup> International BAU-Drug Design Congress (1-3 October 2015) as poster number 4 and 15<sup>th</sup> International Multidisciplinary Symposium on Drug Research & Development (15-17 October 2015) as abstract book page 186.*

Sevda TÜRK, Fatih TOK, Sevgi KARAKUŞ, Bedia KOÇYİĞİT-KAYMAKÇIOĞLU  
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Marmara University, 34668, Istanbul, TURKEY

Hülya ÇELİK  
Department of Pharmaceutical Technology, Faculty of Pharmacy, Agri Ibrahim Cecen University, 04000, Agri, TURKEY

Hayrunnisa NADAROĞLU  
Department of Food Technology, Erzurum Vocational School, Ataturk University, 25240, Erzurum, TURKEY

Kaan KÜÇÜKOĞLU  
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ataturk University, 25240, Erzurum, TURKEY

### Corresponding Author

Sevgi KARAKUŞ  
E-posta: skarakus@marmara.edu.tr

Submitted / Gönderilme: 10.08.2016      Revised / Düzeltilme: 24.08.2016  
Accepted / Kabul: 25.08.2016

### INTRODUCTION

According to the recent studies it has been proved that the solid tumours' extracellular pH is more acidic than the normal tissue. However, the intracellular pH is similar to normal cells or even a bit more basic. To regulate the pH gradient between the intracellular and extracellular compartments the tumour cells excrete ion transport proteins, such as, H<sup>+</sup>-ATPase, Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchanger etc (1). Many tumours also express CAs, the Zn (II) dependent enzymes catalyzing the hydration of carbon dioxide to from bicarbonate and a proton (2-4).

In the animal world, there are 16 isozymes of CA. These enzymes differ in their subcellular localization, catalytic activity and susceptibility to different classes of inhibitors. Some of them are cytosolic (CA I, CA II, CA III, CA VII and CA XIII), others are membrane-bound (CA IV, CA IX, CA XII, CA XIV and CA XV), two are mitochondrial (CA VA and CA VB) and one is secreted in saliva and milk (CA VI) (5-9). It has been reported that CA XV isoform is not expressed in humans or in other primates but it is

plentiful in rodents and other higher vertebrates (10). CAs play an important role in several biological processes: acid-base balance, respiration, carbon dioxide and ion transport, bone resorption, ureagenesis, gluconeogenesis, lipogenesis, etc (11, 12).

Among these subtypes the CA IX and XII are highly secreted in some tumors and mostly associated with oncogenesis (13). The previous studies have also indicated that the CA I and II levels were also higher in several cancer types, such as higher cytosolic erythrocyte levels in stomach, prostate, lung and ovary tumors; also in hematological diseases such as leukemia (14). Also, CA II has come to the forefront by being expressed in the endothelium of neovessels in some cancer tissues, including melanoma, esophageal, renal and lung cancers (15). Furthermore, CA I and II showed significant antiglaucoma effect. It was reported that hCA I and hCA II inhibitors can be used at cerebral edema, glaucoma, altitude sickness (16).

The aromatic/heterocyclic sulfonamide derivatives are broadly known as potent inhibitors of CAs (17). It has been known that acetazolamide (AAZ), methazolamide (MZA), ethoxzolamide (EZA), dorzolamide (DZA), brinzolamide (BRZ), topiramate (TPM), sulpiride (SLP), dichlorophenamide (DCP), indisulam (IND) and zonisamide (ZNS) are clinically used as hCA inhibitors.

In this paper, the synthesis and biological evaluation of a series of *N*-(5-methyl-1,3,4-thiadiazol-2-yl)-4-[(3-substituted)ureido]benzenesulfonamide and *N*-(5-methyl-1,3,4-thiadiazol-2-yl)-4-[(3-substituted)thioureido]benzenesulfonamide derivatives as hCA-I and hCA-II inhibitors has been reported.

## MATERIAL AND METHODS

### Chemistry

All the reagents were obtained commercially and used by further purification using standard procedures. Melting points were determined by Schmelzpunktbestimmer SMP II apparatus. The IR spectra were recorded on a Shimadzu FTIR 8400 S Spectrometer. The <sup>1</sup>H-NMR spectra were recorded (in DMSO-d<sub>6</sub>) with a Varian Mercury 300 MHz spectrometer. The chemical shift values are expressed in ppm (δ scale) using tetramethylsilane as an internal standard. The mass spectral measurements were carried out by Atmospheric Pressure Chemical Ionization (APCI) method on LC-MS-Agilent 1100. Elemental analysis was performed on Leco 215 CHNS-932 analyzer.

### General procedure for the preparation of urea derivatives (1-9)

Sulfamethizole (0.00105 mol, 0.280 g) was solved in acetone, at 80 °C. Then, a solution of the corresponding isocyanate (0.00105mol) in dry acetone was added as two parts, per 30 minutes. After 6 hours the reaction was finalized by TLC control and left overnight. The precipitate was filtered off, dried and purified with ethanol.

#### 4-[(3-Chloro-4-methylphenyl)carbamoyl]amino}-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (1)

Starting from sulfamethizole (0.280 g), 3-chloro-4-methylphenylisocyanate (0.180 g) in dry acetone for 6 h, the title compound **1** was obtained. Yield: 26 %; M.p. 226-228 °C; IR (cm<sup>-1</sup>): 3310, 3275, 3030, 2903, 1692, 1613, 1586, 1526, 1491, 1136. <sup>1</sup>H-NMR (300 MHz), (DMSO-d<sub>6</sub>/TMS) ppm: 2.25 (s, 3H, CH<sub>3</sub>), 2.52 (s, 3H, CH<sub>3</sub>), 7.52-8.60 (m, 7H, Ar-H), 9.62 (s, 2H, NH), 13.85 (s, 1H, -SO<sub>2</sub>NH-). Anal.calc. for C<sub>17</sub>H<sub>16</sub>ClN<sub>5</sub>O<sub>3</sub>S<sub>2</sub>: C, 46.63; H, 3.68; N, 15.99; S, 14.64 %. Found C, 47.14; H, 3.65; N, 16.27; S, 14.35 %. MS (APCI Neg Ion) m/z: 436.06.

#### 4-[(tert-Butylcarbamoyl)amino]-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (2)

Starting from sulfamethizole (0.280 g), *tert*-butylisocyanate (0.120 ml) in dry acetone for 6 h, the title compound **2** was obtained. Yield: 15%; M.p. 215 °C; IR (cm<sup>-1</sup>): 3364, 3129, 3011, 2982, 2899, 2805, 1678, 1522, 1316, 1134. <sup>1</sup>H-NMR (300 MHz), (DMSO-d<sub>6</sub>/TMS) ppm: 3.35 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>C-), 2.51 (s, 3H, -CH<sub>3</sub>), 6.21 (s, 1H, (CH<sub>3</sub>)C-NH), 7.37 (d, 2H, J: 8.7 Hz, Ar-H), 7.58 (d, 2H, J: 8.7 Hz, Ar-H), 8.66 (s, 1H, -NH-), 13.85 (s, 1H, -SO<sub>2</sub>NH-). Anal.calc. for C<sub>14</sub>H<sub>19</sub>N<sub>5</sub>O<sub>3</sub>S<sub>2</sub>: C, 45.51; H, 5.18; N, 18.96; S, 17.36 %. Found C, 45.20; H, 5.16; N, 18.91; S, 17.02 %. MS (APCI Neg Ion) m/z: 368.18.

#### 4-[(Butylcarbamoyl)amino]-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (3)

Starting from sulfamethizole (0.280 g), butylisocyanate (0.121 ml) in dry acetone for 6 h, the title compound **3** was obtained. Yield: 70%; M.p. 259 °C; IR (cm<sup>-1</sup>): 3374, 3129, 3021, 2955, 2895, 1680, 1641, 1557, 1526, 1492, 1402, 1323, 1140. <sup>1</sup>H-NMR (300 MHz), (DMSO-d<sub>6</sub>/TMS) ppm: 0.80 (t, 3H, -CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 1.20-1.85 (m, 4H, -CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 2.50 (s, 3H, -CH<sub>3</sub>), 2.95 (q, 2H, -NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 6.09 (t, 1H, -NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 6.50-7.40 (m, 4H, Ar-H), 8.55 (s, 1H, -NH-), 13.80 (s, 1H, -SO<sub>2</sub>NH-).

Anal.calc. for  $C_{14}H_{19}N_5O_3S_2$ : C, 45.51; H, 5.18; N, 18.96; S, 17.36 %. Found C, 45.86; H, 5.12; N, 18.08; S, 17.02 %. MS (APCI Neg Ion) m/z: 368.20.

***N*-(5-Methyl-1,3,4-thiadiazol-2-yl)-4-[(4-nitrophenyl)carbamoyl]amino}benzenesulfonamide (4)**

Starting from sulfamethizole (0.280 g), 4-nitrophenylisocyanate (0.130 ml) in dry acetone for 6 h, the title compound **4** was obtained. Yield: 55%; M.p. 250-252 °C; IR ( $cm^{-1}$ ): 3308, 3279, 3223, 3102, 3015, 2889, 1690, 1593, 1562, 1530, 1505, 1435, 1400, 1341, 1314, 1134.  $^1H$ -NMR (300 MHz), (DMSO- $d_6$ /TMS) ppm: 2.49 (s, 3H, -CH<sub>3</sub>), 7.61-8.21 (m, 8H, Ar-H), 9.33-9.52 (s, 2H, -NH-), 13.90 (s, 1H, -SO<sub>2</sub>NH-). Anal.calc. for  $C_{16}H_{14}N_6O_5S_2$ : C, 44.23; H, 3.25; N, 19.34; S, 14.76 %. Found C, 44.63; H, 3.32; N, 19.64; S, 15.00 %. MS (APCI Neg Ion) m/z : 433.10.

***4*-[(2,4-Dichlorophenyl)carbamoyl]amino}-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (5)**

Starting from sulfamethizole (0.280 g), 2,4-dichlorophenylisocyanate (0.130 ml) in dry acetone for 6 h, the title compound **5** was obtained. Yield: 60%; M.p. 153-155 °C; IR ( $cm^{-1}$ ): 3308, 3273, 3109, 3034, 2857, 1692, 1584, 1526, 1495, 1470, 1404, 1325, 1153.  $^1H$ -NMR (300 MHz), (DMSO- $d_6$ /TMS) ppm: 2.53 (s, 3H, -CH<sub>3</sub>), 7.48-8.59 (m, 7H, Ar-H), 9.92 (s, 2H, -NH-), 13.88 (s, 1H, -SO<sub>2</sub>NH-). Anal.calc. for  $C_{16}H_{13}Cl_2N_5O_3S_2$ : C, 41.93; H, 2.86; N, 15.28; S, 13.99 %. Found C, 41.39; H, 3.14; N, 16.05; S, 14.26 %. MS (APCI Neg Ion) m/z : 456.08.

***4*-[(2,6-Dichlorophenyl)carbamoyl]amino}-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (6)**

Starting from sulfamethizole (0.280 g), 2,6-dichlorophenylisocyanate (0.130 ml) in dry acetone for 6 h, the title compound **6** was obtained. Yield: 65%; M.p. 190-191 °C; IR ( $cm^{-1}$ ): 3293, 3146, 3042, 2907, 1651, 1530, 1491, 1458, 1437, 1402, 1314, 1128.  $^1H$ -NMR (300 MHz), (DMSO- $d_6$ /TMS) ppm: 2.49 (s, 3H, -CH<sub>3</sub>), 7.30-7.70 (m, 7H, Ar-H), 8.34-9.40 (s, 2H, -NH-), 11.10 (s, 1H, -SO<sub>2</sub>NH-). Anal.calc. for  $C_{16}H_{13}Cl_2N_5O_3S_2$ : C, 41.93; H, 2.86; N, 15.28; S, 13.99 %. Found C, 42.24; H, 2.77; N, 15.73; S, 13.85 %. MS (APCI Neg Ion) m/z : 456.06.

***N*-(5-Methyl-1,3,4-thiadiazol-2-yl)-4-[(2,4,6-trichlorophenyl)carbamoyl]amino}benzenesulfonamide (7)**

Starting from sulfamethizole (0.280 g),

2,4,6-trichlorophenylisocyanate (0.130 ml) in dry acetone for 6 h, the title compound **7** was obtained. Yield: 45%; M.p. 183-184 °C; IR ( $cm^{-1}$ ): 3281, 3256, 3206, 3086, 3015, 2980, 1659, 1574, 1537, 1452, 1134.  $^1H$ -NMR (300 MHz), (DMSO- $d_6$ /TMS) ppm: 2.51 (s, 3H, -CH<sub>3</sub>), 7.74 (s, 6H, Ar-H), 8.63 (s, 2H, -NH-). Anal.calc. for  $C_{16}H_{12}Cl_3N_5O_3S_2$  (492.787): C, 39.00; H, 2.45; N, 14.21; S, 13.01 %. Found C, 39.27; H, 2.40; N, 14.32; S, 13.02 %.

***N*-(5-Methyl-1,3,4-thiadiazol-2-yl)-4-([4-(trifluoromethyl)phenyl]carbamoyl)amino}benzenesulfonamide (8)**

Starting from sulfamethizole (0.280 g), 4-(trifluoromethyl)phenylisocyanate (0.130 ml) in dry acetone for 6 h, the title compound **8** was obtained. Yield: 50%; M.p. 201-203 °C; IR ( $cm^{-1}$ ): 3310, 3283, 3125, 3109, 3084, 3013, 2982, 1690, 1591, 1533, 1516, 1400, 1314, 1140.  $^1H$ -NMR (300 MHz), (DMSO- $d_6$ /TMS) ppm: 2.49 (s, 3H, -CH<sub>3</sub>), 7.39-7.73 (m, 8H, Ar-H), 9.20-9.22 (s, 2H, -NH-), 13.90 (s, 1H, -SO<sub>2</sub>NH-). Anal.calc. for  $C_{17}H_{14}F_3N_5O_3S_2$ : C, 44.63; H, 3.08; N, 15.31; S, 14.02%. Found C, 44.60; H, 3.06; N, 15.34; S, 14.12 %. MS (APCI Neg Ion) m/z : 456.18.

***N*-(5-Methyl-1,3,4-thiadiazol-2-yl)-4-[(2-phenylethyl)carbamoyl]amino}benzenesulfonamide (9)**

Starting from sulfamethizole (0.280 g), 2-phenylethylisocyanate (0.130 ml) in dry acetone for 6 h, the title compound **9** was obtained. Yield: 65%; M.p. 208-209 °C; IR ( $cm^{-1}$ ): 3362, 3096, 3011, 2888, 1670, 1589, 1526, 1441, 1402, 1319, 1134.  $^1H$ -NMR (300 MHz), (DMSO- $d_6$ /TMS) ppm: 2.56 (s, 3H, -CH<sub>3</sub>), 2.85-2.89 (t, 3H, -CH<sub>2</sub>-), 3.46 (t, 3H, -CH<sub>2</sub>NH-) 7.31-7.77 (m, 9H, Ar-H), 9.06 (s, 2H, -NH-), 13.95 (s, 1H, -SO<sub>2</sub>NH-). Anal.calc. for  $C_{18}H_{19}N_5O_3S_2$  (417.505): C, 51.78; H, 4.59; N, 16.77; S, 15.36%. Found C, 51.64; H, 4.48; N, 17.23; S, 15.16 %.

**General procedure for the preparation of thiourea derivatives (10-14)**

Sulfamethizole (0.00105 mol, 0.280 g) was solved in acetone, at 80 °C. Then, a solution of the corresponding isothiocyanate (0.00105 mol) in acetone was added as two parts, per 30 minutes. After 6 hours the reaction was finalized by TLC control and left overnight. The precipitate was filtered off, dried and purified with ethanol.

**4-[[4-(4-Methylphenyl)carbamothioyl]amino]-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (10)**

Starting from sulfamethizole (0.280 g), 4-methylphenylisothiocyanate (0.157 g) in dry acetone for 6 h, the title compound **10** was obtained. Yield: 34%; M.p. 174-177 °C; IR (cm<sup>-1</sup>): 3325, 3275, 3123, 3011, 2861, 1586, 1512, 1346, 1317, 1146, 1132, 1290. <sup>1</sup>H-NMR (300 MHz), (DMSO-d<sub>6</sub>/TMS) ppm: 2.25 (s, 3H, CH<sub>3</sub>), 2.53 (s, 3H, -CH<sub>3</sub>), 7.35-7.86 (m, 8H, Ar-H), 9.17 (s, 2H, -NH-), 13.90 (s, 1H, -SO<sub>2</sub>NH-). Anal.calc. for C<sub>17</sub>H<sub>17</sub>N<sub>5</sub>O<sub>2</sub>S<sub>3</sub>: C, 48.67; H, 4.08; N, 16.69; S, 22.93%. Found C, 48.36; H, 4.16; N, 16.69; S, 22.13 %. MS (APCI Neg Ion) m/z : 418.06.

**N-(5-Methyl-1,3,4-thiadiazol-2-yl)-4-[[4-(4-nitrophenyl)carbamothioyl]amino]benzenesulfonamide (11)**

Starting from sulfamethizole (0.280 g), 4-nitrophenylisothiocyanate (0.077 g) in dry acetone for 6 h, the title compound **11** was obtained. Yield: 65%; M.p. 181-183 °C; IR (cm<sup>-1</sup>): 3318, 3291, 3048, 2916, 2822, 1595, 1537, 1510, 1499, 1427, 1414, 1327, 1134, 1279. <sup>1</sup>H-NMR (300 MHz), (DMSO-d<sub>6</sub>/TMS) ppm: 2.53 (s, 3H, -CH<sub>3</sub>), 7.71-8.31 (m, 9H, Ar-H), 10.59-10.63 (s, 2H, -NH-), 13.90 (s, 1H, -SO<sub>2</sub>NH-). Anal.calc. for C<sub>16</sub>H<sub>14</sub>N<sub>6</sub>O<sub>4</sub>S<sub>3</sub>: C, 42.66; H, 3.13; N, 18.65; S, 21.35%. Found C, 42.56; H, 3.11; N, 18.62; S, 21.22 %.

**4-[[4-(4-Bromophenyl)carbamothioyl]amino]-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (12)**

Starting from sulfamethizole (0.280 g), 4-bromophenylisothiocyanate (0.077 g) in dry acetone for 6 h, the title compound **12** was obtained. Yield: 60%; M.p. 170-172 °C; IR (cm<sup>-1</sup>): 3320, 3264, 3165, 3119, 3102, 3013, 2976, 2922, 2872, 2857, 2814, 1587, 1530, 1487, 1433, 1316, 1146, 1292. <sup>1</sup>H-NMR (300 MHz), (DMSO-d<sub>6</sub>/TMS) ppm: 2.49 (s, 3H, -CH<sub>3</sub>), 7.38-7.75 (m, 8H, Ar-H), 9.93 10.32 (s, 2H, -NH-), 13.90 (s, 1H, -SO<sub>2</sub>NH-). Anal.calc. for C<sub>16</sub>H<sub>14</sub>BrN<sub>5</sub>O<sub>2</sub>S<sub>3</sub>: C, 39.67; H, 2.91; N, 14.46; S, 19.86%. Found C, 40.21; H, 3.02; N, 14.25; S, 19.56 %. MS (APCI Neg Ion) m/z : 482.02.

**4-[[2,6-Difluorophenyl)carbamothioyl]amino]-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (13)**

Starting from sulfamethizole (0.280 g), 2,6-difluorophenylisothiocyanate (0.077 g) in dry acetone for 6 h, the title compound **13** was obtained. Yield: 60%; M.p. 216-218 °C; IR (cm<sup>-1</sup>): 3275, 3092, 2988, 2870 2857, 2822, 1586, 1518, 1506, 1464, 1435, 1350,1339, 1148, 1296. <sup>1</sup>H-NMR (300 MHz), (DMSO-d<sub>6</sub>/TMS) ppm: 2.49 (s, 3H,

-CH<sub>3</sub>), 7.13-7.76 (m, 7H, Ar-H), 9.45-10.41(s, 2H, -NH-), 13.90 (s, 1H, -SO<sub>2</sub>NH-). Anal.calc. for C<sub>16</sub>H<sub>13</sub>F<sub>2</sub>N<sub>5</sub>O<sub>2</sub>S<sub>3</sub>: C, 43.88; H, 2.47; N, 15.21; S, 21.32 %. Found C, 43.53; H, 2.97; N, 15.86; S, 21.79 %. MS (APCI Neg Ion) m/z : 440.12.

**4-[[2,6-Dichlorophenyl)carbamothioyl]amino]-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (14)**

Starting from sulfamethizole (0.280 g), 2,6-chlorophenylisothiocyanate (0.077 g) in dry acetone for 6 h, the title compound **14** was obtained. Yield: 55%; M.p. 221-222 °C; IR (cm<sup>-1</sup>): 3335, 3237, 3032, 2893, 1516, 1499, 1433, 1350, 1150, 1292. <sup>1</sup>H-NMR (300 MHz), (DMSO-d<sub>6</sub>/TMS) ppm: 2.49 (s, 3H, -CH<sub>3</sub>), 7.33-7.77 (m, 7H, Ar-H), 9.69-10.33 (s, 2H, -NH-), 13.90 (s, 1H, -SO<sub>2</sub>NH-). Anal.calc. for C<sub>16</sub>H<sub>13</sub>Cl<sub>2</sub>N<sub>5</sub>O<sub>2</sub>S<sub>3</sub>: C, 40.51; H, 2.76; N, 14.76; S, 20.28%. Found C, 40.86; H, 2.82; N, 15.17; S, 20.26. MS (APCI Neg Ion) m/z : 472.05.

**Biological activity****Purification of carbonic anhydrase from human erythrocytes**

Erythrocytes were purified from human blood obtained from Blood Center of Research Hospital at Atatürk University. Carbonic anhydrases isoenzymes (hCA-I and hCA-II) from human erythrocytes were purified by means of affinity column having a structure of Sepharose 4B-L-tyrosine-sulfonyamide (18) and the study was carried out with these enzymes. The eluates were plotted by doing protein determination at 280 nm and CO<sub>2</sub>-hydratase activity (19).

**Determination of carbonic anhydrase activity and effect of compounds 1-14 on isoenzymes**

Carbonic anhydrase activity and effect of the synthesized compounds were assayed by hydration of CO<sub>2</sub>. Carbonic anhydrase activity was determined using the Wilbur-Anderson Method which was modified by Rickli and Sly (19, 20). CO<sub>2</sub>-Hydratase activity as enzyme unit (EU) was calculated by the equation (t<sub>0</sub>-t<sub>c</sub>/t<sub>c</sub>) where t<sub>0</sub> and t<sub>c</sub> are the times for pH change of the non-enzymatic (buffer) and the enzymatic reaction, respectively.

**Determination of IC<sub>50</sub> values**

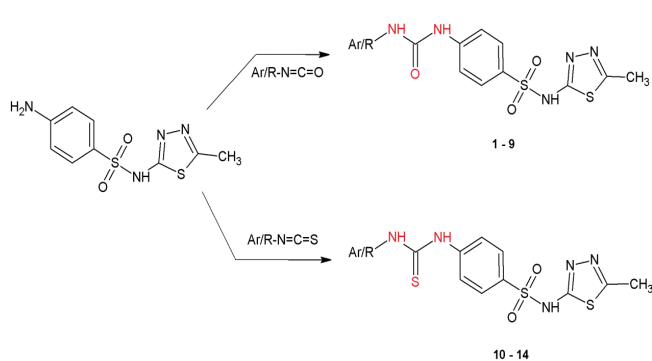
It was studied with compounds **1-14** to calculate values of IC<sub>50</sub> of hCA-I and hCA-II enzymes on the hydratase activity at different concentrations while maintaining constant

the substrate concentration. Activities of enzymes in the medium without inhibitors were used as 100% activity. The activity % values of enzymes were calculated by measuring hydratase activity in the presence of different concentrations of inhibitors. The  $IC_{50}$  value was calculated by utilizing graphs of % activity-[I] for each inhibitor (21, 22).

## RESULTS AND DISCUSSION

The synthetic route to the target compounds is outlined in **Scheme 1**. The urea and thiourea derivatives were prepared by reacting equimolar isocyanates or thiocyanates and sulfamethiazole. Physicochemical and spectroscopic characterization of the urea and thiourea derivatives have been previously described. The structures of the compounds (**1-14**) were confirmed by IR,  $^1H$ -NMR, MS and elemental analysis. IR spectra of the compounds (**1-14**) afforded urea/thiourea and sulfonamide N-H stretching 3374-3102 $cm^{-1}$  and C=O stretching 1692-1651 $cm^{-1}$  bands and aromatic rings' C-H stretching 3096-3011 $cm^{-1}$  bands. The NH protons of urea groups resonated as a singlet or two different singlet

peak because of E/Z isomer at 6.09-10.63 ppm. NH protons of sulfonamide groups appeared as a singlet at 11.10-13.95 ppm. The protons belonging to the aromatic ring and the other aliphatic groups were observed with the expected chemical shift and integral values. Mass spectra (APCI) of compounds showed a (M-1) peak, in line with their molecular formula. Also, the elemental analysis of compounds were in agreement with the proposed structures of the compounds.



**Scheme 1.** The synthesis route of the compounds (**1-14**)

**Table 1.** Results obtained from regression analysis graphs for hCA-I and hCA-II presence of compounds.

Compound	R/Ar	hCA-I Inhibition* ( $IC_{50}$ )	hCA-II Inhibition* ( $IC_{50}$ )	hCA-I / hCA-II
1	3-Cl-4-CH <sub>3</sub> -C <sub>6</sub> H <sub>3</sub> -	2.19	-	-
2	(CH <sub>3</sub> ) <sub>3</sub> -C-	10.25	1.65	6.21
3	CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>3</sub> -	6.08	1.696	3.58
4	4-NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub> -	2.37	6.71	0.35
5	2,4-diCl-C <sub>6</sub> H <sub>3</sub> -	3.48	11.17	0.31
6	2,6-diCl-C <sub>6</sub> H <sub>3</sub> -	4.99	9.34	0.53
7	2,4,6-triCl-C <sub>6</sub> H <sub>2</sub> -	15.65	13.36	1.17
8	4-CF <sub>3</sub> -C <sub>6</sub> H <sub>4</sub> -	6.44	17.95	0.36
9	C <sub>6</sub> H <sub>5</sub> -(CH <sub>2</sub> ) <sub>2</sub> -	ND	ND	ND
10	4-CH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub> -	0.144	0.109	1.32
11	4-NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub> -	3.14	5.60	0.56
12	4-Br-C <sub>6</sub> H <sub>4</sub> -	2.42	1.52	1.59
13	2,6-diF-C <sub>6</sub> H <sub>3</sub> -	8.78	2.33	3.77
14	2,6-diCl-C <sub>6</sub> H <sub>3</sub> -	6.68	8.37	0.80
Sulfamethizole	-	0.304	0.194	1.57
Acetazolamide (AAZ)	-	6.2**	7.4**	0.84**

\*They were determined as nM, \*\* They were determined as  $\mu$ M, ND: Not determined.

All of the compounds had inhibitory activity on hCA-I and except **1** on hCA-II against the reference compound acetazolamide (AAZ) (Table 1). The ureas which have alkyl substitutions (**2**, **3**) and halogen substituted **7**; thioureas which have alkyl substitution (**10**) and halogen substitutions on their aromatic ring (**12**, **13**) were more significant on hCA-II than hCA-I, whereas the inhibitory effects of six derivatives (**4**, **5**, **6**, **8**, **11**, **14**) were more selective on hCA-I than hCA-II.

The inhibitory effects of the compounds **3**, **8**, **14** and **1**, **4**, **12** on hCA-I were fairly close with the IC<sub>50</sub> values between 6.08-6.68 and 2.19-2.42 nM, respectively. Although **4**, **5**, **6**, **8**, **11**, **14** had lower hCA-I/hCA-II ratio than the hCA-I/hCA-II ratio of AAZ and the ratio of **14** was quite close to the hCA-I/hCA-II ratio of AAZ.

## CONCLUSIONS

Here, we synthesized urea and thiourea derivatives and evaluated their ability to inhibit carbonic anhydrase isozymes (hCA-I and hCA-II) against the reference compound AAZ. The compounds **1-3** and **11-14** had remarkable inhibitory activity on hCA-I and **2**, **3**, **10** and all **11-14** series had remarkable inhibitory activity on hCA-II. The compound **2** was the most powerful compound on hCA-II, with the hCA-I/hCA-II ratio value 6.21. On the other hand **5** and **8** showed the most inhibitory activity on hCA-I with the range of 0.31 and 0.36 hCA-I/hCA-II ratio, respectively. These synthesized two compounds (**5** and **8**) may be served as model compounds to design new hCA-I and hCA-II inhibitory agents for further studies.

**Karbonik anhidraz I ve II inhibitörü olan bazı N-(5-metil-1,3,4-tiyadiazol-2-il)-4-[(3-sübstitüe)üreido/tiyöüreido]benzensülfonamitler**

### ÖZ

Bu çalışmada N-(5-metil-1,3,4-tiyadiazol-2-il)-4-[(3-sübstitüe)üreido]benzensülfonamit ve N-(5-metil-1,3,4-tiyadiazol-2-il)-4-[(3-sübstitüe)tiyöüreido]benzensülfonamit türevleri 4-amino-N-(5-metil-1,3,4-tiyadiazol-2-il)benzensülfonamitten (sülfametizol) hareketle sentez edildi. Bütün yeni bileşiklerin

yapıları elemental analiz ve çeşitli spektroskopik yöntemler (FTIR, <sup>1</sup>H-NMR ve MS) yardımıyla aydınlatıldı. Bu yeni sülfonamit türevlerinin, özellikle insan karbonik anhidraz I ve II olmak üzere, karbonik anhidraz inhibitör etkileri incelendi. Bileşiklerin klinikte kullanılan asetazolamide kıyasla insan sitozolik CA I (IC<sub>50</sub> değerleri 0.144-15.65 nM) ve CA II (IC<sub>50</sub> değerleri 0.109-17.95 nM) inhibisyon değerleri ile daha yüksek aktivite gösterdikleri tespit edildi.

**Anahtar kelimeler:** Karbonik anhidraz inhibitörleri, sülfonamit, sülfametizol, üre ve tiyöüre.

## REFERENCES

- Leppialmi M, Koistinen P, Savolainen ER, Hannuksela J, Parkkila AK, Niemelä O, Pastoreková S, Pastorek J, Waheed A, Sly WS, Parkkila S, Rajaniemi H. The expression of carbonic anhydrase II in hematological malignancies. Clin Cancer Res 2002; 8: 2240-5.
- Demir Y, Demir N, Yıldırım S, Nadaroğlu H, Karaosmanoğlu M, Bakan E. The activities of carbonic anhydrase and alkaline phosphatase in ancient human bones. Purification and characterization of outer peripheral, cytosolic, inner peripheral, and integral CA. Prep Biochem Biotech 2001; 31: 291-304.
- Demir N, Demir Y, Nadaroğlu H. Carbonic anhydrase from bovine bone. Prep Biochem Biotech 2001; 31: 33-48.
- Shah GN, Emmett DH, Grubb JH, Migas MC, Fleming RE, Waheed A, Sly WS. Mitochondrial carbonic anhydrase CA VB: Differences in tissue distribution and pattern of evolution from those of CA VA suggest distinct physiological roles. Proc Natl Acad Sci USA 2000; 97: 1677-82.
- Demir Y, Nadaroğlu H, Demir N. Purification and characterization of carbonic anhydrase from bovine stomach and inhibitory effects of some chemical substances on enzyme activities. J Enzym Inhib Med Chem 2005; 20: 75-80.
- Supuran CT. Structure-based drug discovery of carbonic anhydrase inhibitors. J Enzyme Inhib Med Chem 2012; 27: 759-72.
- Supuran CT. Carbonic anhydrases - an overview. Curr Pharm Des 2008; 14: 603-14.
- Supuran CT. Carbonic anhydrase inhibitors. Bioorg Med Chem Lett 2010; 20: 3467-74.
- Supuran CT, Scozzafava A. Carbonic anhydrases as targets for medicinal chemistry. Bioorg Med Chem 2007; 15: 4336-50.
- Hilvo M, Tolvanen M, Clark A, Shen BR, Shah GN, Waheed A, Halmi P, Hanninen M, Hamalainen JM, Vihinen M, Sly WS, Parkkila S. Characterization of CA XV, a new GPI-anchored form of carbonic anhydrase. Biochem J 2005; 392: 83-92.
- Supuran CT. Carbonic anhydrases: Novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov 2008; 7: 168-81.
- Supuran CT. Diuretics: From classical carbonic anhydrase inhibitors to novel applications of the sulfonamides. Curr Pharm Design 2008; 14: 641-48.
- Zolnowska B, Slawinski J, Pogorzelska A, Chojnacki J, Vullo D, Supuran CT. Carbonic anhydrase inhibitors: Synthesis,

- and molecular structure of novel series N-substituted-N'-(2-arylmethylthio-4-chloro-5-methylbenzenesulfonyl) guanidines and their inhibition of human cytosolic isozymes I and II and the transmembrane tumor-associated isozymes IX and XII. *Eur J Med Chem* 2014; 71: 135-47.
14. Arslan A, Demir H, Arslan H. Investigating catalase and carbonic anhydrase enzyme activities and levels of certain trace elements and heavy metals in patients with primary and metastatic hepatic carcinoma. *J Cancer Ther* 2013; 4: 1373-81.
  15. Haapasalo J, Nordfords K, Järvelä S, Bragge H, Rantala I, Parkkila AK, Haapasalo H, Parkkila S. Carbonic anhydrase II in the endothelium of glial tumors: A potential target for therapy. *Neuro Oncol* 2007; 9: 308-13.
  16. Ibrahim DA, Lasheen DS, Zaky MY, Ibrahim AW, Vullo D, Ceruso M, Supuran CT, Ella DA. Design and synthesis of benzothiazole-6-sulfonamides acting as highly potent inhibitors of carbonic anhydrase isoforms I, II, IX and XII. *Bioorg Med Chem* 2015; 23: 4989-99.
  17. Supuran CT, Scozzafava A. Carbonic anhydrase inhibitors: Aromatic sulfonamides and disulfonamides act as efficient tumor growth inhibitors. *Eur J Med Chem* 2000; 35: 867-74.
  18. Arslan O, Nalbantoglu B, Demir N, Ozdemir H, Kufrevioglu OI. A new method for the purification of carbonic anhydrase isoenzymes by affinity chromatography. *Turk J Med Sci* 1996; 26: 163-6.
  19. Rickli EE, Ghazantar SAS, Gibbons BH, Edsall JT. Carbonic anhydrase from human erythrocytes. *J Biol Chem* 1964; 239: 1065-78.
  20. Sly WS, Hu PY. Human carbonic anhydrases and carbonic anhydrase deficiencies. *Annu Rev Biochem* 1995; 64: 375-401.
  21. Hoechster RM, Kates M, Questel JH. *Metabolic inhibitors*. Academic press 1973; 3-4: 71-89 and 66-82.
  22. Christensen GM, Olson D, Riedel B. Chemical effects on the activity of eight enzymes: A review and a discussion relevant to environmental monitoring. *Environ Res* 1982; 29: 247-55.