Synthesis and bioactivities of 1-(4-hydroxyphenyl)-2-((heteroaryl)thio)ethanones as carbonic anhydrase I, II and acetylcholinesterase inhibitors

Cem YAMALI 1,∗, Halise İnci GÜL 1,∗, Yeliz DEMİR 2,∗, Cavit KAZAZ 3,∗, İthami GÜLÇİN 3,∗

1 Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Atatürk University, Erzurum, Turkey
2 Department of Pharmacy Services, Nihat Delibalta Göle Vocational High School, Ardahan University, Ardahan, Turkey
3 Department of Chemistry, Faculty of Science, Atatürk University, Erzurum, Turkey

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Abstract: The discovery of enzyme targeting inhibitors is a popular area of drug research. Biological activities of the compounds bearing phenol and heteroaryl groups make them popular groups in drug design targeting important enzymes such as acetylcholinesterase (AChE, E.C.3.1.1.7) and carbonic anhydrases (CAs, EC 4.2.1.1). 1-(4-hydroxyphenyl)-2-((aryl)thio)ethanones as possible AChE and CAs inhibitors were synthesized, and their chemical structures were confirmed by IR, 1H NMR, 13C NMR, and HRMS. The compounds 2 and 4 were found potent AChE inhibitors with the Ki values of 22.13 ±1.96 nM and 23.71 ±2.95 nM, respectively, while the compounds 2 (Ki = 8.61 ±0.90 nM, on hCA I) and 1 (Ki = 8.76 ±0.84 nM, on hCA II) had considerable CAs inhibitory potency. The lead compounds may help the scientists for the rational designing of an innovative class of drug candidates targeting enzyme-based diseases.

Key words: Carbonic anhydrases, acetylcholinesterase, heterocyclic, phenol

1. Introduction
Acetylcholinesterase (AChE, E.C.3.1.1.7) enzyme plays a vital role in the treatment of Alzheimer’s disease (AD). AChE is also one of the targets for several cholinergic toxicants, plant glycoalkaloids, and drug candidates [1–3]. Acetylcholine (ACH) is converted to choline and acetic acid molecules in synapses via the AChE enzyme. Modern AD therapy targets enhance cholinergic neurotransmission by way of AChE inhibitors (AChEIs). Widely used AChEIs are galantamine, donepezil, and rivastigmine [4,5]. However, these drugs are unfortunately less effective to block AD progression, and they also have side effects [6]. Also, the discovery of novel, effective, and therapeutic drug candidates targeting AChE is one of the widespread issues in medicinal chemistry.

Many heteroaromatic compounds such as benzoxazole [1], triazole [7], tetrazole [8], oxadiazole [6], and benzimidazol [9] which have the potency to make molecular interactions within the ChE enzyme were reported with their ChE inhibitory effects. In addition to these structures, the problematic process of AD directs scientists to search for new therapeutic multifunctional compounds [10,11]. Therefore, phenolic compounds might be a promising structure in the designing of novel AChEIs since the phenolic compounds exhibit cholinesterase inhibitory potency and antioxidant effects [12]. Recently, Sang et al. reported phenolic compounds as AChE inhibitors. In series, the compound, namely TM-3, showed a favorable AChE inhibitory effect with IC50 of 0.69 µM. Molecular docking studies also showed that a 2,4-dihydroxyacetophenone nucleus interacts with the enzyme via intermolecular hydrogen bonds [13].

*Correspondence: c.yamali@yahoo.com

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Carbonic anhydrases (CAs, EC 4.2.1.1) are zinc bearing metalloenzymes which contribute to the controlling of pH balance by the acceleration of the hydration reaction of carbon dioxide in the living cells [14–16]. Regulation of CAs activity by using inhibitors is used in the clinic as antiglaucoma drugs and diuretics [17,18]. Besides, CAIs might have potential as anticancer, antiobesity, and antiinfective agents [19, 20]. To date, 15 α - CA isoforms in mammals have been reported. Among them, the cytosolic CA I and CA II isoforms are well-established targets for several diseases. While CA I is responsible for cerebral edema, CA II inhibition has a role for glaucoma, edema, altitude sickness, and epilepsy [21–23]. The sulfonamides and their bioisosteres (sulfamates, sulfamides, etc.) are the main pharmacophore groups which interact with the active site of CA isoenzymes [24]. For instance, sulfonamide having acetazolamide, methazolamide, ethoxzolamide (Figure 1) are widely used systemic antiglaucoma drugs in the clinic and these drugs have heterocyclic structures in their chemical skeleton. After that, the investigation of CA inhibition capacity and the mechanism of action of the phenol (Figure 1) led to design novel phenolic compounds targeting CA isoenzymes [25–27]. Hence, the substitution of phenol pharmacophore can be considered in the design strategy to have potent compounds with enhanced CA activity.

![Figure 1](image_url). The design strategy of the target compounds.

Based on the reports, different heterocyclic rings were combined with the phenol functional group since the phenol is one of the popular CAIs group and promising potential pharmacophore for new AChEIs. This study aimed to report CAs and AChE enzyme inhibitory potencies of the 1-(4-hydroxyphenyl)-2-((aryl)thio)ethanones 1– 6 to find out CAs and AChE enzyme inhibitors for further studies.

**2. Materials and methods**

**2.1. Chemistry**

NMR spectra were recorded by Bruker AVANCE III 400 MHz (Bruker, Karlsruhe, Germany) and Varian Mercury Plus Spectrometer 400 MHz (Varian Inc., Palo Alto, California, U.S.) in DMSO - d6 (Merck KGaA, Darmstadt, Germany). LCMS - IT - TOF system (Shimadzu, Tokyo, Japan) was used for HRMS spectra. Electrothermal 9100 (IA9100, Bibby Scientific Limited, Staffordshire, UK) device was used to measure melting points (Mp). TLC - Silicagel HF254 (Merck Art 5715) plate was used to check the reaction process under UV lamb (Spectroline, ENF - 240C/ FE, New York, U.S.A). IR spectra of the compounds were recorded as
potassium bromide pellets on a Perkin Elmer 100 Fourier transform (FT)-IR spectrophotometer (PerkinElmer, Inc., Waltham MA, USA)

2.2. A general synthesis method of the compounds 1–6 [28] (Figure 2)
Mercapto-based compounds (9.3 mmol) [Benzo[d]thiazole-2-thiol for 1 (1.6 g), 1H-benzo[d]oxazole-2-thiol for 2 (1.43 g), 1-methyl-1H-imidazole-2-thiol for 3 (1.1 g), 5-methyl-1,3,4-thiadiazole-2-thiol for 4 (1.23 g), 4-methyl-1H-1,2,4-triazole-3-thiol for 5 (1.1 g), 1-methyl-1H-tetrazole-5-thiol for 6 (1.1 g)] was dissolved at room temperature in fresh methanolic NaOH solution (0.4 g, 25 mL methanol) and stirred for 10 min. Then, 2-bromo-4′-hydroxyacetophenone (9.3 mmol, 2 g) was put into the reaction flask. After the final mixture was stirred for several hours at room temperature, it was taken into the water (50 mL). The white solid obtained was filtered, washed with water three times, and then dried. The compounds were purified by crystallization using suitable solvents such as ethanol (1–4, 6) and ethanol: DMF (5).

![Figure 2. Synthesis of the compounds 1–6.](image)

2.2.1. 2-(Benzo[d]thiazol-2-ythio)-1-(4-hydroxyphenyl)ethanone, 1
Mp = 188–189 °C. Yield 72%. IR (KBr) cm⁻¹: 3209, 3061, 3019, 2959, 2914, 1660, 1575, 1414, 1202, 1169, 993, 829, 753, 725. ¹H NMR (400 MHz, DMSO - d₆, δ, ppm) 7.99 - 7.93 (m, 3H, ArH), 7.76 (d, J = 7.7 Hz, 1H, ArH), 7.44 - 7.39 (m, 1H, ArH), 7.35 - 7.31 (m, 1H, ArH), 6.86 (d, J = 8.2 Hz, 2H, ArH), 5.06 (s, 2H, -CH₂-). ¹³C NMR (100 MHz, DMSO - d₆, δ, ppm) 191.5 (C=O), 166.8, 163.4, 153.2, 135.4, 131.9, 127.5, 127.0, 125.1, 122.5, 121.7, 116.1, 41.4 (-CH₂-). Predicted [M + H]⁺ 302.0304; measured [M + H]⁺ 302.0311.

2.2.2. 2-(Benzo[d]oxazol-2-ylthio)-1-(4-hydroxyphenyl)ethanone, 2
Mp = 210–212 °C. Yield 78%. IR (KBr) cm⁻¹: 3051, 2982, 2937, 1668, 1579, 1474, 1451, 1201, 1172, 1146, 1000, 817, 750, 734. ¹H NMR (400 MHz, DMSO - d₆, δ, ppm) 10.5 (s, 1H, -OH), 7.94 (d, J = 8.6 Hz, 2H,
ArH), 7.62 - 7.56 (m, 2H, ArH), 7.30 - 7.27 (m, 2H, ArH), 6.88 (d, J = 8.6 Hz, 2H, ArH), 5.07 (s, 2H, -CH2-).

13C NMR (100 MHz, DMSO - d6, δ, ppm) 191.1 (C=O), 164.7, 163.4, 151.9, 141.9, 131.8, 127.3, 125.3, 124.9, 118.9, 116.1, 110.8, 40.9 (-CH2-). Predicted [M + H]+ 286.0532; measured [M + H]+ 286.0537.

2.2.3. 1-(4-Hydroxyphenyl)-2-((1-methyl-1H-imidazol-2-yl)thio)ethanone, 3

Mp = 214–215 °C. Yield 91%. IR (KBr) cm−1: 3125, 3101, 2947, 2991, 2806, 2680, 2610, 2499, 1655, 1585, 1460, 1277, 1263, 1253, 1130, 846, 772. 1H NMR (400 MHz, DMSO - d6, δ, ppm) 10.5 (s, 1H, -OH), 7.80 (d, J = 8.8 Hz, 2H, ArH), 7.18 (d, J = 1.1 Hz, 1H, ArH), 6.88 (d, J = 1.1 Hz, 2H, ArH), 4.49 (s, 2H, -CH2-), 3.53 (s, 3H, -CH3). 13C NMR (100 MHz, DMSO - d6, δ, ppm) 192.6 (C=O), 162.9, 139.8, 131.5, 128.9, 127.2, 123.9, 115.8, 49.1 (-CH2-), 33.4 (-CH3). Predicted [M + H]+ 249.0692; measured [M + H]+ 249.0682.

2.2.4. 1-(4-Hydroxyphenyl)-2-((5-methyl-1,3,4-thiadiazol-2-yl)thio)ethanone, 4

Mp = 200–202 °C. Yield 70%. IR (KBr) cm−1: 3036, 2948, 2912, 2804, 2688, 2608, 2500, 1655, 1573, 1499, 1389, 1296, 1198, 1173, 988, 829, 700. 1H NMR (400 MHz, DMSO - d6, δ, ppm) 10.5 (bs, 1H, -OH), 7.90 (d, J = 8.4 Hz, 2H, ArH), 6.86 (d, J = 8.4 Hz, 2H, ArH), 4.95 (s, 2H, -CH2-), 2.63 (s, 3H, -CH3). 13C NMR (100 MHz, DMSO - d6, δ, ppm) 191.1 (C=O), 165.9, 164.9, 163.2, 131.6, 127.2, 115.9, 41.8 (-CH2-), 15.6 (-CH3). Predicted [M + H]+ 267.0256; measured [M + H]+ 267.0247.

2.2.5. 1-(4-Hydroxyphenyl)-2-((4-methyl-4H-1,2,4-triazol-3-yl)thio)ethanone, 5

Mp = 270–271 °C. Yield 67%. IR (KBr) cm−1: 3125, 3001, 2960, 2925, 2811, 2694, 2612, 2524, 1670, 1600, 1523, 1460, 1289, 1204, 1174, 826, 695. 1H NMR (400 MHz, DMSO - d6, δ, ppm) 8.52 (s, 1H, ArH), 7.85 (d, J = 8.8 Hz, 2H, ArH), 6.84 (d, J = 8.8 Hz, 2H, ArH), 4.73 (s, 2H, -CH2-), 3.57 (s, 3H, -CH3). 13C NMR (100 MHz, DMSO - d6, δ, ppm) 191.9 (C=O), 163.1, 149.2, 146.6, 131.6, 127.2, 115.8, 41.1 (-CH2-), 31.25 (-CH3). Predicted [M + H]+ 250.0645; measured [M + H]+ 250.0634.

2.2.6. 1-(4-Hydroxyphenyl)-2-((1-methyl-1H-tetrazol-5-yl)thio)ethanone, 6

Mp = 199–201 °C. Yield 74%. IR (KBr) cm−1: 3108, 3031, 2981, 2934, 2818, 2695, 2614, 2542, 1672, 1586, 1383, 1293, 1209, 1185, 1014, 825, 700. 1H NMR (400 MHz, DMSO - d6, δ, ppm) 10.5 (bs, 1H, -OH), 7.89 (d, J = 8.8 Hz, 2H, ArH), 6.86 (d, J = 8.8 Hz, 2H, ArH), 4.98 (s, 2H, -CH2-), 3.97 (s, 3H, -CH3). 13C NMR (100 MHz, DMSO - d6, δ, ppm) 191.0 (C=O), 163.3, 153.9, 131.6, 126.9, 115.9, 41.7 (-CH2-), 34.1 (-CH3). Predicted [M + H]+ 251.0597; measured [M + H]+ 251.0588.

2.3. AChE inhibition assay

AChE inhibitory effects of the newly synthesized compounds were utilized by Ellman’s test [29] as previous studies with minor modifications [30–32]. Briefly, sample solution (750 µL) dissolved in deionized water with Tris/HCl buffer (100 µL, 1 M, pH = 8) at different concentrations, and then AChE solution (50 µL) was added. The final mix was incubated at 21 °C for 64 min. The reaction was initiated by acetylthiocholine iodide (50 µL). Then, 5,5′-dithio-bis(2-nitro-benzoic)acid (50 µL, 0.5 mM) was added. The hydrolysis process
was determined at 412 nm. As a control drug, Tacrine (TAC) was used. The inhibition constants (Ki) were calculated by the Lineweaver–Burk plot [33]. All chemicals were purchased from Sigma-Aldrich Chemie GmbH (Hamburg, Germany).

2.4. CAs inhibition assay
Human CA isoforms (hCAI and hCAII) were purified by the Sepharose - 4B - L - tyrosine - sulfanilamide affinity segregation method as reported [34, 35]. Bradford technique was used to measure protein concentrations at 595 nm [36]. Inhibitory effects of the compounds were investigated by measuring the esterase activity according to Verpoorte et al. [37] as described in previous [38–40]. The hCA activity was determined by measuring the conversion of the p-nitrophenyl acetate substrate to p-nitro phenolate at 348 nm by the spectrophotometer (UV - VIS Spectrophotometer, UVmini-1240, Shimadzu Corporation, Kyoto, Japan) [41]. Acetazolamide (AZA) was used as a control drug. Lineweaver–Burk plot was used to calculate inhibition constants (Ki) of the compounds [33]. All chemicals were purchased from Sigma-Aldrich Chemie GmbH.

3. Results and discussion
3.1. Chemistry
This study reported the synthesis and bioactivities of the compounds having 1-(4-hydroxyphenyl)-2-((aryl)thio) ethanones chemical formula. 2-Bromo-4'-hydroxyacetophenone was attached to the heterocyclic rings via thioether functional group by conducting a single step reaction. The compounds 1, 2, 3, 5, and 6 were found as registered compounds at the Sci Finder database without any article and experimental data, while compound 4 [42] was reported as an intermediate. Therefore, the current study is the first study for the synthesis and bioactivities of 1, 2, 3, 5, and 6.

As a spectral evaluation, in 1H NMR, methylene protons were seen in the range of 5.07–4.49 ppm as expected. In some cases, the phenolic proton was not seen since it is an exchangeable proton. Signal of methyl substituent for the methyl-substituted compounds was seen in the range of 3.97–2.63 ppm. The carbonyl peak of the compounds in 13C NMR was seen in the range of 191.0–192.6 ppm. The carbon peak of the methylene bridge was seen at 49.1–41.1 ppm, while the carbon signal of methyl was in the range of 34.1–15.6 ppm. Further, calculated and measured m/z values of the compounds were also found compatible in HRMS analysis. In the IR spectra, C=O stretching band was recorded in the range of 1655–1672 cm⁻¹.

3.2. Acetylcholinesterase inhibitory effects
In this study, the compounds 1–6 were screened against the AChE enzyme due to significant reports on AD of phenolic natural or synthetic compounds. IC₅₀ and Ki values of the reference drug TAC were 40.76 nM (IC₅₀) and 37.45 ±3.12 nM (Ki) towards AChE, as shown in Table. The compounds inhibited the AChE enzyme in nanomolar concentration in the range of Ki values of 22.13 ±1.96 - 62.11 ±6.00 nM and with IC₅₀ values of 28.76–57.27 nM. The compounds 2 and 4 were found potent AChE inhibitors with the Ki values of 22.13 ±1.96 nM and 23.71 ±2.95 nM, respectively, while the compound 5 was the least inhibiting compound with the highest Ki value of 62.11 ±6.00 nM. On the other hand, the compound 1 was also considered as one of the potent inhibitors with the lowest IC₅₀ value of 28.76 nM against AChE.

The compounds 1 and 2 having a bicyclic ring showed considerable AChE inhibitory effects with low Ki values. When these two compounds compared with each other, it shows that oxygen atom was more favorable
than the sulfur atom. Diazole (3) and tetrazole (6) derivatives were found more effective than triazole (5) derivative against the AChE enzyme. The compound 4 having 5-methyl-1,3,4-thiadiazol ring showed favorable enzyme inhibitory potency with Ki value of 23.71 ±2.95 nM in contrast to other five-membered compounds 3–6. The compounds reported might be potential candidates for designing novel and more powerful AChE inhibitors for future studies. AD drugs such as donepezil, rivastigmine, galantamine have amine moiety in their chemical structure. So, the compounds having nitrogen atom/s may show favorable enzyme-ligand interactions [43]. Also, for the future concept, the most potent amine bearing phenolic compounds can be synthesized with Mannich reaction by the reaction of amine, formaldehyde, and phenolic compound under suitable reaction conditions as mono or bis Mannich bases against AChE based on our previous work regarding Mannich bases as promising AChE inhibitors [30].

3.3. Carbonic anhydrase inhibitory effects
Since the phenol group is an important zinc-binding group, the compounds 1–6 were evaluated towards CAs isoenzymes to show their CA inhibitory potency. The Table showed that the Ki values of reference drug AZA were 21.74 ±5.48 nM and 18.27 ±3.56 nM, whereas the IC_{50} values of AZA were 23.90 nM and 18.73 nM towards hCAI and II, respectively. Ki values of 1–6 were calculated as 8.61 ±0.90 – 42.59 ±7.59 nM (hCAI) and 8.76 ±0.84 – 31.64 ±3.29 nM (hCAII).

Among the compounds having the most common bicyclic rings, the compound 2 (Ki = 8.61 ±0.90 nM) was 2.5 fold more potent inhibitor against hCAI while the compound 1 (Ki = 8.76 ±0.84 nM) was 2.0 fold more potent against hCAII isoenzyme than reference AZA. On the other hand, among the compounds carrying five-membered rings 3–6, the compound 4 (Ki = 13.81 ±2.47 nM) for hCAI, and the compound 5 (Ki = 14.32 ±5.10 nM) for hCAII were potent CA inhibitors. When diazole (3), triazole (5) and tetrazole (6) derivatives were compared, the following results can be made. The tetrazole derivative 6 had good inhibitory potency against hCAI while triazole derivative 5 was effective inhibitor against hCAII isoenzyme. In series, benzothiazole and benzoxazole bearing compounds 1 and 2 were found the most potent CA inhibitor against the hCAs with the lowest Ki values. Also, it can be stated here that linking bicyclic ring with phenol function was found more

| Code | IC_{50} (nM) | hCA I r^2 | hCA II r^2 | AChE r^2 | Ki (nM) | hCA I | hCA II | AChE |
|------|-------------|-----------|------------|----------|--------|-------|-------|------|
| 1    | 35.36       | 0.9743    | 29.36      | 0.9985   | 28.76  | 0.9781| 14.60 ±2.06| 8.76 ±0.84| 26.53 ±5.43 |
| 2    | 32.84       | 0.9678    | 43.58      | 0.9771   | 32.23  | 0.9881| 8.61 ±0.90  | 23.41 ±7.13| 22.13 ±1.96  |
| 3    | 35.18       | 0.9975    | 39.83      | 0.9894   | 47.14  | 0.9900| 17.76 ±2.08| 31.64 ±3.29| 37.79 ±11.41|
| 4    | 48.13       | 0.9868    | 41.75      | 0.9955   | 46.20  | 0.9627| 13.81 ±2.47| 16.95 ±3.99| 23.71 ±2.95  |
| 5    | 65.58       | 0.9928    | 33.97      | 0.9749   | 57.27  | 0.9863| 42.59 ±7.59| 14.32 ±5.10| 62.11 ±6.00  |
| 6    | 62.54       | 0.9777    | 57.27      | 0.9775   | 47.47  | 0.9663| 14.36 ±3.71| 27.38 ±11.79| 35.70 ±2.63  |
| AZA* | 23.90       | 0.9748    | 18.73      | 0.9890   | -      | -     | 21.74 ±5.48| 18.27 ±3.56| -               |
| TAC* | -           | -         | -          | -        | 40.76  | 0.9877| -      | -     | 37.45 ±3.12  |

*AZA: Acetazolamide; TAC: Tacrine; IC_{50}: The half-maximal inhibitory concentration; Ki: Inhibition constant; hCA I, II: Human carbonic anhydrase I, II; AChE: Acetylcholinesterase; r^2: The coefficient of determination; ±: Standard deviation; nM: Nanomolar.
rewarding modification than five-membered rings. Additional aromatic hydrophobic interactions with the active site residues of the enzyme may result in increasing enzyme inhibitory potency of these compounds. For future studies, these phenolic compounds can be used to synthesize novel CAIs by the reaction of phenol group with suitable reagents to obtain new sulfamate-based CAIs to see how this modification affects the CAs inhibition activity.

4. Conclusion
In this study, different heteroaryl mercapto compounds were combined with the phenolic group. The compounds 1-(4-hydroxyphenyl)-2-[(heteroaryl)thio]ethan-1-one 1–6 showed enzyme inhibitory potency at nanomolar concentrations. The compounds 2 and 4 were found potent AChE inhibitors with the Ki values of 22.13 ± 1.96 nM and 23.71 ± 2.95 nM, respectively, among others. On the other hand, based on the IC$_{50}$ values, the compound 1 made attraction with the lowest IC$_{50}$ value of 28.76 nM against AChE. The compounds 2 (Ki = 8.61 ± 0.90 nM, on hCA I) and 1 (Ki = 8.76 ± 0.84 nM, on hCA II) had also considerable CA inhibitory potency. The promising bioactivity results of these compounds can lead to the design of more potent enzyme inhibitors with additional molecular modifications for further studies.

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References
1. Stasiuk M, Bartosiewicz D, Kozubek A. Inhibitory effect of some natural and semisynthetic phenolic lipids upon acetylcholinesterase activity. Food Chemistry 2008; 108 (3): 996-1001. doi: 10.1016/j.foodchem.2007.12.011
2. Bytyqi-Damoni A, Kestane A, Taslimi P, Tuzun B, Zengin M et al. Novel carvacrol based new oxypropanolamine derivatives: design, synthesis, characterization, biological evaluation, and molecular docking studies. Journal of Molecular Structure 2020; 1202 (2020): 127297. doi: 10.1016/j.molstruc.2019.127297
3. Yamali C, Gul HI, Kazaz C, Levent S, Gulcin I. Synthesis, structure elucidation, and in vitro pharmacological evaluation of novel polyfluoro substituted pyrazoline type sulfonamides as multi-target agents for inhibition of acetylcholinesterase and carbonic anhydrase I and II enzymes. Bioorganic Chemistry 2020; 96 (2020): 103627. doi: 10.1016/j.bioorg.2020.103627
4. Li G, Hong G, Li X, Zhang Y, Xu Z et al. Synthesis and activity towards Alzheimer’s disease in vitro: tacrine, phenolic acid and ligustrazine hybrids. European Journal of Medicinal Chemistry 2018; 148: 238-254. doi: 10.1016/j.ejmech.2018.01.028
5. El-Sayed NA, Farag AE, Ezzat MAF, Akincioglu H, Gülçin İ et al. Design, synthesis, in vitro and in vivo evaluation of novel pyrrolizine-basedcompounds with potential activity as cholinesterase inhibitors and anti-Alzheimer’s agents. Bioorganic Chemistry 2019; 93: 103312. doi: 10.1016/j.bioorg.2019.103312
6. Tripathi PN, Srivastava P, Sharma P, Seth A, Shrivastava SK. Design and development of novel N-(pyrimidin-2-yl)-1,3,4-oxadiazole hybrids to treat cognitive dysfunctions. Bioorganic and Medicinal Chemistry 2019; 27 (7): 1327-1340. doi: 10.1016/j.bmc.2019.02.031
7. Yin L, Wang L, Liu XJ, Cheng FC, Shi DH et al. Synthesis and bioactivity of novel C2-glycosyl triazole derivatives as acetylcholinesterase inhibitors. Heterocyclic Communications 2017; 23 (3): 231-236. doi: 10.1515/hc-2016-0163
8. Mehrun N, Munawar MA, Chattha FA, Kousar S, Munir J et al. Synthesis of novel triazoles and a tetrazole of escitalopram as cholinesterase inhibitors. Bioorganic and Medicinal Chemistry 2015; 23 (17): 6014-6024. doi: 10.1016/j.bmc.2015.06.051

9. Acar Cevik U, Saglik BN, Levent S, Osmaniye D, Kaya Cavuşoğlu B et al. Synthesis and AChE-inhibitory activity of new benzimidazole derivatives. Molecules 2019; 24 (5): 861-878. doi: 10.3390/molecules24050861

10. Ivanova L, Karelson M, Dobechev DA. Multitarget approach to drug candidates against Alzheimer’s disease related to AChE, SERT, BACE1 and GSK3β protein targets. Molecules 2020; 25: 1846. doi: 10.3390/molecules25081846

11. Chaves S, Resta S, Rinaldo F, Costa M, Josselin R, Gwizdala K, Piemontese L, Capriati V, Pereira-Santos AR, Cardoso SM, Santos MA. Design, synthesis, and in vitro evaluation of hydroxybenzimidazole-donepezil analogues as multitarget-directed ligands for the treatment of Alzheimer’s disease. Molecules 2020; 25: 985. doi: 10.3390/molecules25040985

12. Cetin Cakmak KC, Gülçin İ. Anticholinergic and antioxidant activities of usnic acid-An activity-structure insight. Toxicology Reports 2019; 6: 1273-1280. doi: 10.1016/j.toxrep.2019.11.003

13. Sang Z, Wang K, Wang H, Wang H, Ma Q et al. Design, synthesis and biological evaluation of 2-acetyl-5-O-(amino-alkyl)phenol derivatives as multifunctional agents for the treatment of Alzheimer’s disease. Bioorganic and Medicinal Chemistry Letters 2017; 27 (22): 5046-5052. doi: 10.1016/j.bmcl.2017.09.057

14. Boztas M, Çetinkaya Y, Topal M, Gülçin İ, Menzek A et al. Synthesis and carbonic anhydrase isoenzymes I, II, IX, and XII inhibitory effects of dimethoxy-bromophenol derivatives incorporating cyclopropane moieties. Journal of Medicinal Chemistry 2015; 58 (2): 640-650. doi: 10.1021/jm501573b

15. Topal M, Gülçin İ. Rosmarinic acid: a potent carbonic anhydrase isoenzymes inhibitor. Turkish Journal of Chemistry 2014; 38 (5): 894-902. doi: 10.3906/kim-1403-5

16. Arabaci B, Gülçin İ, Alwasel S. Capsaicin: a potent inhibitor of carbonic anhydrase isoenzymes. Molecules 2014; 19 (7): 10103-10114. doi: 10.3390/molecules190710103

17. Yıldırım A, Atmaca U, Keskin A, Topal M, Çelik M et al. N-Acylsulfonamides strongly inhibit human carbonic anhydrase isoenzymes I and II. Bioorganic and Medicinal Chemistry 2015; 23 (10): 2598-2605. doi: 10.1016/j.bmc.2014.12.054

18. Scozzafava A, Passaponti M, Supuran CT, Gülçin İ. Carbonic anhydrase inhibitors: guaiacol and catechol derivatives effectively inhibit certain human carbonic anhydrase isoenzymes (hCA I, II, IX, and XII). Journal of Enzyme Inhibition and Medicinal Chemistry 2015; 30 (4): 586-591. doi: 10.3109/14756366.2014.956310

19. Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nature Reviews Drug Discovery 2008; 7 (2): 168-181. doi: 10.1038/nrd2467

20. Aküncoğlu A, Aküncoğlu H, Gülçin İ, Durdağlı S, Supuran CT et al. Discovery of potent carbonic anhydrase and acetylcholine esterase inhibitors: novel sulfamoylcarbamates and sulfamides derived from acetophenones. Bioorganic and Medicinal Chemistry 2015; 23 (13): 3592-3602. doi: 10.1016/j.bmc.2015.04.019

21. Alteriori V, Di Fiore A, D’Ambrosio K, Supuran CT, De Simone G. Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? Chemical Reviews 2012; 112 (8): 4421-4468. doi: 10.1021/cr200176r

22. Özgeriş B, Göksu S, Polat Köse L, Gülçin İ, Salmas RE et al. Acetylcholinesterase and carbonic anhydrase inhibitory properties of novel urea and sulfamide derivatives incorporating dopaminergic 2-aminotetralin scaffolds. Bioorganic and Medicinal Chemistry 2016; 24 (10): 2318-2329. doi: 10.1016/j.bmc.2016.04.002

23. Sujayev A, Talsimi P, Kaya R, Safarov B, Aliyeva L et al. Synthesis, characterization and biological evaluation of N-substituted triazinane-2-thiones and theoretical-experimental mechanism of condensation reaction. Applied Organometallic Chemistry 2020; 34 (2): e5329. doi: 10.1002/aoc.5329
24. Supuran CT. How many carbonic anhydrase inhibition mechanisms exist? Journal of Enzyme Inhibition and Medicinal Chemistry 2016; 31 (3): 345 doi: 10.3109/14756366.2015.1122001

25. Innocenti A, Gülçin İ, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors. Antioxidant polyphenol natural products effectively inhibit mammalian isoforms I–XV. Bioorganic and Medicinal Chemistry Letters 2010; 20 (18): 5050-5053. doi: 10.1016/j.bmcl.2010.07.038

26. Gülçin İ, Beydemir S. Phenolic compounds as antioxidants: carbonic anhydrase isoenzymes inhibitors. Mini-Reviews in Medicinal Chemistry 2013; 13 (3): 408-430. doi: 10.2174/138955751313030009

27. Öztürk Sarkaya SB, Topal F, Şentürk M, Gülçin İ, Supuran CT. In vitro inhibition of α-carbonic anhydrase isozymes by some phenolic compounds. Bioorganic and Medicinal Chemistry Letters 2011; 21 (14): 4259-4262. doi: 10.1016/j.bmcl.2011.05.071

28. Reddy MVR, Pallela VR, Cosenza SC, Malliredigari MR, Patti R et al. Design, synthesis and evaluation of (E)-alpha-benzylthio chalcones as novel inhibitors of BCR-ABL kinase. Bioorganic and Medicinal Chemistry 2010; 18 (6): 2317-2326. doi: 10.1016/j.bmc.2010.01.051

29. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology 1961; 7 (2): 88-95. doi: 10.1016/0006-2952(61)90145-9

30. Ozgun DO, Yamali C, Gül HI, Taslimi P, Gülcin I et al. Inhibitory effects of isatin Mannich bases on carbonic anhydrases, acetylcholinesterase, and butyrylcholinesterase. Journal of Enzyme Inhibition and Medicinal Chemistry 2016; 31 (6): 1498-1501. doi: 10.3109/14756366.2016.1149479

31. Ozgun DO, Gül HI, Yamali C, Sakagami H, Gülcin I et al. Synthesis and bioactivities of pyrazoline benzensulfonamides as carbonic anhydrase and acetylcholinesterase inhibitors with low cytotoxicity. Bioorganic Chemistry 2019; 84: 511-517. doi: 10.1016/j.bioorg.2018.12.028

32. Yamali C, Gül HI, Ece A, Taslimi P, Gülcin I. Synthesis, molecular modeling, and biological evaluation of 4-[5-arylidene-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl] benzensulfonamides toward acetylcholinesterase, carbonic anhydrase I and II enzymes. Chemical Biology and Drug Design 2018; 91 (4): 854-866. doi: 10.1111/cbld.13149

33. Lineweaver H, Burk D. The Determination of enzyme dissociation constants. Journal of the American Chemical Society 1934; 56 (3): 658-666. doi: 10.1021/ja01318a036

34. Kocyigit UM, Budak Y, Gürdere MB, Tekin Ş, Köprüli TK et al. Synthesis, characterization, anticancer, antimicrobial and carbonic anhydrase inhibition profiles of novel (3aR,4S,7R,7aS)-2-(4-(E)-3-(3-aryl)acryloyl) phenyl)-3a,4,7,7a-tetraydro-1H-4,7-methanoisoindole-1,3(2H)-dione derivatives. Bioorganic Chemistry 2017; 70: 118-125. doi: 10.1016/j.bioorg.2016.12.001

35. Taslimi P, Sujayev A, Mamedova S, Kalin P, Gülçin İ et al. Synthesis and bioactivity of several new hetaryl sulfonamides. Journal of Enzyme Inhibition and Medicinal Chemistry 2017; 32 (1): 137-145. doi: 10.1080/14756366.2016.1238367

36. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 1976; 72: 248-254. doi: 10.1016/0003-2697(76)90527-3

37. Verpoorte JA, Mehta S, Edsall JT. Esterase activities of human carbonic anhydrases B and C. The Journal of Biological Chemistry 1967; 242 (18): 4221-4229.

38. Şentürk M, Gülçin I, Daştan A, Küfrevioğlu OI, Supuran CT. Carbonic anhydrase inhibitors. Inhibition of human erythrocyte isozymes I and II with a series of antioxidant phenols. Bioorganic and Medicinal Chemistry 2009; 17 (8): 3207-3211. doi: 10.1016/j.bmc.2009.01.067

39. Akınçoğlu A, Akbaba Y, Göcer H, Göksu S, Gülçin İ et al. Novel sulfamides as potential carbonic anhydrase isoenzymes inhibitors. Bioorganic and Medicinal Chemistry 2013; 21 (6): 1379-1385. doi: 10.1016/j.bmc.2013.01.019
40. Aksu K, Nar M, Tanç M, Vullo D, Gülçin İ et al. Synthesis and carbonic anhydrase inhibitory properties of sulfamides structurally related to dopamine. Bioorganic and Medicinal Chemistry 2013; 21 (11): 2925-2931. doi: 10.1016/j.bmc.2013.03.077

41. Güney M, Coşkun A, Topal F, Daştan A, Gülçin İ et al. Oxidation of cyanobenzocycloheptatrienes: Synthesis, photooxygenation reaction and carbonic anhydrase isoenzymes inhibition properties of some new benzotropone derivatives. Bioorganic and Medicinal Chemistry 2014; 22 (13): 3537-3543. doi: 10.1016/j.bmc.2014.04.007

42. Pradaux-Caggiano F, Su XD, Vicker N, Thomas MP, Smithen D et al. Synthesis and evaluation of thiadiazole derivatives as inhibitors of 11β-hydroxysteroid dehydrogenase type 1. Medicinal Chemistry Communications 2012; 3 (9): 1117-1124. doi: 10.1039/C2MD20091K

43. Li JC, Zhang J, Rodrigues MC, Ding DJ, Longo JP et al. Synthesis and evaluation of novel 1,2,3-triazole-based acetylcholinesterase inhibitors with neuroprotective activity. Bioorganic and Medicinal Chemistry Letters 2016; 26 (16): 3881-3895. doi: 10.1016/j.bmcl.2016.07.017