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Synthesis and Biological Evaluation of Novel Dihydro [2,3D] Pyridine Substituted Enaminosulfonamide Compounds as Potent Human Erythrocyte Carbonic Anhydrase II (hCAII) Inhibitors

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Abstract

Dihydro [2,3D] pyridine substituted enaminosulfonamide compounds have been synthesized and their effects on carbonic anhydrase II (hCAII) have been evaluated. Pyrido [2,3 d] pyrimidines were synthesized from barbituric acid derivatives, malonanitrile, aldehyde derivatives in basic condition and then hydrolyzed with hydrochloric acid. The targeted compounds were synthesized from amino sulfanilamide, dihydro [2,3D] pyridine compounds, and triethylorthoformate. ^{1}H NMR, ^{13}C NMR, FT-IR and elemental analysis were used for the structural analysis of the compounds. The half maximal inhibitory concentration (IC_{50}) values of the compounds were determined to be between 27.03 and 104.39 μM for hCA II and 19.85-76.64 μM for K_i.
Keywords: Barbituric acid, Carbonic anhydrase II, Dihydro [2,3D] pyridine, Enaminosulfonamide

1. INTRODUCTION

Known as the first barbiturate, was synthesized by Conrad and Guthzeit in 1882 [1]. At the 1903, Fischer and von Mehring [2] made a name for themselves in the medical world by investigating hypnotic activity and phenobarbital was introduced in 1912. In fact, when this structure, which does not have physiological effect, is substituted with alkyls from the 5th position, a hypnotic effect is produced and thus numerous compounds are prepared. Also, alkylation or aryl alkylation of position 5 has been disclosed as pharmacodynamics derivatives. This alkylation, molecular physicochemical property defining the pKa and logP value is also used as medicines against many diseases make changes accordingly. There are barbituric acid derivatives used in many treatments.

In some cases, there are many barbituric acid derivatives that do not exert a single effect but enhance the sedative-hypnotic effect when given together [3]. These compounds prevent oxidation during the biotransformation of barbiturates.

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Thus, barbiturates prolong the action time and increase the depth of action.
The pharmacokinetic properties of barbituric and thiobarbituric acid derivatives are determined by enzymatic reactions against acidic and lipophilic properties [4]. These properties are based on structural and physicochemical factors. In general, lipophilic properties increased the amount of metabolization and are known to shorten the duration of action.
Dihydro [3,2-d] pyridopyrimidine compounds are nitrogen-based heterocyclic compounds that have inhibitory and activator properties for many different enzyme types. With this feature, they are compounds that have many bioactive effects. For example, diabetes[5], asthma [6], [7], cancer [8] and aids [9] are among the most important of them. Another feature is that it has shown extremely positive results against p3818 phosphoinositide 3-kinase (PI3K and Epstein-Barr virus (EBV) [10].
Sulfonamides was discovered that it could be used as diuretics in the 1940s [11] and ant diabetic in the 1950s [12]. Computer-aided drug designs that have been developing rapidly in recent years have suggested that sulfonamides may have very different biological activity. As a matter of fact, Sildenafil Citrate (Viagra) [13] was introduced in 1996 and Amprenavir [14], a protease inhibitor used in the treatment of HIV and Celecoxib, which was used for the treatment of pain relief, meniscus and rheumatism, was used in the treatment of HIV [15].
Carbonic anhydrases (CAs; EC 4.2.1.1) are zinc enzymes [16], present in both prokaryotes and eukaryotes,[17] and they catalyze the reversible hydration of CO_2 efficiently to bicarbonate [18], [19]. Abnormal increase or decrease of several CAs activities have been reported to be associated with different human disorders (Alzheimer's disease, edema, epilepsy, altitude sickness, obesity, stroke, several types of cancer) [20] due to their crucial roles in a wide range of pathophysiological processes such as in respiration, pH and CO_2 homeostasis, secretion, gluconeogenesis, and ureagenesis, [19]-[22]. The identification of hCAs in the central nervous system or the choroid plexus, hCA I is expressed in the motor neurons in the human spinal cord and plays role in edema. In contrast, hCA II, plays role in glaucoma, is located both in the choroid plexus and in oligodendrocytes, myelinated tracts, astrocytes, and myelin sheaths in the vertebrate brain [20], [23].

In the present study, we have synthesized and investigated inhibitory effects of Novel Dihydro [2,3D] Pyridine Substituted Enaminosulfonamide Compounds on hCAII.

2. MATERIALS AND METHODS

2.1. Materials and Techniques

FT-IR spectra were measured on a SHIMADZU Prestige-21 (200 VCE) spectrometer with ATR attachment. ^{1}H and ^{13}C NMR spectra were measured on spectrometer at VARIAN Infinity plus 300 and 75 Hz, respectively. ^{1}H and ^{13}C chemical shifts are referenced to the internal deuterated dimethyl sulfoxide (DMSO-$_{d6}$) solvent. The elemental analysis was carried out with a Thermo Scientific Flash 2000 instrument with tin pan. All chemical was purchased from MERCK, Alfa Easer, Sigma-Aldrich and Fluka.

2.1.1. Pyrido [2,3 d] pyrimidines synthesis

Barbituric acid derivative (1 mmol), Malonanitrile (1 mmol,) aldehyde derivates (1 mmol), and 0.1 mL of triethylamine were taken in a reaction flask and stirred in 5 mL of EtOH at rt for 3 hours. The reaction mixture was cooled to room temperature and the Ethanol removed. 3-4 ml of acetic acid was added to the crude product and poured into 50 ml of iced water. The resulting precipitate was filtered through the crucible and left to dry. The structures of the synthesis compounds were confirmed by ^{1}H NMR and ^{13}C NMR spectra.

2.1.2. Dihydro[3,2-d]pyrimidine synthesis

Pyridopyrimidines (2 mmol), 2 mL of concentrated HCl and 4 mL of water were taken up in a reaction flask and stirred overnight at 100°C. The reaction mixture was cooled to room temperature and extracted with dichloromethane (DCM). The crude product was crystallized from Ethyl acetate: Hexane (1:1). The structures of the synthesis compounds were confirmed by ^{1}H NMR and ^{13}C NMR spectra.

2.1.3. Sulfonamide substitute Dihydro[3,2-d] pyrimidine synthesis

Dihydro [3,2-d] pyrimidine (1 mmol), 5 mL of triethylortaformiate and amino sulfonamide (1mmol) were taken up in a reaction flask and stirred 2h at 80°C in Ethanol. The reaction mixture was cooled to room temperature and extracted with Ethyl acetate. The crude product was crystallized from Ethyl acetate: Hexane (1: 1). The structures of the synthesis compounds were confirmed by ^{1}H NMR and ^{13}C NMR spectra.

2.1.4. Preparation of haemolysate and purification from blood red cells

Blood samples (25 ml) were taken from healthy human volunteers. They were centrifuged at 1000 g for 20 min at 4 °C and the supernatant was removed. The packed erythrocytes were washed three times with 0.9% NaCl and then hemolysis in cold water. The pH of the haemolysate was adjusted to pH 8.5 with solid Tris-base. The 25 ml haemolysate was applied to an affinity column containing Sepharose-4B-L-tyrosine-sulfonamide [24]. CA isozymes were then eluted with 0.1 M NaCl/25 mM Na_2HPO_4 (pH 6.3) and 0.1 M CH_3COONa/0.5 M $NaClO_4$ (pH 5.6), which recovered hCAII, respectively.

2.1.5. Esterase activity assay

CA activity was assayed by following the change in absorbance at 348 nm of 4-nitrophenyl-acetate (NPA) to 4-nitrophenylate ion over a period of 3 min at 25°C using a spectrophotometer (Shimadzu UV-Vis 1800) according to the method described in the literature [25]. Inhibitory effects of the compounds (1-10) on enzyme activities were tested under *in vitro* conditions.

2.1.6. *In vitro* inhibition studies

For the inhibition studies of sulfonamides, different concentrations of these compounds were added to the enzyme reaction mixture. CA enzyme activity without a synthesized compounds solution was accepted as to be 100%. Activity percentage values of CA for different concentrations of each sulfonamide were determined by regression analysis using Microsoft Office Excel programme. IC_{50} values were calculated from Lineweaver–Burk [26] graphs and have been given in Table 1 .

2.1.7. Calculation of Inhibition Constants (K_i) by Cheng & Prusoff Equation

The inhibition constants (K_i) of the original synthesis sulfonamide derivatives (1→10) were calculated mathematically using the Cheng & Prusoff equation [27].

3. RESULTS AND DISCUSSION

Dihydro [2,3D] pyridine substituted enaminosulfonamide compounds which is shown in scheme 1 have been synthesized and characterized by ^{1}H NMR, ^{13}C NMR, FT-IR and elemental analysis. In the ^{1}H-NMR, Dihydro [2,3D] pyridine substituted enaminosulfonamide compounds were shown about 11.10 ppm NH peak for sulfanilamide and It can be shown a singlets relating to hydrogens of vinyl proton about 9.05 ppm. All aromatic protons were seen about 8.75 and 7.00 ppm. In addition, Sulfanilamide substituted aromatic proton signals were appeared between from 8.74 to 9.37. Barbituric acid methyl groups can be seen at about 3.50 ppm. In FTIR spectrum of compounds 1-10, it was shown about 3420 cm^{-1} NH vibration. Sulfanilamide SO_2 asymmetric stretching was about 1350 cm^{-1} and SO_2 symmetric stretching was between 1139 to 1150 cm^{-1}. Barbituric acid carbonyl group can be seen between 1520 and 1560 cm^{-1}.

Carbonic anhydrase CA (E.C:4.2.1.1) isoenzyme glaucoma [28]–[30], epilepsy [31] and certain tumor types [32]–[34] such as is one of a number of pharmacological agents designed to prevent or treat the disorder. Therefore, it is of great importance that natural phenolic compounds, which are natural and have no side effects, inhibit CA enzyme activities [35]–[37]. Many natural and synthetic substances can alter the activity of the enzyme, which can affect live metabolism even at low concentrations [38].

Synthesized human carbonic anhydrase II study the effects on enzyme activity previously studied compounds were characterized.

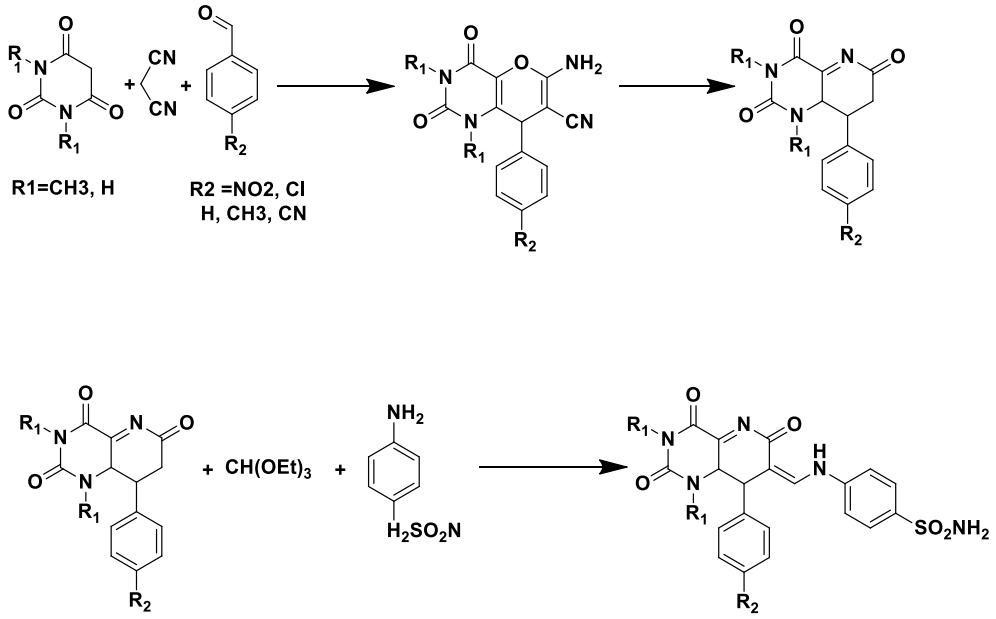
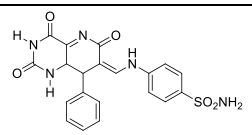
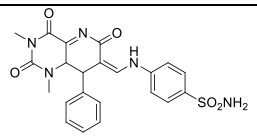
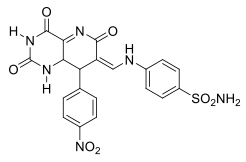
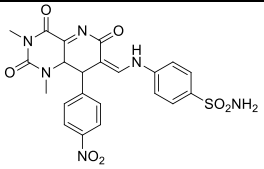


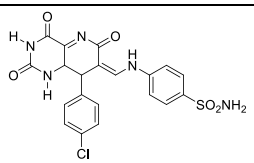
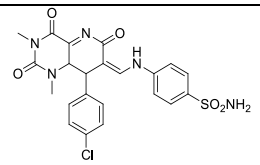
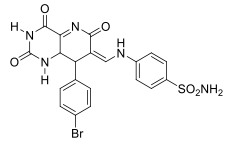
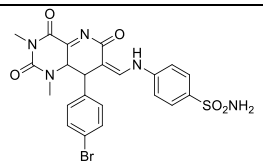
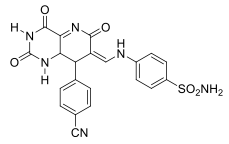
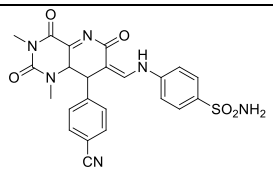
Figure 1. Synthesis of 8-(phenyl)-8,8a-dihydropyrido[3,2-d]pyrimidine-2,4,6(1H,3H,7H)-trione derivatives.

Dihydro [2,3] pyridine compound may isoenzyme activity of CAII inhibitory properties were investigated previously in the literature many times [36]. In our study, the effects of compounds 1-10 on the activity of carbonic anhydrase II isoenzyme purified from human erythrocytes were investigated (Table 1). Compounds 1 and 6 had a strong effect against carbonic anhydrase II isoenzyme activity, while compounds 3 and 8 had a lower effect on CAII activity than other compounds. The IC_{50} values of hCAII for 1 and 6 were observed as 27.03 and 28.63 μM, K_i values for 1 and 6 were 19.85 and 21.02, respectively, whereas those of 3 and 8 were found to be 100.57 and 104.39 μM for the IC_{50} values and 3 and 8 were found to be 73.84 and 76.64 μM for the K_i values (Table 1). When the data obtained are taken into consideration, the highest inhibition shows 1 and the lowest inhibition shows 8.

When looking for the inhibition mechanism with carbonic anhydrase II, it is thought to be the result of the interaction of the zinc atoms in the enzyme and the sulfonamide structure in the structure. Apart from this, the effect of functional groups on the aromatic structure has caused fundamental differences between the results due to the effect of the electron on the sulfonamide.

Table 1. Result of CAII inhibition capacity.

No	Compound	hCAII (IC_{50} μM)	K_i (μM)	No	Compound	hCAII (IC_{50} μM)	K_i (μM)
1		27.03	19.85	6		28.63	21.02
2		82.00	60.21	7		95.87	70.39

3		100.57	73.84	8		104.39	76.64
4		39.95	29.33	9		45.99	33.77
5		48.85	35.87	10		57.14	41.95

4-(((2,4,7-trioxo-5-phenyl-1,2,3,4,4a,5-hexahydropyrido[2,3-d]pyrimidin-6(7H)-ylidene)methyl) amino) benzene sulfonamide 1: Yields: %88. ^{1}H NMR (300 MHz, DMSO-D_6), 11,21 (NH, s), 10,06 (1H, d, N-H), 8.78 (H, d, C=C-H), 7.98 (2H, d, Ar-H), 7.78 (2H, d, Ar-H), 7,76 (2H, s, NH_2), 7.60 (2H, d, Ar-H), 7.41 (2H, d, Ar-H), 7.12 (2H, d, Ar-H), 7.11 (H, t, Ar-H), 4.10 (H, s, C-H), 3.88 (H, s, C-H), ^{13}C NMR (75 MHz, DMSO-D_6), 171.16, 168.20, 155.61, 153.62, 148.76, 141.16, 140.72, 130.10, 130.09, 128.60, 127.48, 126.00, 125.72, 116.16, 45.80, 36.72. FT-IR (ν, cm^{-1}): 1150,36 (O=S=O), 1256.16 (C-HN-C), 1340.53 (S-NH_2), 1616.86 (NH-C=O), 1645.28 (=N-C=O), 1701.22 (=N-C=O), 2191.13 (-CN), 2974.74 (Aliphatic-H), 3026.55 (C=C, Aromatic). Elemental Analysis, $C_{20}H_{17}N_5O_5S$, calculated: C, 54.66; H, 3.90; N, 15.94; O, 18.20; S, 7.30, Found: C, 54.52; H, 3.88; N, 15.87; O, 18.21; S, 7.52.

4-(((5-(4-nitrophenyl)-2,4,7-trioxo-1,2,3,4,4a,5-hexahydropyrido[2,3-d]pyrimidin-6(7H)-yliene) methyl) amino) benzene sulfonamide 2: Yields % 87. ^{1}H NMR (300 MHz, DMSO-D_6), 11,08 (NH, s), 10,16 (1H, d, N-H), 8.74 (H, d, C=C-H), 8,28 (2H, d, Ar-H), 8.01 (2H, d, Ar-H), 7,81 (2H, s, NH_2), 7.80 (2H, d, Ar-H), 7.25 (2H, d, Ar-H), 4.21 (H, s, C-H), 3.87 (H, s, C-H), ^{13}C NMR (75 MHz, DMSO-D_6), 172.06, 168.12, 156.61, 152.02, 149.70, 145.99, 140.16, 140.02, 130.88, 130.79, 128.59, 127.36, 126.12, 116.90, 45.75,

35.02. FT-IR (ν, cm-1): 1161.15 (O=S=O), 1240.23 (NO_2), 1288.45 (C-HN-C), 1340.53 (S-NH_2), 1616.35 (NH-C=O), 1645.28 (=N-C=O), 2972.31 (C=C, Aliphatic), 3026.55 (C=C, Aromatic). 3203.76 (NH), 3273.20 (NH). Elemental Analysis, $C_{20}H_{16}N_6O_7S$; Calculated, C,49.59; H,3.33; N,17.35; O,23.12; S,6.62. Found, C,49.29; H, 3.24; N, 17.59; O, 23.08; S, 6,80.

4-(((5-(4-chlorophenyl)-2,4,7-trioxo-1,2,3,4,4,5-hexahydropyrido[2,3-d]pyrimidin-6(7H)-ylidene) methylamino)benzenesulfonamide 3: Yields %85. ^{1}H NMR (300 MHz, DMSO-D_6), 11,08 (NH, s), 10,24 (1H, d, N-H), 9.06 (H, d, C=C-H), 8,18 (2H, d, Ar-H), 7,96 (2H, s, NH_2), 7.84 (2H, d, Ar-H), 7.44 (2H, d, Ar-H), 7.06 (2H, d, Ar-H), 4.06 (H, s, C-H), 3.82 (H, s, C-H), ^{13}C NMR (75 MHz, DMSO-D_6), 171.53, 167.42, 158.01, 151.88, 148.55, 141.22, 140.12, 130.82, 130.74, 128.60, 127.86, 126.13, 124.32, 116.70, 45.79, 35.48. FT-IR (ν, cm-1), 796.60 (Ar-Cl), 1143.79 (O=S=O), 1286.52 (C-HN-C), 1361.74 (S-NH_2), 1624.06 (NH-C=O), 2920.07 (C=C, Aliphatic), 3078.39 (C=C, Aromatic), 3213.41 (NH), 3354.21 (NH), Elemental Analysis, $C_{20}H_{16}ClN_5O_5S$; Calculated, C,50.69; H,3.40; Cl,7.48; N,14.78; O,16.88; S,6.77. Found, C,50.52; H,3.44; Cl,7,76 N,14.96; O,16.56; S,6,76.

4-(((5-(4-bromophenyl)-2,4,7-trioxo-1,2,3,4,4,5-hexahydropyrido[2,3-d]pyrimidin-6(7H)-ylidene)methyl) amino) benzene sulfonamide 4: Yields: %80. ^{1}H NMR (300 MHz, DMSO-D_6), 12,08 (NH, s), 11,20 (NH, s), 10,64 (1H, d, N-H), 8,80 (H, d, C=C-H), 7.80 (2H, d, Ar-H), 7,66 (2H, s, NH_2), 7.54 (2H, d, Ar-H), 7.48 (2H, d, Ar-H), 7.16 (2H, d, Ar-H), 4.21 (H, s, C-H), 3.99 (H, s, C-H), ^{13}C NMR (75 MHz, DMSO-D_6), 172.63, 167.87, 158.42, 151.00, 148.75, 141.45, 140.12, 130.14, 130.01, 128.78, 127.06, 126.73, 124.32, 115.70, 45.87, 35.88. FT-IR (ν, cm^{-1}): 698.23 (Ar-Br), 1153.43 (O=S=O), 1286.52 (C-HN-C), 1338.60 (S-NH_2), 1593.20(NH-C=O), 1645.28 (=N-C=O), 2900.94 (C=C, Aliphatic), 3066.82 (C=C, Aromatic), 3207.62 (NH), 3361.93 (NH). Elemental Analysis, $C_{20}H_{16}BrN_5O_5S$; Calculated, C,46.34; H,3.11; Br, 15.42; N,13.51; O,15.43; S,6.19. Found, C,46.18; H,3.04; Br,15,66 N,14.58; O,15.68; S,4,86.

4-(((5-(4-cyanophenyl)-2,4,7-trioxo-1,2,3,4,4,5-hexahydropyrido[2,3-d]pyrimidin-6(7H)-ylidene

)methyl)amino)benzene sulfonamide 5: Yields % 81. ^{1}H NMR (300 MHz, DMSO-D_6), 10,57 (1H, d, N-H), 9.37 (H, d, C=C-H), 8.44 (2H, d, Ar-H), 8.21 (2H, d, Ar-H), 8,01 (2H, s, NH_2), 7.81 (2H, d, Ar-H), 7.41 (2H, d, Ar-H), 4.30 (H, s, C-H), 4.10 (H, s, C-H). ^{13}C NMR (75 MHz, DMSO-D_6), 172.99, 1680.70, 159.92, 151.80, 147.86, 145.88, 141.99, 140.78, 130.55, 130.41, 128.74, 127.86, 126.74, 116.41, 45.99, 36.02. FT-IR (ν, cm^{-1}): 1166.73 (O=S=O), 1248.52 (C-HN-C), 1338.88 (S-NH_2), 1599.20 (NH-C=O), 1636.28 (=N-C=O), 2988.94 (C=C, Aliphatic), 3074.03 (C=C, Aromatic), 3223.20 (NH), 3388.99 (NH). Elemental Analysis, $C_{20}H_{16}BrN_5O_5S$; Calculated, C,46.34; H,3.11; N,13.51; O,15.43; S,6.19. Found, C,46.18; H,3.04; N,14.58; O,15.68; S,4,86.

4-(((1,3-dimethyl-2,4,7-trioxo-5-phenyl-1,2,3,4,4,5-hexahydropyrido[2,3-d]pyrimidin-6(7H)-ylidene)methyl)amino)benzenesulfonamide 6: Yields % 81. ^{1}H NMR (300 MHz, DMSO-D_6), 10.62 (1H, d, N-H), 9.02 (H, d, C=C-H), 7,80 (2H, d, Ar-H), 7,81 (2H, s, NH_2), 7.60 (2H, d, Ar-H), 7.24 (2H, d, Ar-H), 7.01 (2H, d, Ar-H), 7.00(H, t, Ar-H), 3.98 (H, s, C-H), 3.79 (H, s, C-H), 3,40 (3H, s, CH_3), 3,36 (3H, s, CH_3), ^{13}C NMR (75 MHz, DMSO-D_6), 173.77, 167.70, 159.01, 150.99, 147.01 140.99, 140.58, 130.78, 130.11, 128.14, 127.75, 126.14,124.58, 115.41, 45.45, 36.99. 28.77, 26.78 FT-IR (ν, cm-1): 1159.22 (O=S=O), 1253.73 (C-HN-C), 1342.46 (S-NH_2), 1381.03, 1548.84 (CH_3-N-C=O), 1589.34 (CH_3-N-C=O), 1641.42 (=N-C=O), 2970.38 (C=C, Aliphatic), 3080.02 (C=C, Aromatic), 3215.34 (NH). Elemental Analysis, $C_{22}H_{21}N_5O_5S$; Calculated, C,56.52; H,4.53; N,14.98; O,17.11; S,6.86. Found, C,56.41; H,4.54; N,14.99; O,17.05; S,7,01.

4-(((1,3-dimethyl-5-(4-nitrophenyl)-2,4,7-trioxo-1,2,3,4,4a,5-hexahydropyrido[2,3-d]pyrimidin-6(7H)-ylidene) methyl) amino) benzene sulfonamide 7: Yields %80. ^{1}H NMR (300 MHz, DMSO-D_6), 10.24 (1H, d, N-H), 9.06 (H, d, C=C-H), 8.18 (2H, d, Ar-H), 8.09 (2H, d, Ar-H), 8.08 (2H, s, NH_2), 7.80 (2H, d, Ar-H), 7.44 (2H, d, Ar-H), 4.01 (H, s, C-H), 3.82 (H, s, C-H), 3,40 (3H, s, CH_3), 3,38 (3H, s, CH_3), ^{13}C NMR (75 MHz, DMSO-D_6), 174.12, 167.50, 159.99, 150.75, 147.25, 145.25, 140.98, 140.08, 130.75, 130.66, 128.72, 127.02, 126.63, 116.74, 46.52, 37.09.

28.57, 26.98. FT-IR (ν, cm^{-1}), 1161.15 (O=S=O), 1257.59 (NO_2), 1338.60 (S-NH_2), 1575.84 (CH_3-N-C=O), 1593.20 (CH_3-N-C=O), 1635.64 (=N-C=O), 2927.78 (C=C, Aliphatic), 3097.98 (C=C, Aromatic), 3313.71 (NH). Elemental Analysis, $C_{22}H_{20}N_6O_7S$; Calculated: C,51.56; H,3.93; N,16.40; O,21.85; S,6.26. Found:C,51,71; H,4,09; N,16.29; O,21.49; S, 6,42.

4-(((5-(4-chlorophenyl)-1,3-dimethyl-2,4,7-trioxo-1,2,3,4,4a,5-hexahydropyrido[2,3-d]pyrimidin-6(7H)-ylidene)methyl)amino) benzene sulfonamide 8: Yields: % 81. ^{1}H NMR (300 MHz, DMSO-D_6), 10.22 (1H, d, N-H), 9.01 (H, d, C=C-H), 8.18 (2H, d, Ar-H), 7.98 (2H, s, NH_2), 7.89 (2H, d, Ar-H), 7.44 (2H, d, Ar-H), 7.06 (2H, d, Ar-H), 4.01 (H, s, C-H), 3.64 (H, s, C-H), 3,60 (3H, s, CH_3), 3,48 (3H, s, CH_3), ^{13}C NMR (75 MHz, DMSO-D_6), 171.12, 166.70, 158.89, 150.99, 147.25, 140.88, 140.08, 130.66, 130.16, 128.71, 127.12, 127,02, 126.43, 116.04, 46.33, 37.99. 28.75, 26.97. FT-IR (ν, cm^{-1}): 754.17 (Ar-Cl), 1147.65 (O=S=O), 1288.45 (C-HN-C), 1348.24 (S-NH_2), 1521.84 (CH_3-N-C=O), 1595.13(CH_3-N-C=O), 1668.43 (=N-C=O), 2964.59 (C=C, Aliphatic), 3009.25 (C=C, Aromatic), 3253.91 (NH). Elemental Analysis, $C_{22}H_{20}ClN_5O_5S$; Calculated, C, 52.64; H,4.02; Cl,7.06; N,13.95; O,15.94; S,6.39. Found, C, 52,71; H, 3.99; Cl,7.50, N,13.99; O,15.72; S, 6,09.

4-(((5-(4-bromophenyl)-1,3-dimethyl-2,4,7-trioxo-1,2,3,4,4a,5-hexahydropyrido[2,3-d] pyrimidin-6(7H)-ylidene) methyl) amino) benzene sulfonamide 9: yields: %80. ^{1}H NMR (300 MHz, DMSO-D_6), 10.41 (1H, d, N-H), 9.21 (H, d, C=C-H), 8.38 (2H, d, Ar-H), 8.12 (2H, s, NH_2), 8.01 (2H, d, Ar-H), 7.68 (2H, d, Ar-H), 7.18 (2H, d, Ar-H), 4.01 (H, s, C-H), 3.98 (H, s, C-H), 3,60 (3H, s, CH_3), 3,52 (3H, s, CH_3), ^{13}C NMR (75 MHz, DMSO-D_6), 172.11, 167.25, 158.78, 150.88, 147.25, 140.78, 140.18, 130.55, 130.45, 128.24, 127.14, 127,04, 126.98, 116.74, 45.73, 36.98. 28.55, 26.72. FT-IR (ν, cm^{-1}): 705.95 (Ar-Br), 1153.43 (O=S=O), 1290.38 (C-HN-C), 1363.67 (S-NH_2), 1489.05 (CH_3-N-C=O), 1556.55 (CH_3-N-C=O), 1579.70 (=N-C=O), 2926.01 (C=C, Aliphatic), 3032.10 (C=C, Aromatic), Elemental Analysis, $C_{22}H_{20}BrN_5O_5S$; Calculated, C, 48.36; H, 3.69; Br, 14.62; N,12.82; O,

14.64; S, 5.87. Found, C, 48,22; H, 3.68; Br, 14.50, N, 12.95; O, 14.22; S, 6,43.

4-(((5-(4-cyanophenyl)-1,3-dimethyl-2,4,7-trioxo-1,2,3,4,4a,5-hexahydropyrido[2,3-d]pyrimidin-6(7H)-ylidene) methyl) amino) benzene sulfonamide 10: Yields: % 82. ^{1}H NMR (300 MHz, DMSO-D_6), 10.16 (1H, d, N-H), 9.01 (H, d, C=C-H), 8.18 (2H, d, Ar-H), 7,86 (2H, s, NH_2), 7.84 (2H, d, Ar-H), 7.64 (2H, d, Ar-H), 7.21 (2H, d, Ar-H), 4.11 (H, s, C-H), 3.98 (H, s, C-H), 3,52 (3H, s, CH_3), 3,42 (3H, s, CH_3), ^{13}C NMR (75 MHz, DMSO-D_6), 173.11, 166.25, 158.75, 150.98, 147.11, 145.99, 140.78, 140.11, 130.99, 130.44, 128.24, 127.78, 127,74, 126.00, 116.74, 45.66, 36.78. 28.65, 26.72. FT-IR (ν, cm^{-1}): 1141.86 (O=S=O), 1288.45 (C-HN-C), 1315.45 (S-NH_2), 1541.12 (CH_3-N-C=O), 1577.77 (CH_3-N-C=O), 1622.13 (=N-C=O), 2977.01 (C=C, Aliphatic), 3078.39(C=C, Aromatic), 3209.55 (NH). Elemental Analysis, $C_{23}H_{20}N_6O_5S$; Calculated, C, 56.09; H, 4.09; N,17.06; O, 16.24, S, 6.51. Found, C, 56.21; H, 4.18; N, 16.95; O, 16.20; S, 6.67.

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The Declaration of Conflict of Interest/ Common Interest

No conflict of interest or common interest has been declared by the authors.

Authors' Contribution

The authors contributed equally to the study.

The Declaration of Ethics Committee Approval

This study does not require ethics committee permission or any special permission

The Declaration of Research and Publicatıon Ethics

The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

REFERENCE

[1] M. Conrad and M. Guthzeit, "Ueber Barbitursäurederivate," *Berichte der Dtsch. Chem. Gesellschaft*, vol. 15, no. 2, pp. 2844–2850, Jul. 1882.

[2] J. T. Mason, J. W. Baker, and F. Pilcher, "Sodium amytal in surgical management," *Am. J. Surg.*, vol. 9, no. 1, pp. 9–15, Jul. 1930.

[3] N. Moussier, L. Bruche, F. Viani, and M. Zanda, "Fluorinated Barbituric Acid Derivatives: Synthesis and Bio-activity," *Curr. Org. Chem.*, vol. 7, no. 11, pp. 1071–1080, Jul. 2003.

[4] A. Barakat *et al.*, "New Diethyl Ammonium Salt of Thiobarbituric Acid Derivative: Synthesis, Molecular Structure Investigations and Docking Studies," *Molecules*, vol. 20, no. 11, pp. 20642–20658, Nov. 2015.

[5] H. R. Bourne, Y. Weinstein, K. L. Melmon, L. M. Lichtenstein, C. S. Henney, and G. M. Shearer, "Modulation of Inflammation and Immunity by Cyclic AMP," *Science (80-.).*, vol. 184, no. 4132, pp. 19–28, Apr. 1974.

[6] P. M. Epstein and R. Hachisu, "Cyclic nucleotide phosphodiesterase in normal and leukemic human lymphocytes and lymphoblasts.," *Adv. Cyclic Nucleotide Protein Phosphorylation Res.*, vol. 16, pp. 303–24, 1984.

[7] M. D. Leibowitz *et al.*, "A Novel Insulin Secretagogue Is a Phosphodiesterase Inhibitor," *Diabetes*, vol. 44, pp. 68–74, 1995.

[8] E. M. Grivsky, S. Lee, C. W. Sigel, D. S. Duch, and C. A. Nichol, "Synthesis and antitumor activity of 2,4-diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-d]pyrimidine," *J. Med. Chem.*, vol. 23, no. 3, pp. 327–329, Mar. 1980.

[9] Y. Hamamoto and N. Yamamoto, "Anti-Fas monoclonal antibody is cytocidal to human," *Proc. Nati. Acad. Sci.*, vol. 87, no. December, pp. 2–6, 1990.

[10] L. K. Wathen, "Method Of Preventing Or Treating Atherosclerosis Or Restenosis," US 2004/0067947 A1, 2004.

[11] W. B. Schwartz, "The Effect of Sulfanilamide on Salt and Water Excretion in Congestive Heart Failure," *N. Engl. J. Med.*, vol. 240, no. 5, pp. 173–177, Feb. 1949.

[12] C. C. L. Quianzon and I. E. Cheikh, "History of current non-insulin medications for diabetes mellitus," *J. Community Hosp. Intern. Med. Perspect.*, vol. 2, no. 3, pp. 19081, Jan. 2012.

[13] N. K. Terrett, A. S. Bell, D. Brown, and P. Ellis, "Sildenafil (VIAGRATM), a potent and selective inhibitor of type 5 cGMP phosphodiesterase with utility for the treatment of male erectile dysfunction," *Bioorg. Med. Chem. Lett.*, vol. 6, no. 15, pp. 1819–1824, Aug. 1996.

[14] C. R. Fischer, Jnos; Ganellin, *Analogue-based Drug Discovery*□. 2006.

[15] S. Dadiboyena and A. T. Hamme II, "Synthesis of Celecoxib and Structural Analogs- A Review," *Curr. Org. Chem.*, vol. 16, no. 11, pp. 1390–1407, May 2012.

[16] J. B. Baell, A. G. Holloway. "New Subst ructure Filters for Removal of Pan Assay Interference Compounds (PAINS) from Screening Libraries and for Their Exclu sion in Bioassays" *Journal Of Medıcınal Chemıstry*, vol. 53, no.7, pp. 2719-2740, Apr 8, 2010.

[17] S. Batra, Y. A. Sabnis, P. J. Rosenthal *et al*. "Structure-based approach to falcipain-2 inhibitors: Synthesis and biological eva luation of 1,6,7-trisubstituted dihydroi soquinolines and isoquinolines," *Bioorga*

nic&Medıcınal Chemıstry, vol.11, no.3, pp. 2293-2299, May 15, 2003.

[18] N. Berber, M. Arslan, C. Bilen *et al*. "Synthesis and evaluation of new phthala zine substituted beta-lactam derivatives as carbonic anhydrase inhibitors," *Russian Journal of Bioorganic Chemistry*, vol. 41, no. 4, pp. 414-420, Jul, 2015.

[19] M. Kalaycı, C. Türkeş, M. Arslan, Y. De mir, S. Beydemir "Novel benzoic acid de rivatives: Synthesis and biological evalua tion as multitarget acetylcholinesterase and carbonic anhydrase inhibitors," *Arch Pharm.*, e2000282, 2020. https://doi.org/10.1002/ardp.202000282

[20] B. Sever, C. Türkes, M. D. Altıntop, Y. Demir, S. Beydemir "Thiazolyl- pyrazo- line derivatives: *In vitro* and *in silico* eva luation as potential acetylcholinesterase and carbonic anhydrase inhibitors," *Inter national Journal of Biological Macromo lecules*, vol. 163, pp. 1970-1988, 2020.

[21] A. Topal, M. Atamanalp, E. Oruç, Y. De mir, S. Beydemir, A. Işık "*In vivo* changes in carbonic anhydrase activity and histo pathology of gill and liver tissues after acute exposure to chlorpyrifos in rainbow trout," *Archives of Industrial Hygiene and Toxicology*, vol. 65, no. 4, pp. 377-385, 2014.

[22] I. Gulcin, S. Beydemir "Phenolic Compo unds as Antioxidants: Carbonic Anhyd rase Isoenzymes Inhibitors," *Mini Re views in Medicinal Chemistry*, vol. 13, no. 3, pp. 408-430, 2013.

[23] M. Tugrak, H. I. Gul, Y. Demir, I. Gulcin "Synthesis of benzamide derivatives with thiourea-substituted benzenesulfonamides as carbonic anhydrase inhibitors," *Arch Pharm.*, e2000230, 2020. https://doi.org/10.1002/ardp.202000230

[24] T. Demirci, M. Arslan, Ç. Bilen, D. Demir, N. Gençer, and O. Arslan, "Synthesis and carbonic anhydrase inhibitory properties of 1,3-dicarbonyl derivatives of methylaminobenzene-sulfonamide," *J. Enzyme Inhib. Med. Chem.*, vol. 29, no. 1, pp. 132–136, 2014.

[25] J. A. Verpoorte, S. Mehta, and J. T. Edsall, "Esterase activities of human carbonic anhydrases B and C.," *J. Biol. Chem.*, vol. 242, no. 18, pp. 4221–9, Sep. 1967.

[26] H. Lineweaver and D. Burk, "The Determination of Enzyme Dissociation Constants," *J. Am. Chem. Soc.*, vol. 56, no. 3, pp. 658–666, Mar. 1934.

[27] C. Yung-Chi and W. H. Prusoff, "Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction," *Biochem. Pharmacol.*, vol. 22, no. 23, pp. 3099–3108, Dec. 1973.

[28] C. T. Supuran, A. S. A. Altamimi, and F. Carta, "Carbonic anhydrase inhibition and the management of glaucoma: a literature and patent review 2013-2019," *Expert Opin. Ther. Pat.*, vol. 29, no. 10, pp. 781–792, Oct. 2019.

[29] E. Masini, S. Sgambellone, and L. Lucarini, "Carbonic anhydrase inhibitors as ophthalmologic drugs for the treatment of glaucoma," in *Carbonic Anhydrases*, Elsevier, 2019, pp. 269–285.

[30] S. Kalinin *et al.*, "Highly hydrophilic 1,3-oxazol-5-yl benzenesulfonamide inhibitors of carbonic anhydrase II for reduction of glaucoma-related intraocular pressure," *Bioorganic Med. Chem.*, vol. 27, no. 21, p. 115086, 2019.

[31] E. Berrino and F. Carta, "Carbonic anhydrase inhibitors for the treatment of epilepsy and obesity," in *Carbonic Anhydrases*, Elsevier, 2019, pp. 311–329.

[32] C. T. Supuran, "Carbonic anhydrase inhibitors as emerging agents for the treatment and imaging of hypoxic tumors,"

Expert Opin. Investig. Drugs, vol. 27, no. 12, pp. 963–970, Dec. 2018.

[33] C. T. Supuran, "Carbonic Anhydrase Inhibition and the Management of Hypoxic Tumors," *Metabolites*, vol. 7, no. 3, p. 48, Sep. 2017.

[34] Y. Zhou, R. B. Mokhtari, J. Pan, E. Cutz, and H. Yeger, "Carbonic Anhydrase II Mediates Malignant Behavior of Pulmonary Neuroendocrine Tumors," *Am. J. Respir. Cell Mol. Biol.*, vol. 52, no. 2, pp. 183–192, Feb. 2015.

[35] Z. Huyut, Ş. Beydemir, and İ. Gülçin, "Inhibition properties of some flavonoids on carbonic anhydrase I and II isoenzymes purified from human erythrocytes," *J. Biochem. Mol. Toxicol.*, vol. 31, no. 9, p. e21930, Sep. 2017.

[36] H. Göcer, A. Akıncıoğlu, S. Göksu, and İ. Gülçin, "Carbonic anhydrase inhibitory properties of phenolic sulfonamides derived from dopamine related compounds," *Arab. J. Chem.*, vol. 10, no. 3, pp. 398–402, 2017.

[37] T. Gokcen, M. Al, M. Topal, I. Gulcin, T. Ozturk, and A. C. Goren, "Synthesis of some natural sulphonamide derivatives as carbonic anhydrase inhibitors," *Org. Commun.*, vol. 10, no. 1, pp. 15–23, 2017.

[38] E. Garibov *et al.*, "Synthesis of 4,5-disubstituted-2-thioxo-1,2,3,4-tetrahydropyrimidines and investigation of their acetylcholinesterase, butyrylcholinesterase, carbonic anhydrase I/II inhibitory and antioxidant activities," *J. Enzyme Inhib. Med. Chem.*, vol. 31, pp. 1–9, 2016.