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Research Article

Synthesis and *In Vitro* Human Carbonic Anhydrase I and II Inhibition Properties of Some Novel Hydrazone Compounds Containing an Aryl Sulfonate Moiety

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Abstract

The aim of this research was to evaluate the enzyme inhibitory potential of some new hydrazone derivatives bearing an aryl sulfonate moiety against the human carbonic anhydrase isoenzymes I and II (hCA I and II), which were attained from commercial suppliers. In the current study, the structures of the target molecules (**5-8**) were confirmed by elemental analysis, FT-IR, ^{1}H NMR and ^{13}C NMR. The inhibition capacities of the compounds on hCA I and II activities were examined by employing the esterase activity method under *in vitro* conditions. The IC_{50} values of the tested molecules were determined in the range of 15.7-135.2 µM against hCA I and in the range of 13.5-76.3 µM against hCA II. Among them, compound **7** exhibited the highest activity against both hCA I and II. The inhibitory activities of all compounds tested were also compared to the standard drug Acetazolamide (AAZ). It was established that some of the tested molecules showed the inhibitory activities close to AAZ.

Keywords: Aryl sulfonate, hydrazone compound, enzyme inhibitory, human carbonic anhydrase

Bir Aril Sülfonat Parçası İçeren Bazı Yeni Hidrazon Bileşiklerinin Sentezi ve *İn Vitro* İnsan Karbonik Anhidraz I ve II İnhibisyon Özellikleri

Öz

Bu çalışmanın amacı, ticari tedarikçilerden elde edilen insan karbonik anhidraz izoenzimleri I ve II'ye (hCA I ve II) karşı bir aril sülfonat parçası taşıyan bazı yeni hidrazon türevlerinin enzim inhibisyon potansiyellerini araştırmaktır. Mevcut çalışmada, hedef moleküllerin yapıları (**5-8**) element analizi, FT-IR, ^{1}H NMR ve ^{13}C NMR ile doğrulanmıştır. Bileşiklerin hCA I ve II aktiviteleri üzerindeki inhibitör etkileri *in vitro* koşullarda esteraz aktivite yöntemi kullanılarak incelenmiştir. Test edilen moleküllerin IC_{50} değerleri, hCA I'e karşı 15.7-135.2 uM aralığında ve hCA II'ye karşı 13.5-76.3 uM aralığında belirlendi. Bunlar arasında, bileşik **7** hem hCA I hem de II'ye karşı en yüksek aktiviteyi göstermiştir. Test edilen tüm bileşiklerin inhibisyon aktiviteleri, standart ilaç Asetazolamid (AAZ) ile de karşılaştırıldı. Test edilen bileşiklerin bazılarının AAZ'a yakın inhibitör aktiviteler gösterdiği belirlendi.

Anahtar Kelimeler: Aril sülfonat, hidrazon bileşiği, enzim inhibitörü, insan karbonik anhidraz

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1. Introduction

Carbonic anhydrases (CAs, E.C.4.2.1.1), first discovered in bovine erythrocytes, are metalloenzymes containing Zn^{2+} ion, which are common in all living organisms [1-3]. These enzymes reversibly catalyze the hydration of carbon dioxide and the dehydration of bicarbonate in living things [4-6]. CAs play an important role in many physiological and pathological processes such as biosynthetic reactions such as gluconeogenesis, lipogenesis and ureogenesis, respiration and transport of CO_2/bicarbonate, electrolyte secretion in various tissues/organs, bone resorption, calcification and carcinogenicity [7,8].

These enzymes are responsible for both accumulation of H^+ and HCO^{3-} in many tissues such as kidney, gastric mucosa and eye lens, while providing metabolic CO_2 transport [9,10]. In the studies conducted in the following years, these enzymes were obtained from human erythrocytes, rat erythrocytes, fish erythrocytes, bovine bone, rat saliva, bovine leukocytes, salivary glands by histochemical methods, brain, muscles, pancreas, nerve myelin sheath, prostate, various bacteria and many herbal sources, and then they were purified and characterized [11-13].

Since CA isoenzymes have been noticed to be effective on many diseases, these enzymes have been seen as potential drugs and research has focused on this subject [14] CA isoenzymes are one of the proteins that have antiglaucoma, antiepileptic, antiobesity, antidiuretic, anti-inflammatory, antitumor and analgesic effects and can be used clinically as therapeutic agents thanks to these effects [15,16]. On the other hand, carbonic anhydrase inhibitors (CAIs) have been employed clinically for a long time as diuretics, antiglaucoma and antiepileptic agents [17,18].

So far, sixteen CA isoenzymes have been identified in mammals. Of these enzymes, carbonic anhydrase I (CA I) and II (CA II) are two major isoenzymes of CA found in high concentrations in erythrocytes and cytosol. CA II is the most active of all CAs [2,19].

The main class of CAIs are sulfonamides, which have long been used clinically as diuretics, antiglaucoma and antiepileptic drugs. Sulfonamides are the earliest discovered organic CAIs. Aromatic or heterocyclic compounds bearing a primary sulfonamide group have been widely used to synthesize new CAIs. Examples of sulfonamide derivatives, which are CAIs, used in the clinic are AAZ, methazolamide, and ethoxazolamide [15, 20-22].

In this research, some new hydrazone derivatives (**5-8**) were synthesized, characterized and assessed for their inhibitory activities against hCA I and II. The structures of targeted compounds were characterized by elemental analysis and some spectroscopic techniques (^{1}H NMR, ^{13}C NMR and FT-IR). In the inhibitory assays, the inhibitory potentials of all molecules tested on hCA I and II were investigated *in vitro* conditions. AAZ as a carbonic anhydrase inhibitor was employed.

2. Material and Method

2.1. Chemicals and instrumentations

All chemicals employed in the current study were procured from commercial suppliers (Sigma Aldrich and Merck Chemical companies). Melting points were determined on a Barnstead IA9100 Electrothermal Digital Melting Points Apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE III 500 MHz spectrometer. FT-IR spectra were taken on a Perkin-Elmer Spectrum 100 FT-IR spectrophotometer. Elemental analysis was performed by a Thermo Scientific Flash 2000 elemental analyzer.

2.2. The preparation of the intermediates

All aryl sulfonate compounds were prepared by the same procedure given below [23-25]. Briefly, a solution of the appropriate phenolic aldehyde (2.3 mmol) in dichloromethane (DCM) (10 mL) was gently stirred for 15 min at room temperature. To this solution, triethylamine (TEA) (4.6 mmol) was carefully added and further stirred for 15 min. Next, tosyl chloride (TsCl) (2.3 mmol) was slowly added to reaction the mixture; and then it was refluxed for about 4 h. After the completion of the reaction, the reaction mixture was cooled; and then extracted two times with 2 M HCI. The obtained organic layer was dried over anhydrous sodium sulfate, and filtered. Then, the solvent was allowed to evaporate at room temperature all night long. Finally, the residue was recrystallized with ethanol to yield an intermediate; and then it was employed for the synthesis of the target molecule

(1) 2-Formylphenyl 4-methylbenzenesulfonate [26]

Off white solid (81%), m.p. 64-65 °C, lit.: m.p. 63-64°C. FT-IR/ATR (cm^{-1}) υ_{max}: 3078, 3041 (aromatic C-H str.), 2926, 2886 (aliphatic C-H str.), 1689 (C=O), 1373 (antisymmetric SO_2 str.), 1175 (symmetric SO_2 str.).

(2) 3-Formylphenyl 4-methylbenzenesulfonate [27]

White solid (80%), m.p. 67-68 °C, lit.: m.p. 64-66°C. FT-IR/ATR (cm^{-1}) υ_{max}: 3092, 3062 (aromatic C-H str.), 2924, 2844 (aliphatic C-H str.), 1694 (C=O), 1365 (antisymmetric SO_2 str.), 1179 (symmetric SO_2 str.).

(3) 4-Formylphenyl 4-methylbenzenesulfonate [28]

Off white solid (83%), m.p. 75-76 °C, lit.: m.p. 72-73°C. FT-IR/ATR (cm^{-1}) υ_{max}: 3102, 3065 (aromatic C-H str.), 2924, 2823 (aliphatic C-H str.), 1704 (C=O), 1358 (antisymmetric SO_2 str.), 1146 (symmetric SO_2 str.).

(4) 1-Formylnaphthalen-2-yl 4-methylbenzenesulfonate [24, 29]

Off white solid (92%), m.p. 137-139 °C, lit.: m.p. 136-138 °C. FT-IR/ATR (cm^{-1}) υ_{max}: 3098, 3061 (aromatic C-H str.), 2976, 2878 (aliphatic C-H str.), 1687 (C=O), 1371 (antisymmetric SO_2 str.), 1171 (symmetric SO_2 str.).

2.3. The synthesis of target molecules

All hydrazone derivatives were successfully synthesized by the same procedure given below [30,31]. To a stirred solution of 4-hydroxybenzhydrazide (4-HBH) (3 mmol) in 10 mL of ethanol, the corresponding aryl sulfonate (3 mmol) in 10 mL of ethanol was slowly added. Then, the reaction mixture was heated under reflux for 4 h. Upon completion, the solution was cooled to room temperature. After this process, the crude product was carefully removed by filtration, washed with diethyl ether; and then recrystallized with ethanol to afford hydrazone derivative.

(5) 2-((2-(4-Hydroxybenzoyl)hydrazono)methyl)phenyl 4-methylbenzenesulfonate

White solid (79%), m.p. 219-210 °C. FT-IR/ATR (cm^{-1}) υ_{max}: 3372 (O-H str.) 3232 (N-H str.), 3152, 3086 (aromatic C-H str.), 2970, 2876 (aliphatic C-H str.), 1612 (C=O), 1564 (C=N str.), 1368 (antisymmetric SO_2 str.), 1181 (symmetric SO_2 str.). ^{1}H NMR (DMSO-d_6): δ 11.64 (s, 1H, NHCO), 10.15 (s, 1H, OH), 8.39 (s, 1H, CH=N), 7.85 (s, 1H, ArH), 7.80 (d, *J* = 7.9 Hz, 2H, ArH), 7.70 (d, *J* = 8.2 Hz, 2H, ArH), 7.41 – 7.35 (m, 4H, ArH), 7.08 (d, *J* = 7.9 Hz, 1H), 6.88 (s, 2H, ArH), 2.30 (s, 3H, -CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ 163.08, 161.28, 147.81, 146.72, 140.39, 131.40, 131.07, 130.62, 130.18, 128.90, 128.50, 128.24, 126.79, 124.06, 123.30, 115.52, 21.57 ppm. Anal. Calcd. for $C_{21}H_{18}N_2O_5S$: C, 61.45; H, 4.42; N, 6.83; S, 7.81%. Found: C, 61.52; H, 4.47; N, 6.74; S, 7.74%.

(6) 3-((2-(4-Hydroxybenzoyl)hydrazono)methyl)phenyl 4-methylbenzenesulfonate

White solid (81%), m.p. 204-205 °C. FT-IR/ATR (cm^{-1}) υ_{max}: 3358 (O-H str.) 3250 (N-H str.), 3072, 3037 (aromatic C-H str.), 2980, 2910 (aliphatic C-H str.), 1665 (C=O), 1578 (C=N str.), 1351 (antisymmetric SO_2 str.), 1178 (symmetric SO_2 str.). ^{1}H NMR (DMSO-d_6): δ 11.72 (s, 1H, NHCO), 10.14 (s, 1H, OH), 8.35 (s, 1H, CH=N), 7.77 (dd, *J* = 15.4, 8.2 Hz, 4H, ArH), 7.61 (d, *J* = 6.9 Hz, 1H, ArH), 7.48 – 7.38 (m, 4H, ArH), 6.99 (s, 1H, ArH), 6.85 (d, *J* = 8.5 Hz, 2H, ArH), 2.39 (s, 3H, -CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ 163.27, 161.25, 149.84, 146.40, 145.58, 137.13, 131.73, 130.97, 130.72, 130.20, 128.69, 126.61, 124.11, 123.53, 120.14, 115.49, 21.62 ppm. Anal. Calcd. for $C_{21}H_{18}N_2O_5S$: C, 61.45; H, 4.42; N, 6.83; S, 7.81%. Found: C, 61.48; H, 4.46; N, 6.87; S, 7.88%.

(7) 4-((2-(4-Hydroxybenzoyl)hydrazono)methyl)phenyl 4-methylbenzenesulfonate

White solid (82%), m.p. 236-237 °C. FT-IR/ATR (cm^{-1}) υ_{max}: 3528 (O-H str.) 3299 (N-H str.), 3064, 2976 (aromatic C-H str.), 2985, 2814 (aliphatic C-H str.), 1634 (C=O), 1587 (C=N str.), 1349 (antisymmetric SO_2 str.), 1176 (symmetric SO_2 str.). ^{1}H NMR (DMSO-d_6): δ 11.68 (s, 1H, NHCO), 10.12 (s, 1H, OH), 8.37 (s, 1H, CH=N), 7.78 (d, *J* = 8.5 Hz, 2H, ArH), 7.73 (d, *J* = 8.2 Hz, 2H, ArH), 7.68 (d, *J* = 7.9 Hz, 2H, ArH), 7.45 (d, *J* = 8.0 Hz, 2H, ArH), 7.08 (d, *J* = 8.5 Hz, 2H, ArH), 6.84 (d, *J* = 8.5 Hz, 2H, ArH), 2.40 (s, 3H, -CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ 163.20, 161.20, 150.19, 146.40, 145.70, 134.16, 131.68, 130.71, 130.16, 128.88, 128.71, 124.16, 123.01, 115.47, 21.62 ppm. Anal. Calcd. for $C_{21}H_{18}N_2O_5S$: C, 61.45; H, 4.42; N, 6.83; S, 7.81%. Found: C, 61.41; H, 4.38; N, 6.89; S, 7.84%.

(8) 1-((2-(4-Hydroxybenzoyl)hydrazono)methyl)naphthalen-2-yl 4-methylbenzenesulfonate

White solid (77%), m.p. 245-246 °C. FT-IR/ATR (cm^{-1}) υ_{max}: 3346 (O-H str.) 3152 (N-H str.), 3062, 3014 (aromatic C-H str.), 2954, 2872 (aliphatic C-H str.), 1657 (C=O), 1593 (C=N str.), 1366 (antisymmetric SO_2 str.), 1167 (symmetric SO_2 str.). ^{1}H NMR (DMSO-d_6): δ 11.66 (s, 1H, NHCO), 10.19 (s, 1H, OH), 9.22 (d, *J* = 7.2 Hz, 1H, ArH), 8.67 (s, 1H, CH=N), 8.03 (d, *J* = 9.0 Hz, 1H, ArH), 7.97 (d, *J* = 8.3 Hz, 1H, ArH), 7.87 (d, *J* = 7.5 Hz, 2H, ArH), 7.72 (d, *J* = 8.2 Hz, 2H, ArH), 7.63 – 7.56 (m, 2H, ArH), 7.34 (t, *J* = 8.1 Hz, 3H, ArH), 6.92 (d, *J* = 7.9 Hz, 2H, ArH), 2.23 (s, 3H, -CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ 163.05, 161.34, 147.39, 146.69, 142.05, 132.56, 132.26, 131.05, 130.58, 130.49, 130.25, 129.02, 128.91, 128.75, 127.34, 127.22, 124.06, 122.75, 121.58, 115.60, 21.57 ppm. Anal. Calcd. for $C_{25}H_{20}N_2O_5S$: C, 65.21; H, 4.38; N, 6.08; S, 6.96%. Found: C, 65.27; H, 4.41; N, 6.71; S, 6.92%.

2.4 *In vitro* Inhibition studies of hCA I and II

In this study, hCA I (C4396) and hCA II (C6165) were procured from Sigma-Aldrich company. The inhibitory activities of all molecules synthesized and AAZ on hCA I and II were determined according to the method described by Verpoorte et al. [7,20,32]. In inhibitory studies, *p*-nitrophenyl acetate was employed as the substrate. On the other hand, AAZ was used as a reference drug. IC_{50} values were calculated for the molecules at different concentrations (5-150 μM).

3. Results and Discussion

3.1 Synthesis and characterization

The new hydrazone derivatives (**5-8**) were obtained by two steps synthetic pathway as outlined in Figure 1. At first, the *O*-sulfonylation of the phenolic aldehydes with TsCl to obtain aryl sulfonate compounds (**1-4**) was performed under reflux conditions for 4 h in DCM medium in the presence of TEA. In the final step, the aryl sulfonate compounds were facilely condensed with 4-HBH in an ethanol medium for 4 h under reflux conditions to obtain hydrazone derivatives. It was determined that all of these aryl sulfonates obtained in this study were synthesized in some studies before [26-29]. These compounds, which were resynthesized and re-characterized in his study [24] by a member of our research group, were employed again to obtain the target compounds in this study. In the aforementioned study, the antioxidant activities of these molecules were determined. On the other hand, it is determined that hydrazone derivatives as the target molecules were obtained for the first time. The structures of the targeted molecules (**5-8**) were characterized by elemental analysis and three spectroscopic techniques.

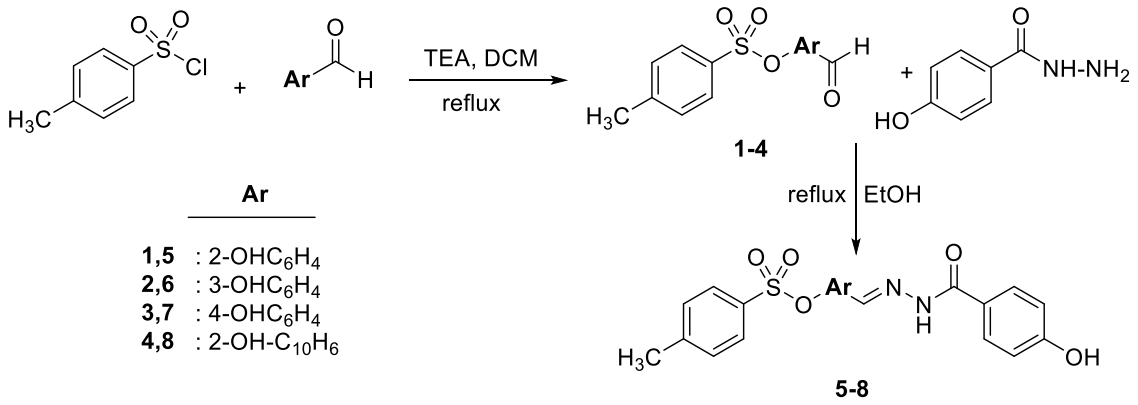

Figure 1. Synthetic route for the preparation of the target molecules

In FT-IR spectra of targeted compounds (**5-8**) showed O-H bands 3528 to 3346 cm^{-1} hydrazone N-H bands in 3299 to 3152 cm^{-1}, C=O bands in 1612 to 1665 cm^{-1} and C=N bands in 1564 to 1593 cm^{-1} region. Asymmetric and symmetric SO_2 bands were seen 1349-1366 cm^{-1} and 1167-1181 cm^{-1}, respectively. In conclusion, the spectral data of all synthesized molecules were in full agreement with the proposed structures. ^1H NMR spectra of hydrazones (**5-8**) showed three signals in the δ 11.64-11.72, δ 10.12-10.15 and δ 8.35-8.67 ppm, which were attributed to the CONH, OH and CH=N proton, respectively. -CH_3 protons in the compounds (**5-8**) were in the range of δ 2.23-2.40 ppm. Also, it was determined that the protons of the aromatic rings resonated between δ 6.84 and 9.22 ppm. In ^{13}C NMR spectra, the resonances of the conjugated C=O and C=N carbon, and -CH_3 carbon atoms were detected at δ 163.05–163.27, δ 147.81–150.19, and δ 21.57-21.62 ppm, respectively. The spectroscopic data agreed with the data presented in the literature [30,31].

3.2 Carbonic anhydrase inhibitory activities

CAIs are employed in the therapy of some disorders including cancer, glaucoma and obesity. Thus, identifying novel and efficacious structures for the treatment of these diseases is a significant approach [33]. The various classes of compounds found in organic chemistry are employed to discover new CAIs. One of these compound classes is hydrazone compounds. Recently, these compounds have received great attention due to their wide spectrum of biological activities including antimicrobial, anticancer and anti-carbonic anhydrase inhibitory activities [30, 31, 34-40]. In this research, it is determined the inhibitory activities of new hydrazone derivatives against hCA I and II. The inhibitory results of all screened molecules and AAZ are presented in Table 1.

Table 1. The inhibition results of eight molecules against hCA I and II

	IC_{50} (μM)[a]	
Inhibitors	**hCA I**	**hCA II**
1	128.6 ± 1.1	73.1 ± 0.7
2	112.4 ± 1.3	68.6 ± 0.7
3	118.7 ± 1.1	70.4 ± 0.8
4	135.2 ± 1.4	76.3 ± 0.9
5	38.1 ± 0.4	24.7 ± 0.3
6	37.6 ± 0.4	23.9 ± 0.3
7	15.7 ± 0.2	13.5 ± 0.2
8	26.4 ± 0.3	18.3 ± 0.2
AAZ[b]	6.07 ± 0.150[c]	4.37 ± 0.15[c]

[a]Mean from 3 different assays
[c]Standard hCA inhibitor for hCA I and II
[c]From Reference [22]

(**i**) In hCA I assay, the tested molecules (**1**-**8**) indicated the inhibitory activities with IC_{50} values ranging from 15.7 ± 0.2 to 135.2 ± 1.4 μM in comparison with the reference drug AAZ showing an IC_{50} value of 6.07 ± 0.150 μM (Table 1). When the results are examined, it was determined that some of the tested molecules exhibited inhibitory activities close to AAZ. Compound **7** (IC_{50} = 15.7 ± 0.2 μM), the 3-hydroxybenzaldehyde-based hydrazone derivative, displayed the highest activity compared to other molecules. On the other hand, it was determined that hydrazone derivatives were more active than aryl sulfonates against this enzyme.

(**ii**) In hCA II assay, it was determined that the tested molecules showed inhibitory activities at different concentrations (13.5 and 76.3 μM) against this enzyme (Table 1). When the inhibition activities of the tested molecules were compared with AAZ, it was determined that some of these molecules showed the inhibitory activities close to AAZ (IC_{50} = 4.37 ± 0.15 μM). Amongst the tested molecules, compound **7** (IC_{50} = 13.05 ± 0.2 μM) showed the closest activity to AAZ. On the other hand, it was determined that aryl sulfonate compounds showed to lower the inhibitory activities than hydrazone derivatives.

4. Conclusion

This research focused on the discovery of some novel hydrazone compounds derived from 4-HBH as potential inhibitors of hCA I and II. In conclusion, four new hydrazone derivatives (**5**-**8**) bearing an aryl sulfonate moiety in this research were successfully obtained for the first time, spectroscopically characterized; and then evaluated for their inhibitory capacities against both enzymes. The activities of these isozymes were determined by employing the esterase activities. IC_{50} values for hCA I and II were calculated as 15.7-135.2 μM and 13.5-76.3 μM, respectively. Amongst the tested molecules, compound **7** for hCA I and II has been determined to be the most efficacious inhibitor. According to the results of the research, it was determined that hydrazone compounds have higher inhibition potential for both enzymes compared to aryl

sulfonate compounds. It is believed that these results may contribute to the development and synthesis of novel hCA inhibitors.

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Author Contributions

R. Çakmak: Design of the study, the synthesis and characterization of the target compounds, evaluation of the study results, writing of the manuscript.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

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