Inhibitory effects of the chalcones towards carbonic anhydrase I, II and acetylcholinesterase enzymes

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Abstract
Chalcones are known as versatile, innovative and bioactive chemical scaffolds in drug development studies. In this study, a series of poly-methoxylated chalcones (1-8) were synthesized by Claisen-Schmidt condensation under the basic condition and their carbonic anhydrase (CA) I/II and acetylcholinesterase (AChE) inhibitory effects were firstly evaluated in this study. CA isoenzymes I and II were inhibited in nanomolar concentration with Ki values of 8.75±0.64 - 37.64±2.38 nM (hCA I) and 11.47±3.31 - 45.97±4.67 nM (hCA II). The compounds inhibited the AChE enzyme in the range of 34.14±20.79 - 53.65±13.25 nM. The compounds 1, 3 and 5 were the best inhibitors against hCA I, hCA II, and AChE, respectively. The bioassay results showed that the compounds can be considered as the main frame to design novel chalcone-based enzyme inhibitors.

Keywords: Chalcone, carbonic anhydrases, acetylcholinesterase, enzyme inhibitor

Şalkonların karbonik anhidraz I, II ve asetilkolinesteraz enzimlerine karşı inhibisyon etkileri

Öz
Şalkonlar ilaç geliştirme çalışmalarında kullanışlı, yenilikçi ve bioaktif kimyasal yapı iskeletleri olarak bilinirler. Bu çalışmada, bir dizi poli-metoksillenmiş şalkon (1-8) bazı koşul altında Claisen-Schmidt kondansasyonu ile sentezlendi ve karbonik anhidraz (CA) I / II ve asetilkolinesteraz (AChE) inhibitör etkileri ilk olarak bu çalışmada değerlendirildi. CA izoenzimleri olan I ve II, 8.75±0.64 - 37.64±2.38 nM (hCA I) ve 11.47±3.31 - 45.97±4.67 nM (hCA II) Ki değerleri ile nanomolar konsantrasyonda inhibe edildi. Bileşikler, AChE enzimini 34.14±20.79 - 53.65±13.25 nM aralığında inhibe etti. Bileşik 1, 3 ve 5, sırasıyla hCA I, hCA II ve AChEye karşı en güçlü inhibitörlerdir. Biyolojik aktivite sonuçları, bileşiklerin yeni şalkon bazlı enzim inhibitörleri tasarlanarak için ana yapı olarak kabul edilebildiğini gösterdi.

Anahtar Kelimeler: Şalkon, karbonik anhidraz, asetilkolin esteraz, enzim inhibitörü
1. Introduction

The chalcone consists of two aromatic rings that are linked by α, β-unsaturated carbonyl system. Their easy synthesis and simple chemistry have increased chalcone's popularity in medicinal chemistry. Synthesis of chalcones was achieved with different kinds of reactions such as Claisen-Schmidt condensation, grinding method, microwave irradiation condition, ultrasound irradiation technique, Heck coupling, Suzuki–Miyaura coupling, and Witting reactions. They are also an innovative class of compounds with therapeutic potential against many diseases (Gomes et al., 2017; Rammohan et al., 2020). Due to their flexible structures, chalcones can effectively bind to different kinds of enzymes or receptors to show their biological effects (Zhang et al., 2018).

The carbonic anhydrases (CAs, EC 4.2.1.1) are widespread metalloenzymes. CAs are required to catalyze the hydration of carbon dioxide (CO$_2$) to bicarbonate (HCO$_3^-$) and protons (H$^+$) for balancing extra- and intra-cellular pH (Supuran, 2016; Supuran, 2017; Supuran, 2018). Inhibition and activation of the CAs seem to be essential for the treatment of many diseases in which the activity of CA isoforms is upregulated. (Supuran et al., 2004; Temperini et al., 2008). Thus, it has become a significant drug target for potential drug discovery. CA isoenzymes are considered drug targets for cancer, glaucoma, obesity, and pain. (Supuran, 2016; Supuran, 2008). Until now, the carbonic anhydrase I and II inhibitors are designed based on sulfonamides and its bioisosteres. But, there is no many current reports regarding chalcones and CA inhibitory effects. Recently, several chalcone type compounds were reported as potent CA inhibitors (Burmaoglu et al., 2019; Mahar et al., 2019). Thus we planned to seek the potential of chalcones against to the most studied carbonic anhydrase I and II enzymes with the expectation to find out new drug candidate molecules.

Many elderly patients undergo Alzheimer's disease (AD), which is the most accepted origin of dementia. AD is a permanent and continuous illness characterized by cognitive deterioration and memory failure (Li et al., 2017). Anti-AD drugs can impermanently develop cognitive and daily actions for patients. FDA approved different drugs for the clinical medication of AD including donepezil, rivastigmine, and galantamine as AChE (AChE, E.C.3.1.1.7) inhibitors (Li et al., 2017). The cholinergic hypothesis was considered as the first theory for AD. According to theory, improvement of the cholinergic function is related to enhancing the amount of the acetylcholine (ACh) by inhibition of AChE enzyme (Alpan et al., 2017; Zengin et al., 2018) using AChE inhibitors. Several chalcone derivatives were reported as successful drug candidates for the AD by targeting AChE and other targets (Zhang et al., 2018).

The biological importance of chalcones inspired our research group to synthesis several poly-methoxylated chalcones and to investigate their cholinesterase inhibitory effects against AChE which is the most popular target for the treatment of Alzheimer’s disease and carbonic anhydrase inhibitory potency against the most studied hCA I/II isoenzymes.

2. Materials and Methods

2.1. Chemistry

Melting points were determined using an Electrothermal 9100/IA9100 (Bibby
2.1.1. A general synthesis method of chalcones 1-8 (Figure)

Chalcones 1-8 were synthesized according to our previous studies (Gul et al., 2018a; Gul et al., 2018b; Yamali et al., 2017; Yamali et al., 2016). Briefly, suitable keton and aldehyde in 1:1 ratio were stirred in ethanol (50 ml) for 15 minutes on ice bath. Then, sodium hydroxide solution (30%, 50 ml) were added and the mixture stirred at room temperature for 24 h. The reaction process were followed by TLC (CHCl₃:MeOH;4.8:0.2). The mixture was poured into ice-water mixture and then neutralized with hydrochloric acid (37%). The crude was filtered, dried and crystallized using suitable solvent or solvent mixtures as ethanol (for 1-3, 5), ethanol:water (6-8) and ethanol:aceton (4). As the compounds were registered, the chemical structure and purity of the chalcones 1-8 were only confirmed by melting point, ¹H and ¹³C NMR spectra.

(E)-1-(4-Fluorophenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one, 1

Cream solid. Yield: 65 %, mp 113-115 °C (from ethanol). ¹H NMR (400 MHz, CDCl₃) δ 8.01 (dd, J = 8.7, 5.4 Hz, 2H), 7.70 (d, J = 15.6 Hz, 1H), 7.36 (d, J = 15.6 Hz, 1H), 7.13 (t, J = 8.7 Hz, 2H), 6.83 (s, 2H), 3.88 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 188.7, 165.5 (d, ¹JC = 252 Hz), 153.5, 145.2, 140.5, 134.5 (d, ¹JC = 3 Hz), 131.0 (d, ¹JC = 9 Hz), 130.2, 120.8, 115.7 (d, ²JC = 22 Hz), 105.7, 60.9, 56.2 (2C).

(E)-1-(4-Chlorophenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one, 2

Light yellow solid. Yield: 72 %, mp 108-110 °C (from ethanol). ¹H NMR (400 MHz, CDCl₃) δ 7.92 (d, J = 8.5 Hz, 2H), 7.68 (d, J = 15.6 Hz, 1H), 7.43 (d, J = 8.5 Hz, 2H), 7.34 (d, J = 15.6 Hz, 1H), 6.85 (s, 2H), 6.83 (so, 6H), 3.88 (so, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 189.1, 153.5, 145.5, 140.6, 139.1, 136.5, 130.1, 129.9, 128.9, 120.8, 105.7, 60.9, 56.2 (2C).

(E)-1-(4-Bromophenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one, 3

Light yellow solid. Yield: 77 %, mp 126-128 °C (from ethanol). ¹H NMR (400 MHz, CDCl₃) δ 7.86 (d, J = 8.5 Hz, 2H), 7.70 (d, J = 15.6 Hz, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.33 (d, J = 15.6 Hz, 1H), 6.85 (s, 2H), 3.91 (s, 6H), 3.89 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 189.4, 153.5, 145.6, 140.6, 136.9, 131.9, 130.1, 130.0, 127.8, 120.8, 105.7, 61.0, 56.2 (2C).

(E)-1-(4-Hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one, 4

Yellow solid. Yield: 50 %, mp 244-246 °C (from ethanol:aceton). ¹H NMR (400 MHz, DMSO-d₆) δ 10.5 (bs, 1H), 8.08 (d, J = 8.7 Hz, 2H), 7.86 (d, J = 15.5 Hz, 1H), 7.63 (d, J = 15.5 Hz, 1H), 7.19 (s, 2H), 6.91 (d, J = 8.7 Hz, 2H), 3.85 (s, 6H), 3.70 (s, 3H); ¹³C NMR (100 MHz, DMSO-d₆) δ 187.5, 162.8, 153.6, 143.6, 139.9, 131.9, 131.7, 130.9, 129.6, 121.8, 115.9, 106.8, 60.6, 56.6 (2C).

(E)-1-(Thiophen-2-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one, 5

Scientific Limited, Staffordshire, UK) instrument and are uncorrected. ¹H and ¹³C NMR spectra of the compounds in CDCl₃ or DMSO-d₆ was recorded by Bruker AVANCE III 400 MHz (Bruker, Karlsruhe, Germany) spectrometer. Chemical shifts (δ) are reported in ppm and coupling constants (J) are expressed in hertz (Hz). The reactions were monitored using silica gel HF254-366 Thin Layer Chromatography (TLC) plates (E. Merck, Germany).
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Yellow solid. Yield: 73 %, mp 151-153 °C (from ethanol). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.85 (dd, \(J = 3.8, 0.9\) Hz, 1H), 7.72 (d, \(J = 15.5\) Hz, 1H), 7.64 (dd, \(J = 4.9, 0.9\) Hz, 1H), 7.29 (d, \(J = 15.6\) Hz, 1H), 7.13 (dd, \(J = 4.9, 3.8\) Hz, 1H), 6.82 (s, 2H), 3.88 (s, 6H), 3.86 (s, 3H); \(^1^3\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 181.9, 153.4, 145.6, 144.2, 140.2, 133.9, 131.9, 130.2, 128.3, 120.8, 105.7, 60.9, 56.2 (2C).

\((E)-1)-(4-Methoxyphenyl)-3-(2,3,4-trimethoxyphenyl)prop-2-en-1-one, 6

Light yellow solid. Yield: 70 %, mp 100-102 °C (from ethanol:water) \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.02 (d, \(J=8.9\) Hz, 2H), 7.97 (d, \(J = 15.8\) Hz, 1H), 7.56 (d, \(J = 15.8\) Hz, 1H), 7.37 (d, \(J = 8.8\) Hz, 1H), 6.95 (d, \(J = 8.9\) Hz, 2H), 6.69 (d, \(J = 8.8\) Hz, 1H), 3.93 (s, 3H), 3.88 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H); \(^1^3\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 189.1, 163.2, 155.6, 153.7, 142.5, 139.2, 131.4, 130.7, 123.8, 122.2, 121.1, 113.8, 107.6, 61.4, 60.9, 56.1, 55.5.

\((E)-1)-(4-Methoxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one, 7

Yellowish solid. Yield: 74 %, mp 119-121°C (from ethanol:water). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.06 (d, \(J = 15.7\) Hz, 1H), 8.00 (d, \(J = 8.9\) Hz, 2H), 7.46 (d, \(J = 15.7\) Hz, 1H), 7.09 (s, 1H), 6.93 (d, \(J = 8.9\) Hz, 2H), 6.48 (s, 1H), 3.90 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H); \(^1^3\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 189.2, 163.1, 154.5, 152.3, 143.2, 139.2, 131.6, 130.7, 119.9, 115.6, 113.7, 111.3, 96.8, 56.5, 56.3, 56.0, 55.4.

\((E)-1)-(4-Methoxyphenyl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-1-one, 8

Light green solid solid. Yield: 76 %, mp 154-156 °C (from ethanol:water). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.24 (d, \(J = 15.8\) Hz, 1H), 8.01 (d, \(J = 8.9\) Hz, 2H), 7.88 (d, \(J = 15.8\) Hz, 1H), 6.93 (d, \(J = 8.9\) Hz, 2H), 6.09 (s, 2H), 3.87 (s, 6H), 3.84 (s, 3H), 3.81 (s, 3H); \(^1^3\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 190.3, 162.9, 162.8, 161.6, 135.2, 132.1, 130.6, 121.6, 113.6, 106.5, 90.5, 55.8 (2C), 55.4, 55.3.

2.2. Bioactivity Assays

CAs inhibitory effects of the chalcones were realised according to Verpoorte et al. (Verpoorte et al., 1967) as described before (Yamali et al., 2018; Taslimi et al., 2017). The inhibitory effect of the compounds on AChE was performed according to Ellman’s method (Ellman et al., 1961) according to previous literature (Yamali et al., 2018; Taslimi et al., 2017).

3. Result and Discussion

3.1. Chemistry

Condensation of acetophenones with suitable benzaldehydes via Claisen-Schmidt reaction afforded the target chalcones 1-8. The chemical structures of the compounds were elucidated with \(^1\)H NMR and \(^1^3\)C NMR. Most of the chalcones reported from either natural origin or synthetic region are in trans (\(E\)) diastereomers only, since the cis form (\(Z\)) of chalcones is thermodynamically not stable (Rammohan et al., 2020). In our study, all chalcones were in \(E\) form with the \(J\) values around 15 Hz. In this new study, CAs and AChE inhibitory effects of the registered compounds were reported for the first time.
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3.2. hCA I and hCA II inhibitory effects of the chalcones

In this current study, poly-methoxylated chalcones were considered as potential CAIs and they were tested on widespread hCA I and hCA II isoenzymes (Ozgun et al., 2018; Yamali et al., 2020; Taslimi et al, 2017). In the bioactivity assay, Acetazolamide (AZA) was used as a reference drug. hCA I and II inhibition results were demonstrated in Table.

The effects of the compounds on slow cytosolic hCA I isoform were as follows. The chalcones showed considerable inhibitory activity and their Ki values were in the range of 8.75±0.64 - 37.64±2.38 nM while IC₅₀ values were ranging from 21.65 - 49.50 nM. AZA had also Ki and IC₅₀ values of 28.75±4.33 nM and 40.13 nM, respectively. The majority of the compounds showed great inhibitory potency against hCA I. The compound 1 was the most potent inhibitor with the lowest Ki value of 8.75±0.64 nM. The compound 8 having 2,4,6-trimethoxyphenyl was the best inhibitor among 6, 7, and 8. It can be stated here position 6 for methoxy group was a preferable position for this type chalcones. On the other hand, it is known that phenol is another popular pharmacophore group for CA inhibitors. In this study, the compound 4 has phenol function and this compounds showed more inhibitory potency with Ki value of 15.21±1.23 nM than AZA. In addition, thiophen bearing compound 5 was found more selective against hCA I than hCA II.

The cytosolic hCA II isoenzyme was inhibited by the compounds with Ki values of 11.47±3.31 - 45.97±4.67 nM while IC₅₀ values were ranging from 24.75 nM to 53.31 nM. On the other hand, IC₅₀ and Ki values of AZA were 45.12 nM and 31.67±3.45,
respectively. Five of the chalcones showed more inhibitory potency than AZA against hCA II. The compound 4-bromophenyl bearing 3 with Ki value of 11.47±3.31 nM was found as a promising inhibitor against hCA II. In contrast to hCA I activity, the phenolic compound 4 did not show satisfactory inhibitory potency with the highest Ki value of 45.97±4.67 nM against hCA II. This situation most probably related to differences of active sides of both enzymes that affects the compounds’ inhibitory ability. As a similar point for hCA II, compound 8 having 2,4,6-trimethoxyphenyl was found the good inhibitor with the lowest Ki value among 6, 7 and 8.

3.3. AChE inhibitory effects of the chalcones

Since chalcones were reported with their significant inhibitory potency on AChE enzyme, which is a well-known therapeutic target of Alzheimer’s disease, the compounds were screened on AChE enzyme according to previous studies (Yamali et al., 2018; Ozgun et al., 2016). Tacrine (TAC) was used as a reference compound. The IC$_{50}$ and Ki values of the compounds were presented in Table. Many of the compounds showed enzyme inhibitory potency in nanomolar concentrations against AChE enzyme with Ki values ranging from 53.65±13.25 nM to 34.14±20.79 nM while the Tacrine’s Ki value was 49.34±3.56 nM. IC$_{50}$ values of the compounds were also in the range of 38.50 - 63.00 nM while Tacrine’s IC$_{50}$ was 55.13 nM.

Five of the compounds showed great inhibitory potency than the reference drug. The thiophene bearing compound 5 with the lowest Ki value of 34.14±20.79 nM was considered the most potent AChE inhibitor among others. Among halogen derivatives 1, 2 and 3, compound 1 having 4-fluorophenyl had the lowest Ki value of 44.90±5.22 nM. When the compounds 6, 7, and 8 were considered, the compound 7 with Ki value of 42.19±10.10 nM which has 2, 4, 5-trimethoxyphenyl was found the best inhibitor. The compounds reported here might be potential leads for designing novel AChE inhibitors.

4. Conclusion

A series of poly-methoxylated chalcones were synthesized and evaluated for their AChE, hCA I and hCA II inhibitory potencies. Many of the compounds exhibited promising CAs enzyme inhibitory potency. The halogenated compounds 1 and 3 showed the great CA inhibitory potency with the lowest Ki values 8.75±0.64 nM (hCA I) and 11.47±3.31 nM (hCA II). Even the compounds moderately inhibited AChE, thiophene bearing compound 5 with the lowest Ki value of 34.14±20.79 nM was considered as the most potent AChE inhibitor. The versatile chemistry and promising biological activities of chalcones made them a hot topic among researchers. Also, we hope that the results reported here lead to the design and development of novel chalcone-based enzyme inhibitors.
Table. AChE and CAs enzyme inhibition results of the chalcones 1-8

| Code | IC₅₀ (nM) | AChE | Ki (nM) |
|------|----------|------|---------|
|      | hCA I    | hCA II |        | hCA I | hCA II | AChE |
| 1    | 21.65    | 33.00  | 9870   | 46.20 | 0.9802 | 8.75±0.64 | 20.62±3.80 | 44.90±5.22 |
| 2    | 25.67    | 40.76  | 9738   | 49.50 | 0.9861 | 22.00±3.15 | 27.46±0.11 | 53.65±13.25 |
| 3    | 31.50    | 24.75  | 9740   | 46.20 | 0.9853 | 13.35±3.26 | 11.47±3.31 | 46.58±9.18  |
| 4    | 36.37    | 46.20  | 9694   | 38.50 | 0.9779 | 15.21±1.23 | 45.97±4.67 | 42.40±3.29  |
| 5    | 40.76    | 53.31  | 9803   | 63.00 | 0.9617 | 18.71±1.02 | 37.93±4.94 | 34.14±20.79 |
| 6    | 46.20    | 34.65  | 9811   | 57.75 | 0.9770 | 37.64±2.38 | 31.67±5.73 | 49.17±5.07  |
| 7    | 49.50    | 38.50  | 9833   | 40.76 | 0.9738 | 22.58±0.52 | 22.69±1.31 | 42.19±10.10 |
| 8    | 27.50    | 43.31  | 9618   | 43.31 | 0.9746 | 16.79±2.93 | 14.65±0.57 | 51.31±5.27  |
| AZA  | 40.13    | 45.12  | 9875   | -     | 28.75±4.33 | 31.67±3.45 | -           |
| TAC  | -        | -      | -      | 55.13 | 0.9811 | -           | -           | 49.34±3.56  |

Table. AChE and CAs enzyme inhibition results of the chalcones 1-8

AZA: Acetazolamide

TAC: Tacrine

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6. Conflicts of Interest

There are no known conflicts of interest relevant to this paper.

7. Table Caption and Figure Legend

Figure. Synthesis and chemical formula of the chalcones 1-8

8. References

Alpan, A.S., Sarikaya, G., Coban, G., Parlar, S., Armagan, G., Alptuzun, V. 2017. “Mannich-Benzimidazole Derivatives as Antioxidant and Anticholinesterase Inhibitors: Synthesis, Biological Evaluations, and Molecular Docking Study”. Arch Pharm (Weinheim), 350(7), e1600351.

Burmaoglu, S., Yilmaz, A.O., Polat, M.F., Kaya, R., Gulcin, I., Algul, O. 2019. “Synthesis and Biological Evaluation of Novel Tris-Chalcones as Potent Carbonic Anhydrase, Acetylcholinesterase,
Butyrylcholinesterase and Alpha-Glycosidase Inhibitors”. Bioorg Chem, 85, 191-197.

Ellman, G.L., Courtney, K.D., Andres, V., Jr., Feather-Stone, R.M. 1961. “A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity”. Biochem Pharmacol, 7, 88-95.

Gomes, M.N., Muratov, E.N., Pereira, M., Peixoto, J.C., Rosseto, L.P., Cravo, P.V.L., Andrade, C.H., Neves, B.J. 2017. “Chalcone Derivatives: Promising Starting Points for Drug Design”. Molecules, 22(8), 1210

Gul, H.I., Yamali, C., Bulboller, M., Kirmizibayrak, P.B., Gul, M., Angeli, A., Bua, S., Supuran, C.T. 2018a. “Anticancer Effects of New Dibenzenesulfonamides by Inducing Apoptosis and Autophagy Pathways and Their Carbonic Anhydrase Inhibitory Effects on hCA I, hCA II, hCA IX, hCA XII Isoenzymes”. Bioorg Chem, 78, 290-297.

Gul, H.I., Yamali, C., Sakagami, H., Angeli, A., Leitans, J., Kazaks, A., Tars, K., Ozgun, D.O., Supuran, C.T. 2018b. “New Anticancer Drug Candidates Sulfonamides as Selective hCA IX or hCA XII Inhibitors”. Bioorg Chem, 77, 411-419.

Li, Y., Qiang, X., Luo, L., Yang, X., Xiao, G., Zheng, Y., Cao, Z., Sang, Z., Su, F., Deng, Y. 2017. “Multitarget Drug Design Strategy Against Alzheimer’s Disease: Homoisoﬂavonoid Mannich Base Derivatives Serve As Acetylcholinesterase and Monoamine Oxidase B Dual Inhibitors with Multifunctional Properties”. Bioorg Med Chem, 25(2), 714-726.

Mahar, J., Saeed, A., Belfield, K.D., Ali Larik, F., Ali Channar, P., Ali Kazi, M., Abbas, Q., Hassan, M., Raza, H., Seo, S.Y. 2019. “1-(2-Hydroxy-5-((trimethylsilyl)ethynyl)phenyl)ethanone Based Alpha, Beta-Unsaturated Derivatives An Alternate To Non-Sulfonamide Carbonic Anhydrase II Inhibitors, Synthesis Via Sonogashira Coupling, Binding Analysis, Lipinski's Rule Validation”. Bioorg Chem, 84, 170-6.

Ozgun, D.O., Gul, H.I., Yamali, C., Sakagami, H., Gulcin, I., Sukuroglu, M., Supuran, C.T. 2019. “Synthesis and Bioactivities of Pyrazoline Benzensulfonamides as Carbonic Anhydrase and Acetylcholinesterase Inhibitors With Low Cytotoxicity”. Bioorg Chem, 84, 511-517.

Ozgun, D.O., Yamali, C., Gul, H.I., Taslimi, P., Gulcin, I., Yanik, T., Supuran, C.T. 2016. “Inhibitory Effects of Isatin Mannich Bases On Carbonic Anhydrases, Acetylcholinesterase, and Butyrylcholinesterase”. J Enzyme Inhib Med Chem, 31(6), 1498-501.

Rammohan, A., Reddy, J.S., Sravya, G., Rao, C.N., Zyryanov, G.V. 2020. “Chalcone Synthesis, Properties and Medicinal Applications: A Review”. Environ Chem Lett, 18, 433-458.

Supuran, C.T. 2016. “Structure and Function of Carbonic Anhydrases”. Biochem J, 473(14), 2023-32.

Supuran, C.T. 2017. “Advances In Structure-Based Drug Discovery of Carbonic Anhydrase Inhibitors”. Expert Opin Drug Discov, 12(1), 61-88.

Supuran, C.T. 2018. “Carbonic Anhydrase Inhibitors and Their Potential In A Range of
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Therapeutic Areas”. *Expert Opin Ther Pat*, 28(10), 709-712.

Supuran, C.T., Vullo, D., Manole, G., Casini, A., Scozzafava, A. 2004. “Designing of Novel Carbonic Anhydrase Inhibitors and Activators”. *Curr Med Chem Cardiovasc Hematol Agents*, 2(1), 51-70.

Supuran, C.T. 2008. “Carbonic anhydrases: Novel Therapeutic Applications for Inhibitors and Activators”. *Nat Rev Drug Discov*, 7(2), 168-181.

Taslimi, P., Sujayev, A., Mamedova, S., Kalin, P., Gulcin, I., Sadeghian, N., Beydemir, S., Kufrevioglu, O.I., Alwasel, S.H., Farzaliyev, V., Mamedov, S. 2017. “Synthesis and Bioactivity of Several New Hetaryl Sulfonamides”. *J Enzym Inhib Med Chem*, 32(1), 137-145.

Temperini, C., Scozzafava, A., Supuran, C.T. 2008. “Carbonic Anhydrase Activation and The Drug Design”. *Curr Pharm Des*, 14(7), 708-715.

Verpoorte, J.A., Mehta, S., Edsall, J.T. 1967. “Esterase Activities of Human Carbonic Anhydrases B and C”. *J Biol Chem*, 242(18), 4221-4229.

Yamali, C., Gul, H.I., Ece, A., Taslimi, P., Gulcin, I. 2018. “Synthesis, Molecular Modeling, and Biological Evaluation of 4-[5-aryl-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]benzenesulfonamides Toward Acetylcholinesterase, Carbonic Anhydrase I and II Enzymes”. *Chem Biol Drug Des*, 91(4), 854-866.

Yamali, C., Gul, H.I., Kazaz, C., Levent, S., Gulcin, I. 2020. “Synthesis, Structure Elucidation, and In Vitro Pharmacological Evaluation of Novel Polyfluoro Substituted Pyrazoline Type Sulfonamides as Multi-Target Agents for Inhibition of Acetylcholinesterase and Carbonic Anhydrase I and II Enzymes”. *Bioorg Chem*, 96, 103627.

Yamali, C., Gul, H.I., Ozgun, D.O., Sakagami, H., Umemura, N., Kazaz, C., Gul, M. 2017. “Synthesis and Cytotoxic Activities of Difluoro-Dimethoxy Chalcones”. *Anticancer Agents Med Chem*, 17(10), 1426-1433.

Yamali, C., Gul, H.I., Sakagami, H., Supuran, C.T. 2016. “Synthesis and Bioactivities of Halogen Bearing Phenolic Chalcones and Their Corresponding Bis Mannich Bases”. *J Enzyme Inhib Med Chem*, 31(sup4), 125-131.

Zhang, X., Rakesh, K.P., Bukhari, S.N.A., Balakrishna, M., Manukumar, H.M., Qin, H.L. 20118. “Multi-targetable Chalcone Analogs To Treat Deadly Alzheimer's Disease: Current View and Upcoming Advice”. *Bioorg Chem*, 80, 86-93.

Zengin, M., Genc, H., Taslimi, P., Kestane, A., Guclu, E., Ogutlu, A., Karabay, O., Gulcin, I. 2018. “Novel Thymol Bearing Oxypropanolamine Derivatives As Potent Some Metabolic Enzyme Inhibitors - Their Antidiabetic, Anticholinergic and Antibacterial Potentials”. *Bioorg Chem*, 81, 119-126.