Future Visions Journal DOI: 10.29329/futvis.2021.404.5 Gelecek Vizyonlar Dergisi (fvj: Future Visions Journal) 5(4): 2021, 40-49

New Carbonic Anhydrase Inhibitors: Investigation of The Inhibition Effects of Some Synthesis Products on Human Carbonic Anhydrase-I

Fatma Yaylacı Karahalil[1*], Nurhan Gümrükçüoğlu[2] and İmdat Aygül[3]

[1] Karadeniz Technical University, Maçka Vocational School, Department of Chemistry and Chemical Processing Technologies, Biochemistry Program, 61750, Maçka, Trabzon, Turkey; fyaylaci@ktu.edu.tr

[2] Karadeniz Technical University, Vocational School of Health Services, Department of Medical Services Technology, Medical Imaging Techniques Program, 61080, Trabzon, Turkey; ngumrukcuoglu@ktu.edu.tr

[3] Gümüşhane University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Gümüşhane, Turkey; imdat_aygul25@hotmail.com

*Correspondence: fyaylaci@ktu.edu.tr

Abstract

In this study, inhibition of human carbonic anhydrase (h CAI) enzyme was investigated in four different organic synthesis compounds. Two of these compounds are [(4-amino-3-phenyl-5-p-tolyl-4*H*-1,2,4-triazole) (1) and (2,5-diphenyl-1,3,4-oxadiazol) (2)] was previously synthesized by our group, while the other two were newly synthesized [(4-methyl-N-(1-tosyl-1*H*-1,2,4-triazol-3yl)benzene sulfonamide) (3) and (4-((5-) bromo-2-hydroxy benilidene)amino)-5-(4-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one) (4)]. In this study, carbonic anhydrase enzyme inhibition was examined and inhibition values were measured using the Ki and IC_{50} values of the enzyme. IC_{50} values measured by esterase activity were detected in the range of 0.023 to 0.095 mM for h CAI, while compound 4 showed the highest inhibition value. Ki values for h CA I was observed between 0.046 and 0.056 mM, and the highest Ki value was also measured for again compound 4.

Keywords: Enzyme inhibition, Benzene sulfonamide, Triazol, Oxadiazole, Shift base, Carbonic anhydrase

Yeni Karbonik Anhidraz İnhibitörleri: Bazı Sentez Ürünlerinin İnsan Karbonik Anhidraz-I Üzerindeki İnhibisyon Etkilerinin İncelenmesi

Özet

Bu çalışmada, dört farklı organik sentez bileşiğinde insan karbonik anhidraz (h CAI) enziminin inhibisyonu araştırıldı. Bu bileşiklerden ikisi [(4-amino-3-fenil-5-p-tolil-4*H*-1,2,4-triazol) (1) ve (2,5-difenil-1,3,4-oksadiazol) (2)] daha önce grubumuz tarafından sentezlendi, diğer ikisi ise yeni sentezlendi [(4-metil-N-(1-tosil-1*H*-1,2,4-triazol-3yl)benzen sülfonamid) (3) ve (4 - ((5-) bromo-2-hidroksi beniliden) amino) -5- (4-klorofenil) -2,4-dihidro-3*H*-1,2,4-triazol-3-on) (4)]. Esteraz aktivitesi ile ölçülen IC_{50} değerleri h CAI için 0.023 ila 0.095 mM aralığında tespit edilirken, bileşik 4 en yüksek inhibisyon değerini gösterdi. h CA I için Ki değerleri 0,046 ile 0,056 mM arasında gözlendi ve en yüksek Ki değeri yine bileşik 4 için ölçüldü.

Anahtar Kelimeler: Enzim inhibisyonu, Benzen sülfonamid, Triazol, Oksadiazol, Shift bazı, Karbonik anhidraz

1 Introduction

Carbonic anhydrases (CA, carbonate hydrolysis, E.C.4.2.1.1), one of the most studied enzymes present in all species, form a family of enzymes involved in regulating pH, water, electrolyte, and ion transport. Carbonic anhydrase is an enzyme from a zinc metallo-enzyme family and has a structure in which a single peptide chain ion is coordinated via Zn^{2+} ion in the active region. Physiologically, it catalyzes the hydration of carbon dioxide and dehydration of bicarbonate in a reversible manner (Innocenti, Hilvo, Scozzafava, Parkkila, & Supuran, 2008; Innocenti, Gülçin, Scozzafava, & Supuran, 2010).

The CA enzyme, commonly found in organisms, has different isoenzymes that vary according to environmental conditions and requirements. To date, sixteen isozymes have been identified (Esbaugh,& Tufts, 2007). The most widely available and studied isozymes are CA I and CA II.

The enzyme exhibits esterase activity and hydratase activity, but hydratase activity is important in terms of physiology. This plays an important role in regulating the acid-base balance of organisms. In cases where this balance is impaired, such as when intraocular pressure is compromised, interfering with carbonic anhydrase activity is a frequently applied treatment. In this respect, carbonic anhydrase inhibitors are clinically significant compounds (Puscas, Puscas, Coltau, Baican, & Domuta, 2000; Supuran, Ilies, & Scozzafava,1998; Supuran, 2008).

Systemic carbonic anhydrase inhibitors are one of the most powerful agents used to lower intraocular pressure. However, their use is associated with many undesirable side effects.

Acetazolamide, methazolamide, and sulfonamide derivatives have been used for 40 years to reduce intraocular pressure in glaucoma. Inhibitors reduce the concentration of bicarbonate, leading to a flow of water and Na+ ions to the posterior part of the eye, causing a decrease in the aqueous environment and intraocular pressure. Acetazolamide carbonic anhydrase inhibitor is used to reverse metabolic alkalosis when fluid and potassium replacements are insufficient to correct blood alkalinity (Faisy, Mokline, , Sanchez, Tadié, & Fagon, 2010). Acetazolamide has been shown to be effective in reducing the frequency of both vertigo and headache attacks (Çelebisoy, Gökçay, Karahan, Bilgen, Kirazlı, Karapolat, & Köse, 2016).

Numerous studies have investigated natural products and synthetic products associated with the inhibition of CA I. Natural compounds are attractive in terms of permitting the design of novel chemotypes acting as carbon anhydrase inhibitors (Durdagi, Scozzafava, Vullo, Sahin, Kolayli, & Supuran, 2014). Many studies have focused on the biotechnological use of various CAs, some of which were isolated from less common vertebrates (Özensoy, Arslan, & Supuran, 2011; Kolayli, Karahalil, Sahin, Dincer, & Supuran, 2011; Sahin, Can, Yildiz, Kolayli, Innocenti, Scozzafava, & Supuran, 2012)investigated the inhibition of carbonic anhydrase isozymes I and II with natural products extracted from plants, mushrooms and honey. They reported IC_{50} values in the range of 0.11–66.50 µg/mL against h CA I.

The common feature of these inhibitory substances is the free sulfonamide (-SO_2 NH_2) group attached to the aromatic rings, causing inhibition of the enzyme by bonding to the active region. The undesirable side effects of drugs used as CA II inhibitors in glaucoma led to the synthesis of more effective sulfonamide derivatives (Becker, 1954). At the same time, the intense side effects of these substances resulted in the synthesis of new class compounds as precursors in carbonic anhydrase inhibitors. Therefore, the purpose of this study was to investigate the effects of various synthesis compounds, amine, Schiff base and oxadiazole, sharing the common property of nitrogen content, on the human carbonic anhydrase I (h CA-I) isoforms involved in crucial physiological and pathological processes.

Triazole derivatives have been reported to possess pharmacological, insecticidal, fungicidal, and herbicidal activities (Sokmen, Gumrukcuoglu, Ugras, Ugras, & Yanardag, 2013). Compounds with triazole moieties, such as vorozole, letrozole and anastrozole have also been used as nonsteroidal aromatase inhibitors for treating breast cancer. 1, 2, 4-triazoles are also important in various areas of chemistry owing to their biological activities (Gumrukcuoglu, Sokmen, Sahin, Ugras, Sagkal, & Ugras, 2016).

Oxadiazole is a versatile heterocyclic nucleus, which has attracted considerable interest from medicinal chemists in developing new drugs. Oxadiazole is a cyclic compound containing one oxygen atom and two nitrogen atoms in a five-membered ring with the general formula $C_2H_2ON_2$. There are four isomers of oxadiazoles, which are known as 1,2,4-oxadiazole, 1,3,4-oxadiazole, 1,2,5-oxadiazole, and 1,2,3-oxadiazole, but 1,2,3 is unbalanced and reverse to the diazoketone tautomers (Kavitha, Gnanavel, & Kannan, 2014). Oxadiazole is derived from furan by substitution of two methylene groups with two pyridine-type nitrogen atoms. 1, 3, 4-oxadiazole undergoes a number of reactions, including electrophilic substitution, nucleophilic substitution, and thermal and photochemical reactions.

A number of interesting developments have been observed in the biological activities of oxadiazole derivatives in recent years. Various derivatives of oxadiazole have been synthesized for their pharmacological activities.

1,3,4-oxadiazoles are a biologically important group of compounds with bactericidal, antifungal, analgesic, anti-inflammatory and wheat growth-promoting properties (Khan, & Akhtar, 2003; Zheng, Li, Wang, Chen, Huang, Liu, & Song, 2003). Previous studies reported that some 1,3,4-oxadiazoles exhibited antimicrobial activity (Gümrükçüoğlu, Serdar, Celik, Sevim, & DEMİRBAŞ, 2007). We now report the synthesis and carbonic anhydrase enzyme inhibition of substitute 1, 3, 4 oxadiazole.

Antibacterial sulphonamides were the first effective chemotherapeutic agents used for to teart bacterial infections in humans. The term sulphonamide is usually employed as a generic name for the derivatives of para amino benzene sulphonamides. Sulphonamides inhibit gram-positive and gram-negative bacteria, Nocardia, Chlamydia trachomatis and some Protozoa. Some enteric bacteria, such as Escherichia coli, Klebsiella, Salmonella, Shigella and Enterobacter, are also inhibited. Sulphonamides are used in the treatment of tonsillitis, septicemia, meningococcal meningitis, bacillary dysentery and several urinary tract infections.

Sulphonamides are bacteriostatic in nature. Sulphonamide-sensitive micro-organisms require p-Amino benzoic acid (PABA) to synthesize folic acid, which is essential for the synthesis of DNA and RNA. Sulphonamides block the biosynthesis of this folate coenzyme, resulting in the arrest of bacterial growth and cell division.

Recent studies have reported that benzylidene-4-(5-benzylsulphonyl-4H-(1,2,3) triazol- 3yl-phenyl)-amine derivatives possess numerous biological activities, such as antibacterial, anticonvulsant and antituberculoid properties, while exhibiting no deleterious effects on the nervous system (Gumrukcuoglu, Sokmen, Sahin, Ugras, Sagkal, & Ugras, 2016). In previous studies, we reported that 1, 2 4-triazole ligands exhibited anticancer, antitumor, and antimicrobial activities (Gumrukcuoglu, Sokmen, Sahin, Ugras, Sagkal, & Ugras, 2016; Gümrükçüoğlu, Serdar, Celik, Sevim, & DEMİRBAŞ, 2007).

Schiff bases and complexes are used in medicine, the plastics industry, water treatment, biochemical processes and many other areas due to their specific and important features (MOHAMED, Omar, & Hindy, 2006). Many catalysts which are industrially used are essentially coordination compounds. In addition, the working mechanisms of the enzymes (Tuna, 2004) and the C = N double bond, Schiff base formation, are also involved during visual processing (Solomons, & Fryhle, 2002). The metal complexes obtained from the groups of Schiff bases are used as pigment materials in the paint industry, especially in textile dyeing, since they are colored materials (Zishen, Ziqi, & Zhenhuan, 1990). Some Schiff bases are also used in the construction of ion-selective electrodes.

In particular, the gradual realization of the effectiveness of various metal complexes in living organisms has led to the intensification of studies of these compounds (Abbaspour, Esmaeilbeig, Jarrahpour, Khajeh, & Kia, 2002). The clarification of the structures of Schiff bases and complexes has therefore become increasingly important.

Schiff bases derived from 1, 2, 4-triazoles are known to exhibit excellent biological properties. Particularly, these include antibacterial, antifungal, antituberculous, antioxidant, anticancer (Gumrukcuoglu, Sokmen, Sahin, Ugras, Sagkal, & Ugras, 2016; Gümrükçüoğlu, Serdar, Celik, Sevim,, & DEMİRBAŞ, 2007); 2013) antimalarial, anticonvulsant, anti-inflammatory (Bhandari, Bothara, Raut, Patil, Sarkate, & Mokale, 2008; Sujith, Rao, Shetty, & Kalluraya, 2009) and pesticidal properties. They are important molecules in the medicinal and pharmaceutical fields, and it has been suggested that the azomethine linkage may be responsible for the biological activities displayed by Schiff bases.

Salicylic aldehyde derivatives, with one or more halo-atoms in the aromatic ring, have been reported to exhibit a variety of biological properties, including antibacterial and antifungal activities (Felton, & Brewer, 1947). These investigations led to the idea that the Schiff base of 5-bromo-salicylaldehyde would possess potential enzyme inhibition properties. This study describes the synthesis and properties of the Schiff base of 5-bromo-salicylaldehyde. The results of this study may be useful to researchers in providing a greater understanding of the enzyme inhibition activity of Schiff base compounds.

2 Material and Methods

2.1 Reagents

Analytical grade solvents (methanol and ethanol) were obtained from Merck Co. (Merck, Darmstadt, Germany). Buffer and other reagents were of the highest purity grade and were purchased from Sigma-Aldrich (Milan, Italy). CA isozyme was isolated from human blood cells using affinity chromatography. Our own study group synthesized all compounds for CA inhibition.

2.2 CA Esterase Activity and Inhibition

IC_{50} and Ki values of the inhibitors were determined on human erythrocyte h CAI. Carbonic anhydrase hydrolyzes the p-nitrophenylacetate substrate to p-nitrophenol or p-nitrophenolate. The activity is determined by the decrease in absorbance at 348 nm (Khalifah, 1971; Verpoorte, Mehta, & Edsall, 1967; Armstrong, J. M., Myers, Verpoorte, & Edsall,1966). The *p*-nitro phenyl acetate (*p*-NFA) used as the substrate was freshly prepared on a daily basis. The enzyme-free part was used as a control during activity measurement. During pipetting, a mixture solution of 0.470 µL 0.05 M, pH 7.4 Tris-SO_4 buffer, 3mM 0.350 µL of *p*-NPA, and 180 µL of pure water was used as the control. Instead of reducing the amount of water in the control sample, a control sample was prepared using 50 µL of enzyme solution. Absorbances at minutes 0 and 3 were read at 348 nm, and the difference between them was calculated. The difference between the control values obtained with or without enzyme was calculated, and this was regarded as corresponding to 100% activity. This differential value needs to be higher than the value measured with the addition of the inhibitor. The inhibitions were expressed as IC_{50} values, representing the concentration of the compound producing 50% inhibition of the enzymes. An IC_{50} value was calculated for each inhibitor by taking an inhibitor at a specific concentration, diluting it with five different concentrations, and then drawing a concentration % activity graph.

In order to determine the Ki values of the inhibitors, activity measurements were made with the concentration of inhibitor which radically reduced the carbonic anhydrase I isoenzyme activity, and a concentration of the two stable inhibitors, below and above this value, at five different fixed substrate concentrations. Concentrations of five different substrates were determined by pre-testing using stock solutions. Lineweaver-Burk graphs were drawn for each inhibitor using the obtained values. Ki values were calculated using the formula Vmax=VImax(1+[I]/Ki) for noncompetitive inhibition in the graphical equation and KMI = KM (1+ [I]/Ki) for competitive inhibition. Ki values of these compounds were obtained by calculating using the Lineweaver–Burk graphs (Hisar, Beydemir, Bülbül, & Yanik, (2006). (Figures 1,2,3).

2.3 Synthesis compounds and mechanisms

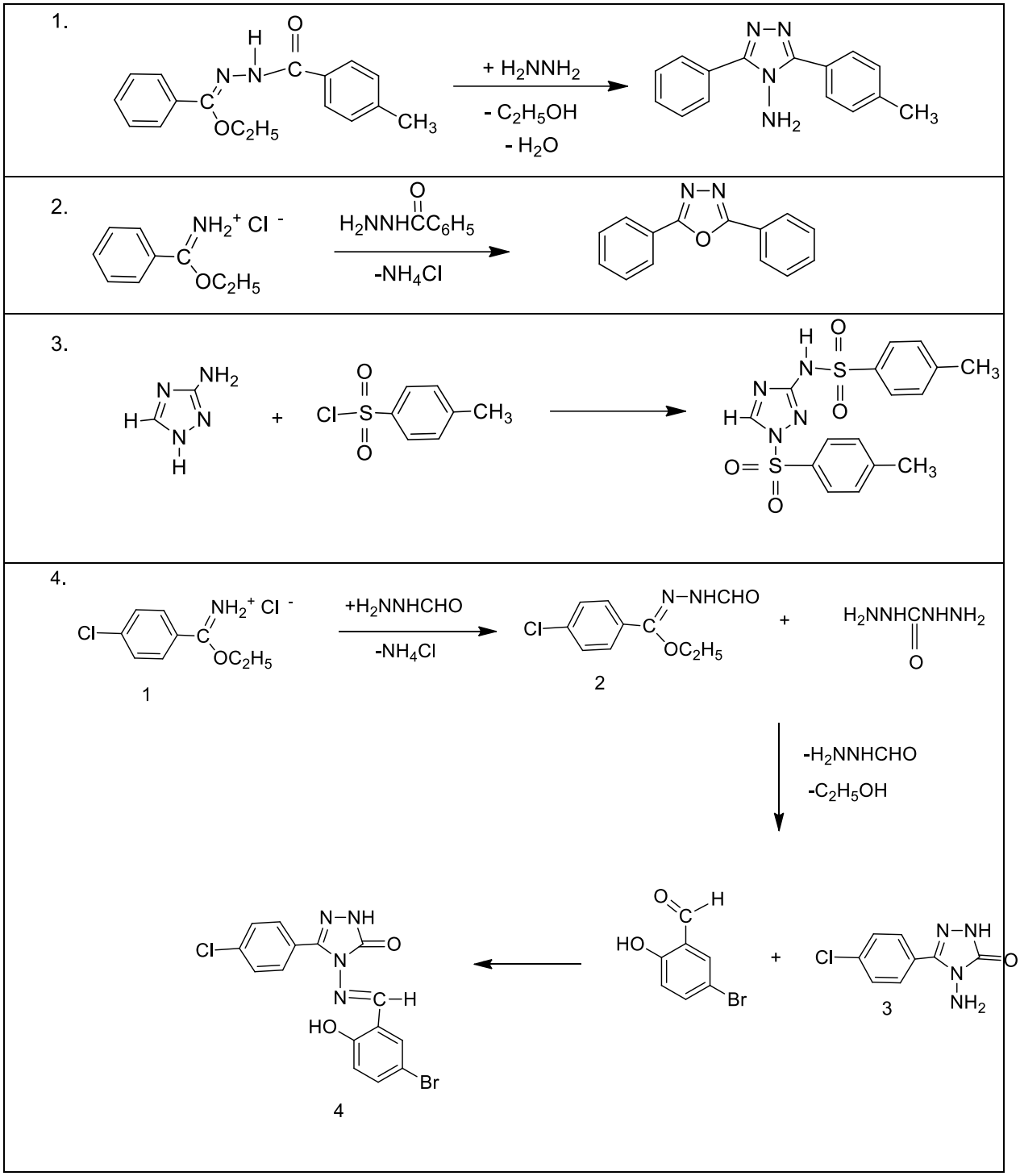
2.3.1 4-amino-3-phenyl-5-p-tolyl-4H-1, 2, 4-triazole and Experimental Procedure

In recent years, the chemistry of heterocyclic compounds containing a five-membered 1, 2, 4-triazole nucleus has been an interesting field of study. First, acylhydrazone was synthesized by the condensation of iminoester hydrochloride with acyl hydrazine. The treatment of acylhydrazone with hydrazine hydrate afforded 4-amino-(3, 5-diaryl-4-yl)-4*H*-1, 2, 4-triazole (Sokmen, Gumrukcuoglu, Ugras, Ugras, & Yanardag, 2013). The compound was characterized by elemental analyses, IR, 1H NMR and 13C NMR spectral data.

The amino compound was synthesized following the method described in the literature (Serdar, Gümrükcüoğlu, Karaoğlu, & Demirbaş, 2007). Ethyl p-methylbenzoate benzoylhydrazone (0.005mol) was added to the solution of hydrazine hydrate (0.01 mol) in 50 mL of 1-propanol, and the mixture was refluxed for 24 h. On cooling, a precipitate formed. This product was filtered and washed with 20 mL of benzene after drying. The insoluble part in benzene was recrystallized from 1-propanol to afford a pure compound. Yield 85%, m.p. 283-284 ◦C, IR (KBr) cm−1: 3345, 3250 (vNH_2), 1619 (v C=N), 824, 768, 696 (v arm.ring), 1H NMR (DMSO-d6) δ 2.38 (s, 1H, CH3), 6.30 (s, 2H, NH_2), Ar-H: [7.30 (d, 2H), 7.50 (m, 3H), 7.98 (d, 2H), 8.10 (m, 2H, Ar-H)], 13C NMR (DMSO-d6) δ 154.15 (triazole C3), 154.02 (triazole C5), Ar-C: [139.11, 129.42, 128.94 (2C), 128.36 (2C), 128.18 (2C), 128.09 (2C),

127.10, 124.31], 20.87 (CH3), UV [λmax, nm (ε×10−3)]: 256 (19.9), 210 (16.0), Anal. Calcd. for (C15H14N4): C: 71.96, H: 5.64, N: 22.39. Found: C: 72.11, H: 5.23, N: 22.66.

Table1. Synthesis of the compounds (triazole, oxadiazole, sulfonamide and schiff base)



2.3.2 2,5 -diphenyl-1, 3, 4-oxadiazole and Experimental Procedure

Compound 2 was synthesized by the reaction of corresponding iminoester hydrochloride (benziminoethylester), which was obtained using a previously published method (Gümrükçüoğlu, Serdar, Celik, Sevim, & Demirbaş, 2007), with acyl hydrazine (benzoylhydrazine). The structure was established by IR, 1H-NMR, and 13C-NMR and elemental analyses techniques. The formed acylhydrazones (Khan and Akhtar, 2003) underwent partial intramolecular cyclization; thus, 1, 3, 4-oxadiazole (2) formed in the same reaction media. Oxadiazole gave spectral data consistent with its structure.

The oxadiazole compound was synthesized according to the method described in the literature (Gümrükçüoğlu, Serdar, Celik, Sevim, & Demirbaş, 2007).

2.3.3 4-methyl-N-(1-tosyl-1H-1,2,4-triazole-3-yl-) benzenesulphonamide and Experimental Procedure

In this study, 3-amino-1*H*-1, 2, 4-triazole compound has been prepared with biologically active 'sulphonamide' moiety as the side chain. The structure of the newly synthesized compound has been established on the basis of its spectral data and elemental analysis.

The amide compound was synthesized according to the method described in the literature (Bıyıklıoğlu, Kantekin, & Özil, 2007). 3-amino-1*H*-1, 2, 4-triazole compound (0.015 mol) was dissolved in pyridine (30 ml) under nitrogen, and powdered *p*- toluene-sulfonyl chloride (0.037 mol) was added portion-wise over 0.5 h. This part was stirred and cooled in an ice-salt bath at 10 C. Stirring and cooling of the reaction mixture were continued for 1.5 h at 10 °C, then the mixture was stirred at room temperature overnight. The solution was poured slowly onto ice (100 g) and diluted with water (100 ml). The precipitated ditosylate was filtered off and washed with cold water and diethyl ether. The product was dried in vacuum and obtained as a yellow solid. Yield 63 %, m.p. 213-214 ◦C, IR (KBr) cm−1: 3238 (vNH), 3020 (ν Ar-H), 1599 (νN-H bending), 1588 (νC=N), 1472, 1320–1160 (SO2); 1H NMR (DMSO-d6) δ ppm 2.42 (s, 3H, CH3), 2.44 (s, 3H, CH3), 6.30 (s, 1H, NH), Ar-H: 7.57-7.64 (m, 2H), 7.68 (s, 1H), 7.70-7.78 (m, 2H), 7.80-7.85 (m, 4H); 13C-NMR (DMSO-d6) δ ppm 151.43 (C=N), Ar-C: [159.48 (2C), 156.80 (4CH), 151.61 (4CH), 143.15 (2C)], 21.32 (CH3), 21.19 (CH3). Anal. calculated for (C16H16N4O4S2): C: 48.97, H: 4.11, N: 14.28. Found: C: 48.89, H: 4.07, N: 14.29.

2.2.1. 4-(5-bromo-2-hydroxybenylidene)amino)-5-(4-chlorophenyl)-2,4-dihidro-3H-1,2,4-triazol-3-one and Experimental Procedure

A Schiff base compound was synthesized by reacting 2-hydroxy-5-bromobenzaldehyde and 3-(p-cholorophenyl)-4-2-hydroxy-5-bromobenzylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one .The chemical structure of this compound was confirmed by means of IR, 1H NMR, 13C NMR and elemental analyses.

The corresponding 3-p-cholorophenyl-4-amino-4,5-dihydro-1*H*-1, 2, 4-triazol-5-one (1) (0.01 mol) was heated in an oil bath with 2-hydroxy-5-bromobenzaldehyde (0.01 mol) at 150–160 °C for 2 h. After cooling the mixture to room temperature, a white solid appeared, and this was recrystallized from ethyl acetate to afford the desired compound.

(Yield 1.32 g, 76.17 %). M.p. 175–176 °C; Analysis (% calculated/found): for C15H10O2N4ClBr C: 69.84/69.80, H: 5.52/5.42, N: 19.17/18.63; IR (KBr) cm−1 3308 (ν OH), 3246 (ν NH), 1726 (ν C=O), 1598 (νC=N), 889-795 (trisubstitue arom. ring), 826 (1,4 disubstitue arom. ring); 1H-NMR (DMSO-d6) δ (ppm) Ar–H [6.67 (d, 2H), 6.95 (d, 2H), 7.47–7.51 (m, 1H), 7.62–7.69 (m, 1H), 7.78 (s, 1H)], 8.81 (s, 1H, N=CH), 11.68 (s, 1H, NH), 12.44 (s, 1H, OH); 13C-NMR (DMSO-d6) δ (ppm) 172.36 (C=O), 171.06 (N=CH), 150.39 (triazole C3), Ar–C [141.31 (C), 139.10 (C), 131.53 (2CH), 130.84 (C), 130.72 (CH), 130.17 (C), 129.62 (2CH), 128.97 (C), 128.26 (CH), 113.25 (CH)].

3 Results and Discussion

This study evaluated the inhibition effects of some synthesized compounds of h CAI isoenzymes. The results showed that all the synthesized compounds inhibited h CAI enzyme activity. Some benzene sulfonamide, triazol, oxadiazole, and Schiff base compounds investigated in this study exhibited effective h CAI inhibitory activity in the low micromolar range by the esterase method. The IC_{50} values for h CAI inhibition are summarized in Table II. The IC_{50} values were between 23 and 95 µM for h CAI, and Ki values were between 0.047 and 0.056 mM, respectively.

Inhibition values against CAs were reported by Erzengin (2014). In that study, the inhibition value ranges were 5.37–3868.00 mM for hCA I and 4.81–3390.00 mM for hCA II (Erzengin, Bilen, Ergun, & Gencer, 2014). Another report on carbonic anhydrase inhibition is by Ekinci et al. (2012). In that study, three compounds behaved as weak inhibitors of the slow cytosolic h CAI, with Ki values in the range of 0.215–4 mM. Catechol 10 was an ineffective h CAI inhibitor (Ki of 4003 µM). The three derivatives of the second group compounds showed better inhibitory activity than the previously mentioned compounds, with Ki values of 50.11–134.6 µM. Acetazolamide and sulphanilamide were also described as medium inhibitors with this assay and substrates against h CA I (Ki-s of 26.23 and 36.22 µM, respectively). Our results are in agreement with those of the second group in the above-mentioned study. These compounds appear to be medium potency inhibitors. In our study, two of the investigated compounds acted as non-competitive inhibitors, and one acted as a competitive inhibitor with *p*-NPA as a substrate.

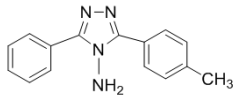
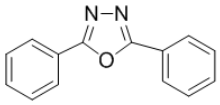
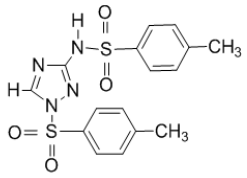
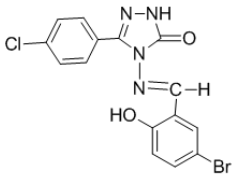
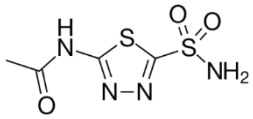
According to another study (Balaydin, Durdağı, Ekinci, Şentürk, Göksu, & Menzek, 2012), when inhibitions against *p*-nitrophenylacetate substrate were investigated according to esterase activity, Ki values for h CA I ranged between 6.83 and 892.109µM. Our results are in this range. In our study, derivative 1 exhibited a better inhibitory profile than compounds 3 and 4. It must be stressed that Ki measured using the esterase method is always in the micromolar range because h CAI and II are weak esterases (Supuran, Mincione, Scozzafava, Briganti, Mincione, & Ilies, 1998).; Akkemik, Şentürk, Özgeriş, Taşer, & Ciftci, 2011).

A previous study synthesized (3, 4-dihydroxyphenyl)(2,3,4-trihydroxyphenyl) methanone and a series of its derivatives (Nar, Çetinkaya, Gülçin, & Menzek, 2013). The synthesized compounds exhibited an inhibitory effect on h CA I. The results showed that synthesized compounds (5, 13–16) demonstrated the best inhibitory activity against h CA I (IC_{50}: 3.22–54.28 µM). Our findings also range from 23 µM to 95 µM.

Another study investigated the inhibitory effects of synthesized compounds (2a-f) on h CA I and h CA II isozymes. The esterase activity and Ki values were determined. None of the synthesized compounds (2a-f) exhibited any inhibition effects on the esterase activity of h CA I (Büyükkıdan, Büyükkıdan, Bülbül, Özer, & Gonca Yalçın, 2013). Acetazolamide (AAZ), a control compound, was investigated in vitro using esterase assays. Concentrations (IC_{50}) representing half-maximal inhibition were determined in terms of esterase activities. The IC_{50} value of AAZ was calculated as 5.9 µM.

Very weak or no activity was observed against h CAI in another previous study (Awadallah, El-Waei, Hanna, Abbas, Ceruso, Oz, & Supuran, 2015). The authors also reported that most new sulfonamides exhibited weak inhibitory activity against the off-target cytosolic isoforms, h CAI and h CAII.

Table2. Ki and IC_{50} values which were obtained from in vitro studies for synthesized compounds on esterase activity of carbonic anhydrase I isoenzyme.

Structure	Compound	Ki(µM)	IC_{50} (µM)
	1	46	40µM
	2	38	95 µM
	3	47	48 µM
	4	56	23 µM
Asetazolamide 			49 M

In the literature, three distinct series of isoxazole-based primary mono- and bis-sulfonamides have been synthesized via direct sulfochlorination. One study described the synthesis and inhibitory activity against four isoforms of human carbonic anhydrase of three distinct series of primary mono- and bis-sulfonamides (Krasavin, Korsakov, Zvonaryova, Semyonychev, Tuccinardi, Kalinin, & Supuran, 2017). Nanomolar levels of inhibition were achieved in all three series. However, in eight of the 18 synthesis compounds, the Ki value exceeded 10000 nM.

Acetylcholine esterase inhibitors and antioxidant sulfonamides 1–4 were evaluated in terms of their CA inhibitory properties. One study observed micromolar levels of Ki and IC_{50} values (Göcer, Akıncıoğlu, Göksu, & Gülçin, 2017). The inhibitory effects of phenolic sulfonamides 1–4 were tested on human carbonic anhydrase isoenzymes h CA I. Among compounds 1–4, compound 1 exhibited the best inhibitory effects. According to their data, IC_{50} values of compound 1 were 3.55 M for h CA I.

Of the 27 carbohydrazones synthesized in one study, only eight exhibited carbonic anhydrase inhibition (Iqbal, Saleem, Azim, Taha, Salar, Khan, & Choudhary, 2017). Carbohydrazones as new class of carbonic anhydrase inhibitors: Synthesis, kinetics, and ligand docking studies. These synthesized compounds exhibited an inhibitory effect on the h CAI micromolar rate.

The enzyme inhibition activities of sulfonylhydrazones have also been investigated for carbonic anhydrase I (hCA I) isoenzyme (Gündüzalp, Parlakgümüş, Uzun, Özmen, Özbek, Sarı, & Tunç, 2016).). When the inhibition activities of the compounds are compared with the standard (AAZ) used as a strong inhibitor in glaucoma treatment, they exhibit good inhibitory properties. The values determined were lower than the results in our own study. In fact, our synthesis compounds have a generally higher level of

inhibition of acetazolamide, the standard inhibitor of carbonic anhydrase, than in that study. Many of the investigated compounds in our study were more effective or similar to acetazolamide in terms of the inhibition of this isoform.

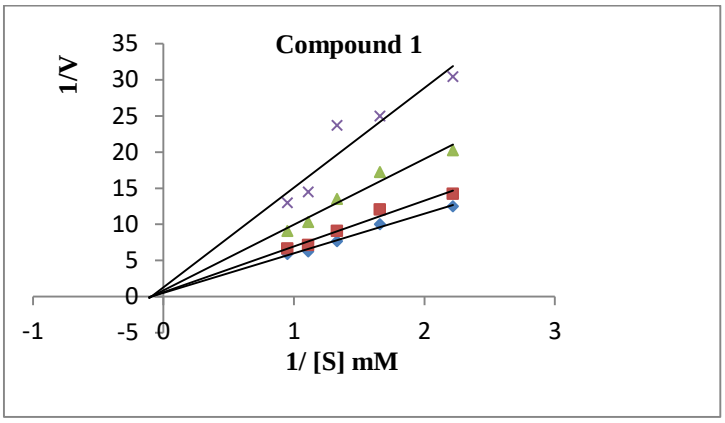


Figure1. (for hCA-I): Lineweaver–Burk graph of compound 1using five different sample concentrations for the determination of Ki and inhibition type (Noncompetitive inhibition).

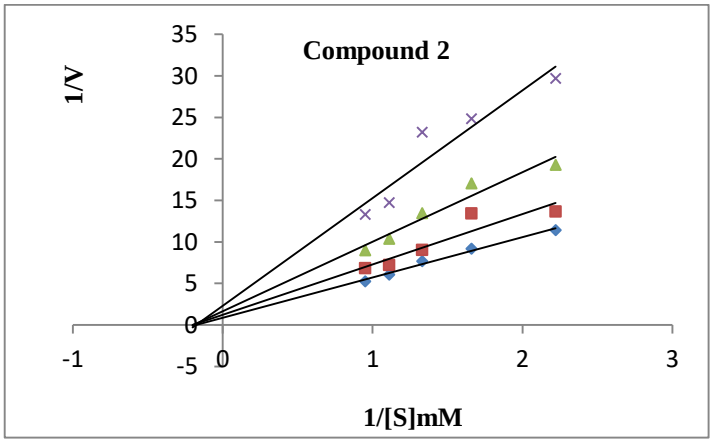


Figure 2. (for hCA-I): Lineweaver–Burk graph of compound 2 using five different sample concentrations for the determination of Ki and inhibition type (Noncompetitive inhibition).

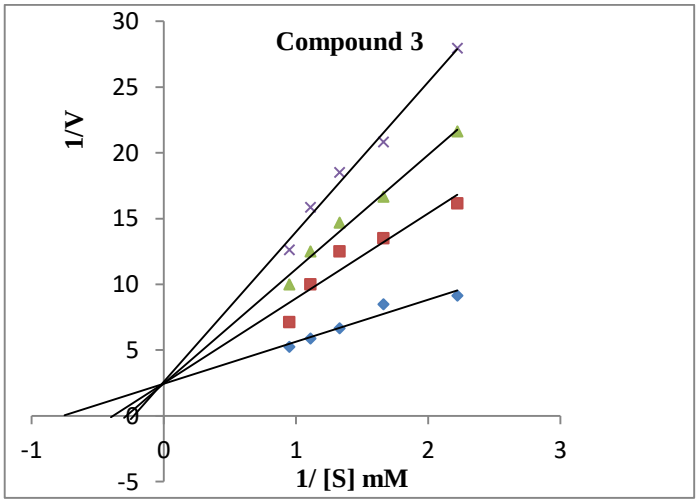


Figure 3. (for hCA-I): Lineweaver–Burk graph of compound 3 using five different sample concentrations for the determination of Ki and inhibition type (Competitive inhibition).

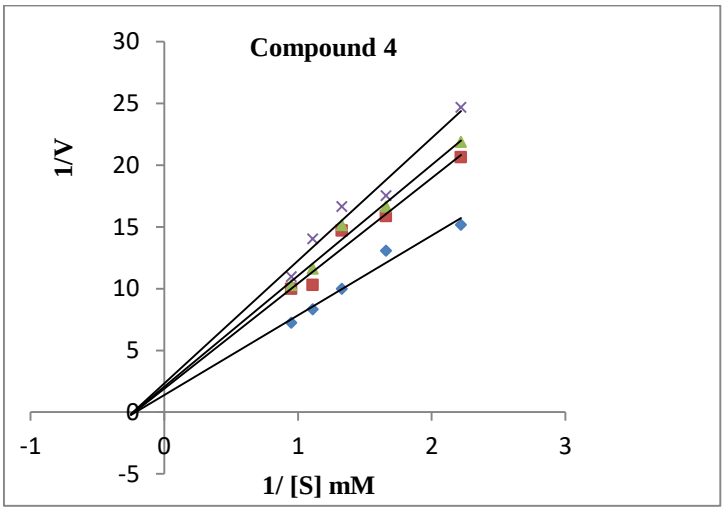
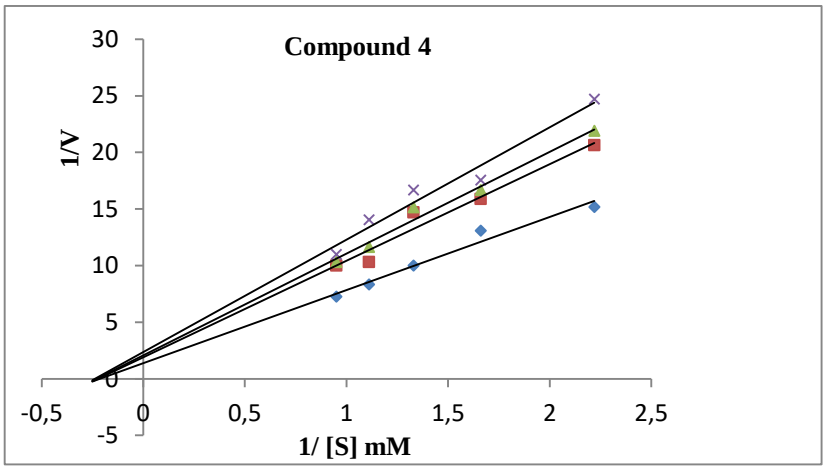


Figure 4. (for hCA-I): Lineweaver–Burk graph of compound 4 using five different sample concentrations for the determination of Ki and inhibition type (Noncompetitive inhibition).



4 Conclusion

The discovery and development of carbonic anhydrase inhibitors are crucial for clinical use as antiepileptic, diuretic and anti-glaucoma agents. The structural characterizations of synthesized compounds were made using elemental analyses and spectroscopic methods. In this study, two new organic compounds were synthesized, and their biological activities in terms of hCA I were measured. Inhibition values were calculated in terms of Ki and IC_{50} values. The enzyme inhibitory effects of the compounds were evaluated using activity parameters Ki and IC_{50} values determined by the spectrophotometric method. When the inhibition activities of compounds are compared with the standard in the literature, acetazolamide, all synthesized compounds exhibited moderate inhibitory properties. Schiff base, compound four, exhibited the highest inhibition with an IC_{50} value of 0.023 µM and Ki of 56 µM. Therefore, newly synthesized modifications of this compound need to be investigated for h CA inhibition. Although classical sulfonamide inhibitors are still widely used today, we think that newly synthesized compounds are suitable for clinical use.

5 References

Abbaspour, A., Esmaeilbeig, A. R., Jarrahpour, A. A., Khajeh, B., & Kia, R. (2002). Aluminium (III)-selective electrode based on a newly synthesized tetradentate Schiff base. *Talanta, 58*(2), 397-403.

Akbaba, Y., Türkeş, C., Polat, L., Söyüt, H., Şahin, E., Menzek, A., & Beydemir, Ş. (2013). Synthesis and paroxonase activities of novel bromophenols. *Journal of enzyme inhibition and medicinal chemistry, 28*(5), 1073-1079.

Akkemik, E., Şentürk, M., Özgeriş, F. B., TAŞER, P., & Ciftci, M. (2011). In vitro effects of some drugs on human erythrocyte glutathione reductase. *Turkish Journal of Medical Sciences, 41*(2), 235-241.

Armstrong, J. M., Myers, D. V., Verpoorte, J. A., & Edsall, J. T. (1966). Purification and properties of human erythrocyte carbonic anhydrases. *Journal of biological chemistry, 241*(21), 5137-5149.

Awadallah, F. M., El-Waei, T. A., Hanna, M. M., Abbas, S. E., Ceruso, M., Oz, B. E., ... & Supuran, C. T. (2015). Synthesis, carbonic anhydrase inhibition and cytotoxic activity of novel chromone-based sulfonamide derivatives. *European journal of medicinal chemistry, 96*, 425-435.

Balaydin, H. T., Durdağı, S., Ekinci, D., Şentürk, M., Göksu, S., & Menzek, A. (2012). Inhibition of human carbonic anhydrase isozymes I, II and VI with a series of bisphenol, methoxy and bromophenol compounds. *Journal of enzyme inhibition and medicinal chemistry, 27*(4), 467-475.

Becker, B. (1954). Decrease in intraocular pressure in man by a carbonic anhydrase inhibitor, Diamox: a preliminary report. *American journal of ophthalmology, 37*(1), 13-15.

Bhandari, S. V., Bothara, K. G., Raut, M. K., Patil, A. A., Sarkate, A. P., & Mokale, V. J. (2008). Design, synthesis and evaluation of antiinflammatory, analgesic and ulcerogenicity studies of novel S-substituted phenacyl-1, 3, 4-oxadiazole-2-thiol and Schiff bases of diclofenac acid as nonulcerogenic derivatives. *Bioorganic & medicinal chemistry, 16*(4), 1822-1831.

Bıyıklıoğlu, Z., Kantekin, H., & Özil, M. (2007). Microwave-assisted synthesis and characterization of novel metal-free and metallophthalocyanines containing four 14-membered tetraaza macrocycles. *Journal of organometallic chemistry, 692*(12), 2436-2440.

Büyükkıdan, N., Büyükkıdan, B., Bülbül, M., Özer, S., & Gonca Yalçın, H. (2013). Synthesis and characterization of phenolic Mannich bases and effects of these compounds on human carbonic anhydrase isozymes I and II. *Journal of enzyme inhibition and medicinal chemistry, 28*(2), 337-342.

Çelebisoy, N., Gökçay, F., Karahan, C., Bilgen, C., Kirazlı, T., Karapolat, H., & Köse, T. (2016). Acetazolamide in vestibular migraine prophylaxis: a retrospective study. *European Archives of Oto-Rhino-Laryngology, 273*(10), 2947-2951.

Durdagi, S., Scozzafava, G., Vullo, D., Sahin, H., Kolayli, S., & Supuran, C. T. (2014). Inhibition of mammalian carbonic anhydrases I-XIV with grayanotoxin III: solution and in silico studies. *Journal of enzyme inhibition and medicinal chemistry, 29*(4), 469-475.

Ekinci, D., Kurbanoglu, N. I., Salamcı, E., Şentürk, M., & Supuran, C. T. (2012). Carbonic anhydrase inhibitors: inhibition of human and bovine isoenzymes by benzenesulphonamides, cyclitols and phenolic compounds. *Journal of enzyme inhibition and medicinal chemistry, 27*(6), 845-848.

Erzengin, M., Bilen, C., Ergun, A., & Gencer, N. (2014). Antipsychotic agents screened as human carbonic anhydrase I and II inhibitors. *Archives of physiology and biochemistry, 120*(1), 29-33.

Esbaugh, A. J., & Tufts, B. L. (2007). Evidence for a carbonic anhydrase-related protein in the brain of rainbow trout (Oncorhynchus mykiss). *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 2*(4), 287-294.

Faisy, C., Mokline, A., Sanchez, O., Tadié, J. M., & Fagon, J. Y. (2010). Effectiveness of acetazolamide for reversal of metabolic alkalosis in weaning COPD patients from mechanical ventilation. *Intensive care medicine, 36*(5), 859-863.

Felton, L. C., & Brewer, J. H. (1947). Action of substituted salicylaldehydes on bacteria and fungi. Science, 105(2729), 409-410.

Göcer, H., Akıncıoğlu, A., Göksu, S., & Gülçin, İ. (2017). Carbonic anhydrase inhibitory properties of phenolic sulfonamides derived from dopamine related compounds. *Arabian journal of chemistry, 10*(3), 398-402.

Gümrükçüoğlu, N., Serdar, M., Celik, E., Sevim, A., & DEMİRBAŞ, N. (2007). Synthesis and antimicrobial activities of some new 1, 2, 4-triazole derivatives. *Turkish Journal of Chemistry, 31*(3), 335-348.

Gumrukcuoglu, N., Sokmen, B. B., Sahin, H., Ugras, S., Sagkal, Y., & Ugras, H. I. (2016). Synthesis of New 3, 5-disubstituted-1, 2, 4-Triazoles and Evaluation of Antibacterial, Antiurease and Antioxidant Activities. *Journal of the Chemical Society of Pakistan, 38*(5).

Gündüzalp, A. B., Parlakgümüş, G., Uzun, D., Özmen, Ü. Ö., Özbek, N., Sarı, M., & Tunç, T. (2016). Carbonic anhydrase inhibitors: Synthesis, characterization and inhibition activities of furan sulfonylhydrazones against carbonic anhydrase I (hCA I). *Journal of Molecular Structure, 1105*, 332-340.

Hisar, O. L. C. A. Y., Beydemir, Ş., Bülbül, M., & Yanik, T. (2006). Kinetic properties of carbonic anhydrase purified from gills of rainbow trout (Oncorhynchus mykiss). *Journal of applied animal research, 30*(2), 185-188.

Innocenti, A., Gülçin, I., Scozzafava, A., & Supuran, C. T. (2010). Carbonic anhydrase inhibitors. Antioxidant polyphenols effectively inhibit mammalian isoforms I–XV. *Bioorganic & medicinal chemistry letters, 20*(17), 5050-5053.

Innocenti, A., Hilvo, M., Scozzafava, A., Parkkila, S., & Supuran, C. T. (2008). Carbonic anhydrase inhibitors: Inhibition of the new membrane-associated isoform XV with phenols. *Bioorganic & medicinal chemistry letters, 18*(12), 3593-3596.

Iqbal, S., Saleem, M., Azim, M. K., Taha, M., Salar, U., Khan, K. M., & Choudhary, M. I. (2017). Carbohydrazones as new class of carbonic anhydrase inhibitors: Synthesis, kinetics, and ligand docking studies. *Bioorganic chemistry, 72,* 89-101.

Kavitha, S., Gnanavel, S., & Kannan, K. (2014). Biological aspects of 1, 3, 4-oxadiazole derivatives. *Asian Journal of Pharmaceutical and Clinical Research, 7*(4), 11-20.

Khalifah, R. G. (1971). The carbon dioxide hydration activity of carbonic anhydrase: I. Stop-flow kinetic studies on the native human isoenzymes B and C. *Journal of Biological Chemistry, 246*(8), 2561-2573.

Khan, M. S. Y., & Akhtar, M. (2003). Synthesis of some new 2, 5-disubstituted 1, 3, 4-oxadiazole derivatives and their biological activity. *ChemInform, 42b*, 900-904.

Kolayli, S., Karahalil, F., Sahin, H., Dincer, B., & Supuran, C. T. (2011). Characterization and inhibition studies of an α-carbonic anhydrase from the endangered sturgeon species Acipenser gueldenstaedti. *Journal of enzyme inhibition and medicinal chemistry, 26*(6), 895-900.

Krasavin, M., Korsakov, M., Zvonaryova, Z., Semyonychev, E., Tuccinardi, T., Kalinin, S., ... & Supuran, C. T. (2017). Human carbonic anhydrase inhibitory profile of mono-and bis-sulfonamides synthesized via a direct sulfochlorination of 3-and 4-(hetero) arylisoxazol-5-amine scaffolds. *Bioorganic & medicinal chemistry, 25*(6), 1914-1925.

Mohamed, G. G., Omar, M. M., & Hindy, A. M. (2006). Metal complexes of Schiff bases: preparation, characterization, and biological activity. *Turkish Journal of Chemistry, 30*(3), 361-382.

Nar, M., Çetinkaya, Y., Gülçin, İ., & Menzek, A. (2013). (3, 4-Dihydroxyphenyl)(2, 3, 4-trihydroxyphenyl) methanone and its derivatives as carbonic anhydrase isoenzymes inhibitors. *Journal of enzyme inhibition and medicinal chemistry, 28*(2), 402-406.

Özensoy, Ö., Arslan, M., & Supuran, C. T. (2011). Carbonic anhydrase inhibitors: purification and inhibition studies of pigeon (Columba livia var. domestica) red blood cell carbonic anhydrase with sulfonamides. *Journal of enzyme inhibition and medicinal chemistry, 26*(5), 749-753.

Puscas, I., Puscas, C., Coltau, M., Baican, M. ve Domuta, G. (2000). The serum of Carcinoma Patients Powerfully Activates Carbonic Anhydrase II. *Exp Oncol, 22*, 162-164.

Sahin, H., Can, Z., Yildiz, O., Kolayli, S., Innocenti, A., Scozzafava, G., & Supuran, C. T. (2012). Inhibition of carbonic anhydrase isozymes I and II with natural products extracted from plants, mushrooms and honey. *Journal of enzyme inhibition and medicinal chemistry, 27*(3), 395-402.

Serdar, M., Gümrükcüoğlu, N., KARAOĞLU, Ş. A., & DEMİRBAŞ, N. (2007). Synthesis of some novel 3, 5-diaryl-1, 2, 4-triazole derivatives and investigation of their antimicrobial activities. *Turkish Journal of Chemistry, 31*(3), 315-326.

Sokmen, B. B., Gumrukcuoglu, N., Ugras, S., Ugras, H. I., & Yanardag, R. (2013). Synthesis, antibacterial, antielastase, antiurease and antioxidant activities of new methoxy substitued bis-1, 2, 4-triazole derivatives. *Journal of enzyme inhibition and medicinal chemistry, 28*(1), 72-77.

Solomons, G. T. W., & Fryhle, B. C. (2002). *Organik Kimya* (Çev. G. Okay ve Y. Yıldırır). Literatür Yayıncılık.

Sujith, K. V., Rao, J. N., Shetty, P., & Kalluraya, B. (2009). Regioselective reaction: synthesis and pharmacological study of Mannich bases containing ibuprofen moiety. *European journal of medicinal chemistry, 44*(9), 3697-3702.

Supuran, C. T. (2008). Diuretics: from classical carbonic anhydrase inhibitors to novel applications of the sulfonamides. *Current pharmaceutical design, 14*(7), 641-648.

Supuran, C. T., Ilies, M. A., & Scozzafava, A. (1998). Carbonic anhydrase inhibitors—Part 29 1: Interaction of isozymes I, II and IV with benzolamide-like derivatives. *European journal of medicinal chemistry, 33*(9), 739-751.

Supuran, C. T., Mincione, F., Scozzafava, A., Briganti, F., Mincione, G., & Ilies, M. A. (1998). Carbonic anhydrase inhibitors—part 52. Metal complexes of heterocyclic sulfonamides: a new class of strong topical intraocular pressure-lowering agents in rabbits. *European journal of medicinal chemistry, 33*(4), 247-254.

Tuna, S. (2004). *Sübstitüe imin bileşikleri ve bunların Co (II), Ni (II), Cu (II) ve Zn (II) komplekslerinin sentezi ve karakterizasyonu/Synthesis and characterization of ligands containing sübstitüe imine conpounds and thier Co (II), Ni (II), Cu (II) and Zn (II) complexes*. Unpublished Master Thesis, Fırat University, Elazığ.

Verpoorte, J. A., Mehta, S., & Edsall, J. T. (1967). Esterase activities of human carbonic anhydrases B and C. *Journal of biological chemistry, 242*(18), 4221-4229.

Zheng, X., Li, Z., Wang, Y., Chen, W., Huang, Q., Liu, C., & Song, G. (2003). Syntheses and insecticidal activities of novel 2, 5-disubstituted 1, 3, 4-oxadiazoles. *Journal of Fluorine Chemistry, 123*(2), 163-169.

Zishen, W., Ziqi, G., & Zhenhuan, Y. (1990). Synthesis, characterization and anticancer activity of L-alanine Schiff base complexes of copper (II), zinc (II), nickel (II) and cobalt (II). *Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry, 20*(3), 335-344.