



Inhibitory effects of novel benzamide derivatives towards acetylcholinesterase enzyme

Cem YAMALI^{1,2*}  , Halise Inci GUL^{2*}  , Serkan LEVENT³  , Yeliz DEMIR⁴  

¹Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, Cukurova University, Adana, Turkey

²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ataturk University, Erzurum, Turkey

³Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey

⁴Department of Pharmacy Services, Nihat Delibalta Gole Vocational High School, Ardahan University, Ardahan, Turkey

Abstract: Alzheimer's disease is one of the diseases which is identified by progressive memory loss and cognitive deficits leading to a decline in the lifespan of the patients. The drugs used in the clinic show palliative properties and they are unable to modify disease progression. In this study, *N*-(4-(*N*-(diaminomethylene)sulfamoyl)phenyl)-2-(substituted-benzamido)benzamide derivatives were synthesized and evaluated towards acetylcholinesterase (AChE, E.C.3.1.1.7) enzyme which is the most studied enzyme regarding Alzheimer's disease. The inhibition constants (K_i) of the compounds synthesized towards the AChE enzyme were in the range of 15.51 ± 1.88 - 41.24 ± 10.13 nM. The most effective compound with the lowest $K_i = 15.51 \pm 1.88$ nM, 2-benzamido-*N*-(4-(*N*-(diaminomethylene)sulfamoyl)phenyl)benzamide **6**, can be reported as a lead compound of this study. Bioactivity results obtained by this study may provide useful information on the development of novel and potent inhibitors targeting Alzheimer's disease.

Keywords: Acetylcholinesterase, benzamide, sulfaguanidine, Alzheimer's disease.

Submitted: December 17, 2020. **Accepted:** February 08, 2021.

Cite this: Yamali C, Gul HI, Levent S, Demir Y. Inhibitory effects of novel benzamide derivatives towards acetylcholinesterase enzyme. JOTCSA. 2021;8(2):429-34.

DOI: <https://doi.org/10.18596/jotcsa.842465>.

***Corresponding authors. E-mail:** (c.yamali@yahoo.com) (incigul@atauni.edu.tr)

INTRODUCTION

Alzheimer's disease (AD) is identified by progressive memory loss and cognitive deficits leading to declining in the lifespan of the patients. Despite its complicated molecular pathogenesis, several treatments have been used at the clinic (1).

Manipulation of cholinergic activity by decreased production of acetylcholine (ACh) or abnormal acetylcholinesterase (AChE) activity is the most-studied clinical approach. Two types of cholinesterases (ChEs) were identified as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE, pseudo-cholinesterase). Both ChEs have a role in the breakdown of ACh into choline and acetate (2). Cholinesterase inhibitors (ChEIs) as drug or drug candidates enhance the synaptic level of ACh by inhibiting ChEs in a dose-dependent manner (3).

According to the cholinergic hypothesis, to design inhibitors as antagonists of AChE and to discover molecules to bind with ACh receptors as agonists are the major strategies to control the amount of ACh in the synaptic cleft (4). Even drugs used in the clinic could improve the cognitive symptoms, they are unable to modify disease progression and they also show a different mode of actions, pharmacokinetics, and side effects. To date, several ChEIs are approved for the treatment of AD such as donepezil, rivastigmine, and galantamine. Even tacrine was the first drug used in AD, it is no longer used due to severe side effects. Notwithstanding scientific efforts, there is still no effective therapeutics for the prevention and treatment of AD (5-7).

A variety of chemical scaffolds has been reported with anti-AD effects. Benzamide derivative incorporating isoquinoline moiety was reported as the most potent candidate against human AChE with

Ki of 6.47 nM (4). As another attractive pharmacophoric group for AD, guanidine skeleton shows promising biological activities (8). Hydrazones having guanidine core as butyrylcholinesterase inhibitors (8), and cyclic acyl guanidine carbamate type compounds as ChEIs were reported with promising inhibitory potencies (6).

Our research group focused on primary sulfonamide derivatives and their bioactivities on CA I, CA II, CA IX, CA XII, and/or cholinesterase enzymes and their anticancer effects as potent drug candidates (9-12). This is the first study regarding some novel secondary sulfonamides to extend our earlier investigations. Novel sulfaguanidine bearing benzamide derivatives having the chemical structure of *N*-(4-(*N*-(diaminomethylene)sulfamoyl)phenyl)-2-(substituted-benzamido)benzamides **6-9** were designed, synthesized, and evaluated towards AChE enzyme to find out novel and potent AChE enzyme inhibitors for further experiments.

EXPERIMENTAL SECTION

Chemistry

NMR spectra of the compounds were recorded by Bruker AVANCE III 400 MHz (Bruker, Karlsruhe, Germany) in DMSO-*d*₆ (Merck KGaA, Darmstadt, Germany). LCMS-IT-TOF system (Shimadzu, Tokyo, Japan) was used for HRMS spectra. Electrothermal 9100 (IA9100, Bibby Scientific Limited, Staffordshire, UK) device was used to measure melting points (Mp). TLC-Silicagel HF254 (Merck Art 5715) plate was used to check the reaction process using UV lamp (Spectrolite, ENF-240C/ FE, New York, U.S.A). DCM:MeOH mixture was used as a TLC solvent system.

Synthesis of 2-(4-substituted phenyl)-4*H*-benzo(d)(1,3)oxazin-4-ones, **2-5** (13, 14)

Methyl anthranilate (33 g) was stirred in NaOH solution (2N, 150 mL) at room temperature for 30 hours. The mixture was poured into 100 mL of ice water and neutralized by HCl (37%). The white solid 2-aminobenzoic acid (**1**) was filtered, washed with water, and dried. To a solution of 2-aminobenzoic acid (**1**, anthranilic acid) (14.6 mmol) in pyridine (30) at 0-5 °C, a suitable benzoyl chloride (21.9 mmol) was added while stirring. After 30 min, the mixture was stirred at room temperature for 3-7 hours. The mixture was then treated with NaHCO₃ solution (10%, 50 mL) to remove the unreacted acid. Then the white solid obtained was filtered and washed with water several times to remove excess pyridine. The white color intermediates were used for the next step without further purification since the compounds are single spots on TLC plates. Experimental data for the raw intermediates were given below. Yield 60%, mp = 120-122 °C. HRMS (ESI-MS) C₁₄H₉NO₂ *m/z* Calc. (M+H)⁺ 224.0706; Found: 224.0695. Compound **3**: Yield 65%, mp = 150-151 °C. Compound **4**: Yield 71%, mp = 169-171

°C. HRMS (ESI-MS) C₁₄H₈NO₂F *m/z* Calc. (M+H)⁺ 242.0612; Found: 242.0623. Compound **5**: Yield 64%, mp = 90-92 °C. HRMS (ESI-MS) C₁₅H₈NO₂F₃ *m/z* Calc. (M+H)⁺ 292.0580; Found: 292.0574.

Synthesis of benzamide derivatives **6-9**, Figure 1

Sulfaguanidine (1 mmol) was added into a suitable 2-(4-substituted phenyl)-4*H*-benzo(d)(1,3)oxazin-4-ones (**2-5**) (1 mmol) solution in hot acetic acid (20 mL). The mixture was refluxed for 24 hours. After completion of the reaction, it was poured into cold water (100 mL). The solid product was precipitated out and filtered. The crude was washed with water and dried. The compounds were purified by crystallization using suitable solvents mentioned below. The chemical structures were confirmed by ¹H NMR, ¹³C NMR, and HRMS.

2-Benzamido-*N*-(4-(*N*-(diaminomethylene)sulfamoyl)phenyl)benzamide, **6**

White solid (methanol:DMF:water), 42% yield, mp = 294-295 °C. ¹H NMR (DMSO-*d*₆, 400 MHz, ppm) δ 11.50 (s, 1H, -CONH-), 10.78 (s, 1H, -CONH-), 8.42 (d, *J* = 8.3 Hz, 1H, ArH), 7.94-7.90 (m, 3H, ArH), 7.86 (d, *J* = 8.7 Hz, 2H, ArH), 7.76 (d, *J* = 8.7 Hz, 2H, ArH), 7.64-7.53 (m, 4H, ArH), 7.29 (t, *J* = 7.5 Hz, 1H, ArH), 6.74 (bs, 4H, -NH₂ x 2). ¹³C NMR (DMSO-*d*₆, 100 MHz, ppm) δ 168.1, 165.2, 158.6, 141.6, 140.2, 138.9, 134.9, 132.9, 132.5, 129.6, 129.3, 127.6, 126.9, 123.9, 123.8, 122.1, 120.8. HRMS (ESI-MS) C₂₁H₁₉N₅O₄S *m/z* Calc. (M+H)⁺ 438.1231; Found 438.1232.

N-(4-(*N*-(diaminomethylene)sulfamoyl)phenyl)-2-(4-methoxybenzamido) benzamide, **7**

White solid (methanol:acetone:water), 33% yield, mp = 235-236 °C. ¹H NMR (DMSO-*d*₆, 400 MHz, ppm) δ 11.43 (s, 1H, -CONH-), 10.76 (s, 1H, -CONH-), 8.43 (d, *J* = 8.3 Hz, 1H, ArH), 7.91-7.87 (m, 3H, ArH), 7.83 (d, *J* = 8.7 Hz, 2H, ArH), 7.75 (d, *J* = 8.6 Hz, 2H, ArH), 7.61 (t, *J* = 7.7 Hz, 1H, ArH), 7.28 (t, *J* = 7.7 Hz, 1H, ArH), 7.09 (d, *J* = 8.3 Hz, 2H, ArH), 6.71 (bs, 4H, -NH₂ x 2), 3.82 (s, 3H, -OCH₃). ¹³C NMR (DMSO-*d*₆, 100 MHz, ppm) δ 168.2, 164.6, 162.7, 158.6, 141.5, 140.2, 139.3, 129.6, 129.5, 129.4, 126.9, 126.8, 123.6, 123.2, 121.9, 120.9, 114.6, 55.9. HRMS (ESI-MS) C₂₂H₂₁N₅O₅S *m/z* Calc. (M+H)⁺ 468.1336; Found 468.1340.

N-(4-(*N*-(diaminomethylene)sulfamoyl)phenyl)-2-(4-fluorobenzamido)benzamide, **8**

White solid (methanol:DMF:water), 34% yield, mp = 285-287 °C. ¹H NMR (DMSO-*d*₆, 400 MHz, ppm) δ 11.39 (s, 1H, -CONH-), 10.76 (s, 1H, -CONH-), 8.32 (d, *J* = 8.2 Hz, 1H, ArH), 7.97 (t, *J* = 6.4 Hz, 2H, ArH), 7.88 (d, *J* = 7.8 Hz, 1H, ArH), 7.81 (d, *J* = 8.5 Hz, 2H, ArH), 7.74 (d, *J* = 8.5 Hz, 2H, ArH), 7.62 (t, *J* = 7.6 Hz, 1H, ArH), 7.39 (d, *J* = 8.5 Hz, 2H, ArH), 7.30 (t, *J* = 7.3 Hz, 1H, ArH), 6.69 (bs, 4H, -NH₂ x 2). ¹³C NMR (DMSO-*d*₆, 100 MHz, ppm) δ 167.9, 164.7 (d, *J*_{CF} = 248 Hz), 163.4, 158.6, 141.6, 140.1, 138.7, 132.8, 131.4, 130.3 (d, *J*_{CF} = 9 Hz), 129.6, 126.9, 124.3,

124.1, 122.4, 120.8, 116.3 (d, $J_{CF} = 22$ Hz). HRMS (ESI-MS) $C_{21}H_{18}N_5O_4FS$ m/z Calc. (M+H)⁺ 456.1136; Found 456.1136.

***N*-(4-(*N*-(diaminomethylene)sulfamoyl)phenyl)-2-(4(trifluoromethyl) benzamido)benzamide, 9**

White solid (methanol:DMF:water), 30% yield, mp = 297-299 °C. ¹H NMR (DMSO-*d*₆, 400 MHz, ppm) δ 11.49 (s, 1H, -CONH-), 10.77 (s, 1H, -CONH-), 8.28 (d, $J = 8.2$ Hz, 1H, ArH), 8.09 (d, $J = 8.2$ Hz, 2H, ArH), 7.94 (d, $J = 8.3$ Hz, 2H, ArH), 7.89 (d, $J = 7.8$ Hz, 1H, ArH), 7.82 (d, $J = 8.7$ Hz, 2H, ArH), 7.73 (d, $J = 8.7$ Hz, 2H, ArH), 7.63 (t, $J = 7.5$ Hz, 1H, ArH), 7.33 (t, $J = 7.5$ Hz, 1H, ArH), 6.69 (bs, 4H, -NH₂ x 2). ¹³C NMR (DMSO-*d*₆, 100 MHz, ppm) δ 167.8, 164.2, 158.6, 141.6, 140.1, 138.8, 138.2, 132.7, 132.2 (d, $J_{CF} = 32$ Hz), 129.6, 128.6, 126.9, 126.3 (d, $J_{CF} = 3$ Hz), 124.9, 124.5, 124.3 (d, $J_{CF} = 271$ Hz), 122.7, 120.7. HRMS (ESI-MS) $C_{22}H_{18}N_5O_4F_3S$ m/z Calc. (M+H)⁺ 506.1111; Found 506.1104.

AChE inhibition assay

5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB, D8130), acetylthiocholine iodide (AChI, 01480) and AChE from *Electrophorus electricus* (C2888, Type V-S), were acquired from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). *In vitro* effects on AChE activity of the compounds were evaluated according to the Ellman's assay (15) as previously reported (16, 17). AChI was used as the substrate at 412 nm. Tacrine (TAC) was used as a control drug. One enzyme unit (EU) was defined as the hydrolysis of 1.0 μ mol acetylcholine to choline and acetate per minute at pH = 8 at 37 °C. All the measurements were repeated three times. The analysis results were expressed as means of triplicate assays \pm SEM.

RESULTS and DISCUSSION

The novel compounds **6-9** were synthesized in three steps by the method outlined in Figure 1. Firstly, a starting compound methyl anthranilate was hydrolyzed under basic conditions to afford 2-aminobenzoic acid, **1**. The chemical structure was confirmed with NMRs. Methyl peak belonging ester moiety of methyl anthranilate did not appear in ¹H NMR spectra. This data showed that methyl anthranilate turned into its acid derivative. In the second step, compound **1** was reacted with several benzoyl chlorides in pyridine and the cyclic intermediate compounds, 2-(4-substituted phenyl)-4*H*-benzo(d)(1,3)oxazin-4-ones (**2-5**), were isolated from the reaction media. Finally, compounds **2-5** reacted with sulfaguanidine in acetic acid solution by heating to obtain final compounds **6-9**.

Different benzoxazine derivatives of intermediates were transformed into the respective quinazolinone derivatives by a condensation reaction with several amine derivatives in pyridine (14) and acetic acid *via* microwave heating (18). Herein, we isolated and confirmed open-chain forms of the compounds under the conditions applied. Chemical structures of the compounds were elucidated successfully by the spectral methods. According to NMRs and HRMS results of the representative compound **7**, signals of two amide protons were seen at 11.43 ppm (s, 1H, -CONH-) and 10.76 ppm (s, 1H, -CONH-). These two protons testified that the isolated compound was not a quinazolinone derivative. Besides, -NH₂ protons belong to guanidine moiety was seen as a broad singlet at 6.71 ppm. Furthermore, ¹³C NMR spectra confirmed the chemical structure of compound **7** as it showed a total of 18 carbon peaks as expected. Calculated and measured m/z values are also compatible in HRMS spectra as reported in the experimental section.

According to our preliminary experiments based on the different projects (unpublished data), we obtained a series of quinazolinone-type benzenesulfonamides in considerable high yields under reflux conditions in acetic acid. In this study, we used sulfaguanidine as another kind of starting compound having primary amine. In the previous study, we easily isolated the ring-closed product after cooled the reaction medium. However, in this study, we isolated open-chain compounds in the same conditions. Reaction time was extended to increase products' yield, however, starting material did not consume totally. Moreover, the reaction medium had also more than three spots which have very similar R_f values. After isolation of the open-chain target products 6-9, side products left in the acetic acid. We tried some extraction processes to isolate them, but it was not successful. Therefore, we are planning to use the microwave irradiation technique and using different mole ratios to improve the reaction's yield and time for future study.

Benzamide derivative compounds **6-9** were evaluated towards the AChE enzyme that is one of the targets for the treatment of AD. Inhibition results were shown in Table 1. All of the compounds were more effective inhibitors than reference drug Tacrine according to K_i values. K_i values were in the range of 15.51 \pm 1.88 - 41.24 \pm 10.13 nM in Table 1. The compounds **6-9** were 1.2-3.2 times more potent than Tacrine (K_i = 49.23 \pm 2.67 nM) in terms of K_i values.

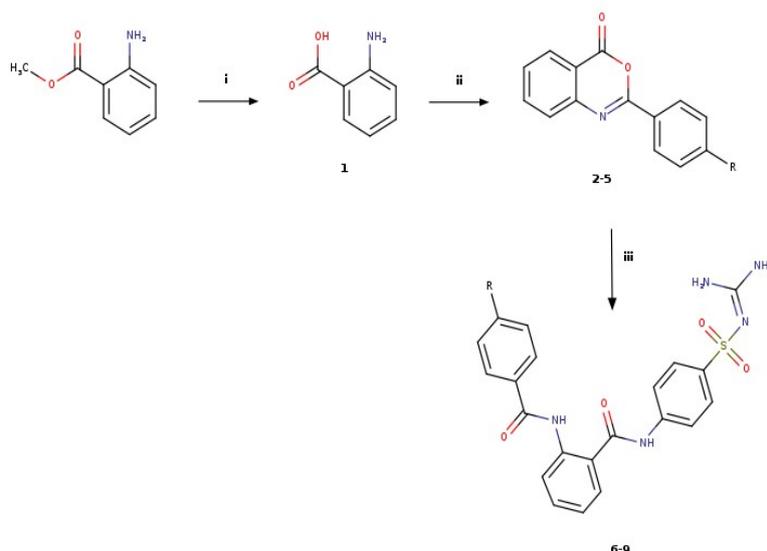


Figure 1. Synthetic method of the target compounds **6-9**.

Reagents and conditions. i : NaOH solution (2 N), HCl (37%), rt. ii: Benzoyl chloride derivative (R = H- (6), -OCH₃ (7), -F (8), -CF₃ (9)), pyridine, 0 - 5 °C, NaHCO₃ (10%). iii: Sulfaguandine, acetic acid, reflux.

The nonsubstituted compound **6** was the most promising AChE inhibitor among others. Substitution of methoxy (for **7**), fluoro (for **8**), and trifluoromethylene (for **9**) groups on phenyl ring decreased the enzyme inhibitory potency in 2.4, 2.6, and 2.7 times compared to parent compound **6**, respectively. Substitution of the *para* position of phenyl ring with different groups led to decrease inhibition potency. Fluorine substitution is one of the

useful modifications in medicinal chemistry to increase bioactivity, bioavailability, stability, and lipophilicity of the compounds (19-21). However, in our study, using halogen substituents did not improve bioactivity significantly when compared to compound **6**. Even three of the compounds moderately inhibited, they are effective at the nanomolar level towards AChE enzyme when compared to the reference drug.

Table 1. AChE enzyme inhibitory results of the compounds.

Code	Chemical Formula	AChE, Ki (nM)
6		15.51 ± 1.88
7		37.39 ± 6.38
8		39.55 ± 10.23
9		41.24 ± 10.13
Tacrine		49.23 ± 2.67

CONCLUSION

Here we reported synthesis, structure elucidation, and AChE enzyme inhibitory studies of a small series of novel benzamide derivatives having sulfaguanidine moiety. According to spectroscopic results, we obtained an open-chain structure of the compounds instead of quinazolinone derivatives. The compounds showed nanomolar inhibition level towards the AChE enzyme. The results indicated that compound **6** had remarkable inhibitory potency with the lowest K_i value compared to reference drug Tacrine. Therefore, the lead compound **6** can be evaluated on different targets of AD in future studies.

ACKNOWLEDGEMENTS

We would like thank to Prof. Dr. Ilhami Gulcin for his valuable scientific supports.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Wajid S, Khatoon A, Khan MA, Zafar H, Kanwal S et al. Microwave-assisted organic synthesis, structure-activity relationship, kinetics and molecular docking studies of non-cytotoxic benzamide derivatives as selective butyrylcholinesterase inhibitors. *Bioorganic and Medicinal Chemistry*. 2019;27(18): 4030-40. doi: 10.1016/j.bmc.2019.07.015.
2. Kratky M, Stepankova S, Vorcakova K, Svarcova M, Vinsova J. Novel cholinesterase inhibitors based on O-aromatic N,N-disubstituted carbamates and thiocarbamates. *Molecules*. 2016;21(2): 191-201. doi: 10.3390/molecules21020191.
3. Oliveira C, Bagetta D, Cagide F, Teixeira J, Amorim R, Silva T, et al. Benzoic acid-derived nitrones: A new class of potential acetylcholinesterase inhibitors and neuroprotective agents. *European Journal of Medicinal Chemistry*. 2019;174:116-29. doi: 10.1016/j.ejmech.2019.04.026.
4. Peng DY, Sun Q, Zhu XL, Lin HY, Chen Q, Yu NX, et al. Design, synthesis, and bioevaluation of benzamides: novel acetylcholinesterase inhibitors with multi-functions on butylcholinesterase, A β aggregation, and β -secretase. *Bioorganic and Medicinal Chemistry*. 2012;20(22):6739-50. doi: 10.1016/j.bmc.2012.09.016.
5. <https://www.nia.nih.gov/health/how-alzheimers-disease-treated>, December 2020.
6. Darras FH, Kling B, Sawatzky E, Heilmann J, Decker M. Cyclic acyl guanidines bearing carbamate moieties allow potent and dirigible cholinesterase inhibition of either acetyl- or butyrylcholinesterase. *Bioorganic and Medicinal Chemistry*. 2014;22(17):5020-34. doi: 10.1016/j.bmc.2014.06.010.
7. Yiannopoulou KG, Papageorgiou SG. Current and future treatments for Alzheimer's disease. *Therapeutic Advances in Neurological Disorders*. 2013;6(1):19-33. doi: 10.1177/1756285612461679.
8. Sekutor M, Mlinaric-Majerski K, Hrenar T, Tomic S, Primozic I. Adamantane-substituted guanylhydrazones: novel inhibitors of butyrylcholinesterase. *Bioorganic Chemistry*. 2012;41-42:28-34. doi: 10.1016/j.bioorg.2012.01.004.
9. Gul HI, Yamali C, Bulbuller M, Kirmizibayrak PB, Gul M, Angeli A, et al. 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. *Bioorganic Chemistry*. 2018;78:290-97. doi: 10.1016/j.bioorg.2018.03.027.
10. Gul HI, Yamali C, Sakagami H, Angeli A, Leitans J, Kazaks A, et al. New anticancer drug candidates sulfonamides as selective hCA IX or hCA XII inhibitors. *Bioorganic Chemistry*. 2018;77:411-19. doi: 10.1016/j.bioorg.2018.01.021.
11. Yamali C, Gul HI, Ece A, Taslimi P, Gulcin I. 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. *Chemical Biology and Drug Design*. 2018;91(4):854-66. doi: 10.1111/cbdd.13149.
12. Yamali C, Gul HI, Kazaz C, Levent S, Gulcin I. Synthesis, structure elucidation, and in vitro pharmacological evaluation of novel polyfluoro substituted pyrazoline type sulfonamides as multi-target agents for inhibition of acetylcholinesterase and carbonic anhydrase I and II enzymes. *Bioorganic Chemistry*. 2020;96:103627. doi: 10.1016/j.bioorg.2020.103627.
13. Zhu J, Yang HY, Chen Y, Lin HZ, Li Q, Mo J, et al. Synthesis, pharmacology and molecular docking on multifunctional tacrine-ferulic acid hybrids as cholinesterase inhibitors against Alzheimer's disease. *Journal of Enzym Inhibition and Medicinal Chemistry*. 2018;33(1):496-06. doi: 10.1080/14756366.2018.1430691.
14. Asundaria ST, Patel NS, Patel KC. Synthesis, characterization, and antimicrobial studies of novel 1,3,4-thiadiazolium-5-thiolates. *Medicinal Chemistry Research*. 2012;21:1199-06. doi: 10.1007/s00044-011-9632-2.
15. Ellman GL, Courtney KD, Andres V, Jr., Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity.

- Biochemical Pharmacology. 1961;7:88-95. doi: 10.1016/0006-2952(61)90145-9.
16. Yamali C, Gul HI, Demir Y, Kazaz C, Gulcin I. Synthesis and bioactivities of 1-(4-hydroxyphenyl)-2-((heteroaryl) thio) ethanones as carbonic anhydrase I, II and acetylcholinesterase inhibitors. Turkish Journal of Chemistry. 2020; 44(4), 1058-67. doi:10.3906/kim-2004-36.
17. Yamali C, Gul HI, Cakir T, Demir Y, Gulcin I. Aminoalkylated Phenolic Chalcones: Investigation of Biological Effects on Acetylcholinesterase and carbonic anhydrase I and II as potential lead enzyme inhibitors. Letters in Drug Design and Discovery. 2002;17(10): 1283-92. doi: 10.2174/1570180817999200520123510.
18. Selvam P, Vijayalakshimi P, Smee DF, Gowen BB, Julander JG, Day CW, et al. Novel 3-sulphonamido-quinazolin-4(3H)-one derivatives: microwave-assisted synthesis and evaluation of antiviral activities against respiratory and biodefense viruses. Antiviral Chemistry and Chemotherapy. 2007;18(5):301-5. doi: 10.1177/095632020701800506.
19. Gillis EP, Eastman KJ, Hill MD, Donnelly DJ, Meanwell NA. Applications of fluorine in medicinal chemistry. Journal of Medicinal Chemistry. 2015;58(21):8315-59. doi: 10.1021/acs.jmedchem.5b00258.
20. Yamali C, Gul HI, Ozgun DO, Sakagami H, Umemura N, Kazaz C, et al. Synthesis and cytotoxic activities of difluoro-dimethoxy chalcones. Anticancer Agents in Medicinal Chemistry. 2017;17(10):1426-33. doi: 10.2174/1871520617666170327123909.
21. Yamali C, Ozgun DO, Gul HI et al. Synthesis and structure elucidation of 1-(2,5/3,5-difluorophenyl)-3-(2,3/2,4/2,5/3,4-dimethoxyphenyl)-2-propen-1-ones as anticancer agents. Medicinal Chemistry Research 2017;26:2015-23. doi: 10.1007/s00044-017-1911-0.