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Determination of Anticholinergic Effects of *p*-Nitroaniline

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Abstract: Cholinesterases allow the nervous system to function properly by catalyzing the breakdown of the neurotransmitter acetylcholine (ACh), a naturally occurring neurotransmitter, into choline and acetic acid. Cholinesterases in the human body are of two types: acetylcholinesterase (AChE; E.C.3.1.1.7) and butyrylcholinesterase (BChE; E.C.3.1.1.8). In this study, the anticholinergic effect of p-Nitroaniline was investigated experimentally and theoretically and compared with tacrine, which is known as an AChE inhibitor. As a result of experimental inhibition studies, IC_{50} values were calculated as 17.77 nm against AChE and 18.73 nm against BCE for p-nitroaniline, and 15.06 nM against AChE and 15.75 nM against BChE for tacrine. In addition, K_i values; for p-nitroaniline it was calculated as 1.80 ± 0.16 nm against AChE, 6.49 ± 1.63 nm against BCHE, and for tacrine it was calculated as 2.59 ± 0.90 nM against AChE and 7.08 ± 0.90 nM against BChE. Docking Score values were -4.631 against AChE and -3.779 against BChE for *p*-nitroaniline and -12.908 against AChE and -6.090 against BChE for tacrine. The results of the study showed that *p*- nitroaniline has an effective inhibition against ACHE and BCHE enzymes associated with global metabolic AD disease.

Keywords: Alzheimer's disease, enzyme inhibition, acetylcholinesterase, butyrylcholinesterase.

p-Nitroanilinin antikolinerjik etkilerinin belirlenmesi

Özet: Kolinesterazlar, doğal olarak oluşan bir nörotransmiter olan nörotransmitter asetilkolinin (ACh) kolin ve asetik aside parçalanmasını katalize ederek sinir sisteminin düzgün çalışmasına izin verir. İnsan vücudunda kolinesterazlar, asetilkolinesteraz (AChE; EC3.1.1.7) ve butirilkolinesteraz (BChE; EC3.1.1.8) dahil olmak üzere iki tiptir. Bu çalışmada, p-Nitroanilin'in antikolinerjik etkisi deneysel ve teorik olarak araştırıldı ve AChE inhibitörü olarak bilinen takrin ile karşılaştırıldı. Deneysel inhibisyon çalışmaları sonucunda IC_{50} değerleri *p*-nitroanilin için AChE'ye karşı 17.77 nm ve BCE'ye karşı 18.73 nm, takrin için AChE'ye karşı 15.06 nM ve BChE'ye karşı 15.75 nM olarak hesaplanmıştır. Ayrıca K_I değerleri; *p*-nitroanilin için AChE'ye karşı 1.80 ± 0.16 nm, BCHE'ye karşı 6.49 ± 1.63 nm, takrin için AChE'ye karşı 2.59 ± 0.90 nM ve BChE'ye karşı 7.08 ± 0.90 nM olarak hesaplandı. Yerleştirme skoru değerleri *p*-nitroanilin için AChE'ye karşı -4.631, BChE'ye karşı -3.779 ve takrin için AChE'ye karşı -12.908, BChE'ye karşı -6.090 idi. Çalışmanın sonuçları, *p*-nitroanilin'in global metabolik AD hastalığı ile ilişkili ACHE ve BCHE enzimlerine karşı etkili bir inhibisyona sahip olduğunu göstermiştir.

Anahtar Kelimeler: Alzheimer hastalığı, enzim inhibisyonu, asetilkolinesteraz, butirilkolinesteraz

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1. INTRODUCTION

Alzheimer's disease (AD) is a slowly progressive neurodegenerative brain disease that affects approximately 5-10% of the elderly population, for which no current treatment has yet been found and the mechanism of which is not fully known [1, 2]. According to the cholinergic hypothesis, the cerebral cortex's acetylcholine level decreases with cholinergic neurons' loss in AD [3]. Characteristic pathological symptoms with decreased acetylcholine (ACH), β-protein amyloid aggregation, and tau-hyperphosphorylation are observed in patients, and studies show that the cholinergic hypothesis is a clinically appropriate strategy [4]. The level of acetylcholine (an important neurotransmitter associated with memory) in the hippocampus and cortex region of the brain has been reported to have a direct effect on AD [5]. Cholinesterase enzymes including acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) can convert acetylcholine (ACh) and butyrylcholine (ACh) to acetate, butyrate and choline (Ch). Therefore, Alzheimer's disease can be treated with the use of pharmaceuticals that restore acetylcholine levels through the inhibition of cholinesterase enzymes [6-8]. N-forms heterocyclic compounds that are widely distributed in nature, have physiological and pharmacological properties, and are components of many biologically important molecules, including many vitamins, nucleic acids, drugs, and antibiotics [9]. They also form an integral part of many pharmacologically active molecules. Base pairs of DNA and RNA (guanine, cytosine, adenine, and thymine), purines, and pyrimidines are also heterocyclic compounds containing N [10].
It was determined that p-nitraniline has high inhibitory activity on AChE and BChE enzymes by preliminary in silico molecular chelation studies. As a result, in this study, the inhibitory effects of p-nitraniline on AChE and BChE enzymes, whose inhibitory effects have not been studied before, on these enzymes were investigated experimentally and theoretically.Tacrine, known as an AChE inhibitor, used to treat Alzheimer's disease, was used as a reference molecule in studies.

2. MATERIALS AND METHOD

2.1. AChE and BChE enzymes inhibition assays

The inhibition effects of p-nitroaniline on the activities of AChE and BChE enzymes were determined according to the Ellman method [11]. Acetylthiocholine iodide (AChI) for AChE enzyme activity, butyryl thiocholine iodide (BChI) for BChE enzyme activity, and 5,5'- Dithio-bis(2-nitro-benzoic) acid (DTNB) were used as a substrate for activity measurements. Briefly, by mixing 100 mL of Tris/HCl buffer (1.0 M and pH 8.0), 10 mL of p-nitroaniline and tacrine solution dissolved in deionized water at different concentrations, and 50 mL of AChE/BChE (5.32 ± 10–3 EU) solution. It was incubated for 10 minutes at 25 °C. Then, the reaction was started by adding 50 mL, 0.5 mM DTNB and 50 mL, 10 mM AChI/BChI. The formation of yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholine released by enzymatic hydrolysis of AChI/BChI was monitored spectrophotometrically at 412 nm wavelength and the results were recorded [12-14]. From the recorded results, activity (%)-concentration and Line weaver-Burk plots were drawn for tacrine and p-nitroaniline. IC_{50}, r^2 and K_i values were calculated and inhibition types were determined.

2.2. Molecular modeling studies

Theoretically, there are many programs in bioinformatic chemistry for the design of new drugs and active substances. Molecular placement is one of the methods developed for this (15). Experimental inhibition studies have also been supported theoretically by molecular placement studies. The Schrödinger Molecular Modeling Suite (the docking program of Maestro 20.3) was used to predict the binding affinity and possible interactions between ligands (p-Nitroaniline and tacrine) and corresponding proteins (AChE and BChE) [16]. In the first phase of molecular studies, the molecular structure of p-Nitroaniline was drawn with the chem-draw program, and the pdb extension file was created. Crystal structures of acetylcholinesterase and butyrylcholine esterase with 2 Å resolution (TJH (PDB ID: 4TVK), 3F9 (PDB ID: 4TVK)) were obtained from the protein database [17, 18]. The ligands were prepared using Lig Prep at pH 7.0. The energy minimization was done by using an OPLS3e force field. Based on the calculated Glide score the best pose was ranked [19].

3. RESULTS AND DISCUSSION

Alzheimer's disease (AD) is one of the most common forms of dementia for older people. A chronic disease that causes a decline in central nervous system, language ability, and cognitive function [20]. As a result of irregularities in acetylcholine metabolism, AChE activity increases. These increases increase amyloid protein formation and hydrolysis of acetylcholine, resulting in neurodegenerative diseases such as AD. Synthesis and detection of molecules that reduce AChE activity are extremely important to prevent these diseases caused by damage to the cholinergic system [21]. Enzyme inhibition studies are effective therapeutic approaches for the pharmaceutical and cosmetic industries [22]. When the Ki values were compared as a result of experimental studies conducted for this purpose, it was determined that *p*-nitroaniline had a stronger inhibitory effect against the AChE and BCHE enzymes associated with global metabolic AD disease than the positive control compound tacrine (Table 1).

The results of the molecular insertion simulations are given in Table 2. When the experimental inhibition results and free binding energy scores were evaluated, it was observed that the results were compatible. The results showed that p-Nitroaniline had a weaker insertion score and higher free binding energy than tacrine, the positive control compound.

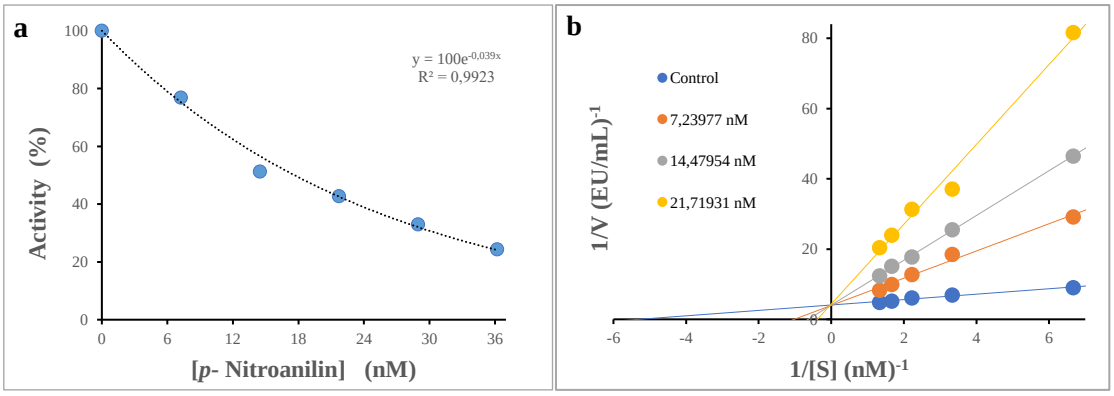

Figure 1. Plotted to determine the inhibitory effect of p-nitroaniline on AChE enzyme at different concentrations; a) Activity (%)-[p-Nitroaniline] and b) Lineweaver-Burk (Ki) plots

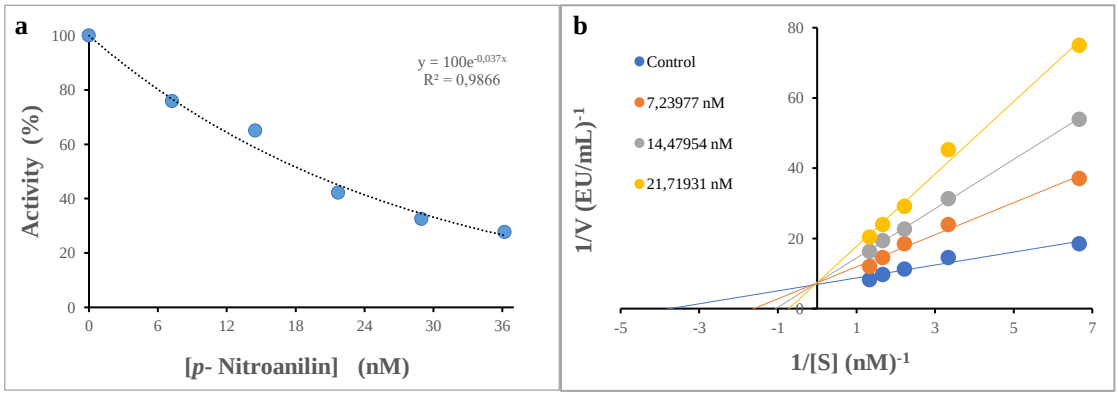

Figure 2. Plotted to determine the inhibitory effect of p-nitroaniline on BChE enzyme at different concentrations; a) Activity (%)-[p-Nitroaniline] and b) Lineweaver-Burk (Ki) plots

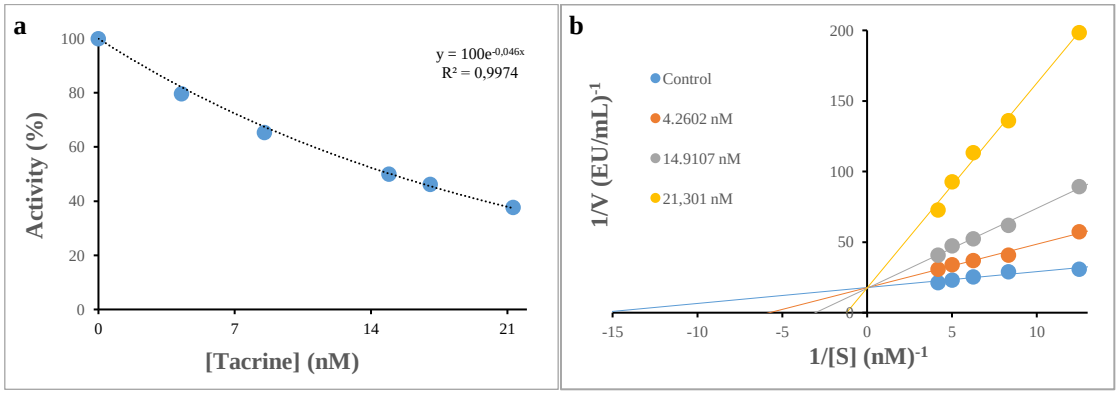

Figure 3. Plotted to determine the inhibitory of p-nitroaniline on AChE enzyme at different concentrations; a) Activity (%)-[Tacrine] and b) Lineweaver-Burk (Ki) plots

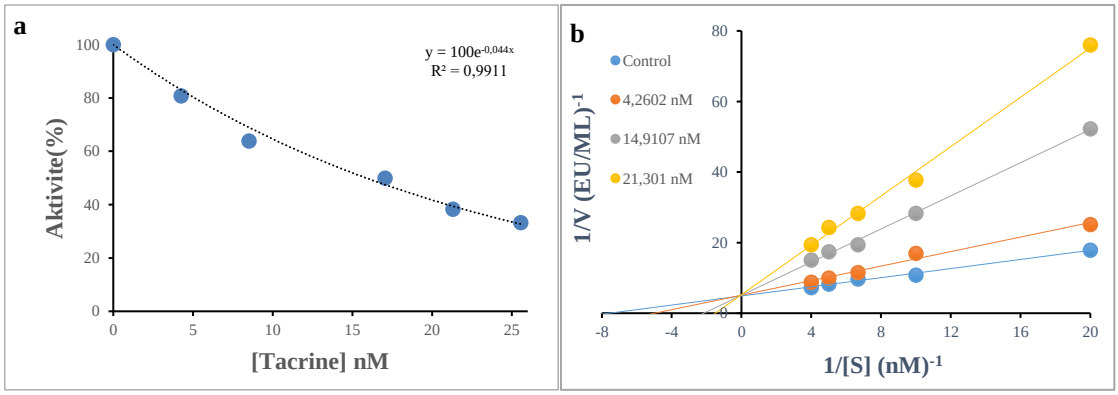

Figure 4. Plotted to determine the inhibitory of p-nitroaniline on BChE enzyme at different concentrations; a) Activity (%)-[Tacrine] and b) Lineweaver-Burk (Ki) plots

Table 1. The enzyme inhibition results of p-Nitroaniline against acetylcholinesterase (AChE), and butyrylcholine esterase (BChE) enzymes

Compounds	IC_{50} (nM)				K_i (nM)	
	AChE	r^2	BChE	r^2	AChE	BChE
***p* Nitroaniline**	17.77	0.9918	18.73	0.9840	1.80 ± 0.16	6.49 ± 1.63
Tacrine	15.06	0.9965	15.75	0.9890	2.59 ± 0.90	7.08 ± 0.90

*Tacrine (TAC) was used as a positive control for AChE and BChE enzymes.

Table 2. Docking scores and binding energies of the compounds for acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) enzymes

Enzymes	Compounds	Docking Score	XP GScore	Glide gscore	Glide emodel
Acetylcholinesterase (PDB: 4TVK)	*p*-Nitroaniline	-4.631	-4.631	-4.631	-35.412
	Tacrine	-12.908	-12.909	-12.909	-62.350
Butyrylcholinesterase (PDB: 4TPK)	*p*-Nitroaniline	-3.779	-3.779	-3.779	-31.096
	Tacrine	-6.090	-6.091	-6.091	-42.653

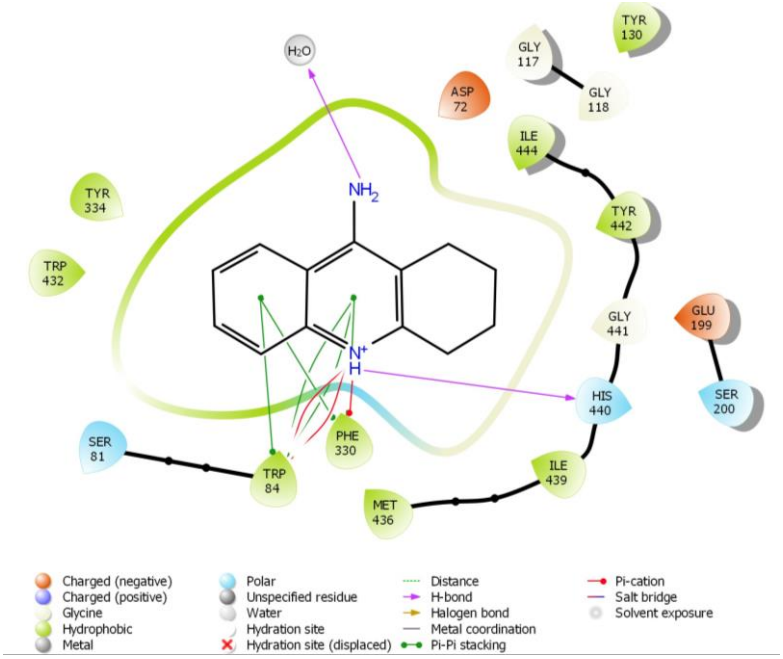

Figure 5. Two-dimensional (2D) ligand interactions of tacrine with AChE enzyme

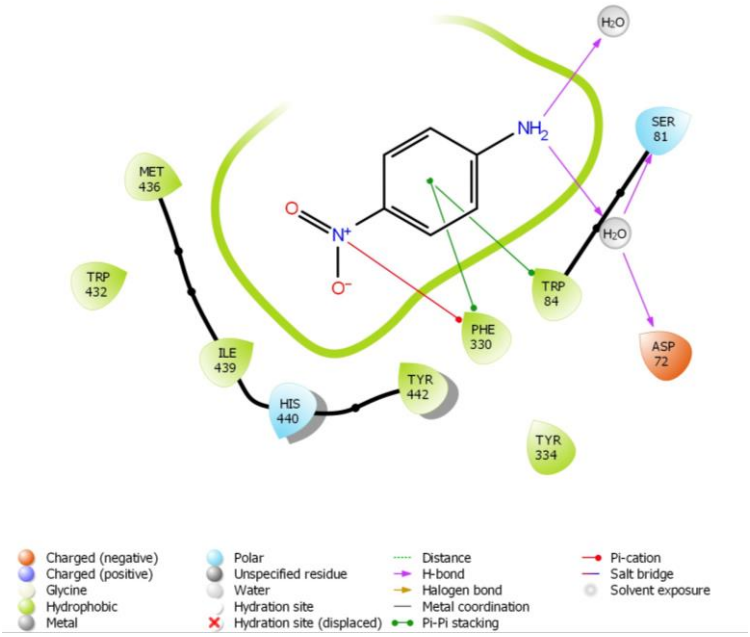

Figure 6. Two-dimensional (2D) ligand interactions of p-nitroaniline with AChE enzyme

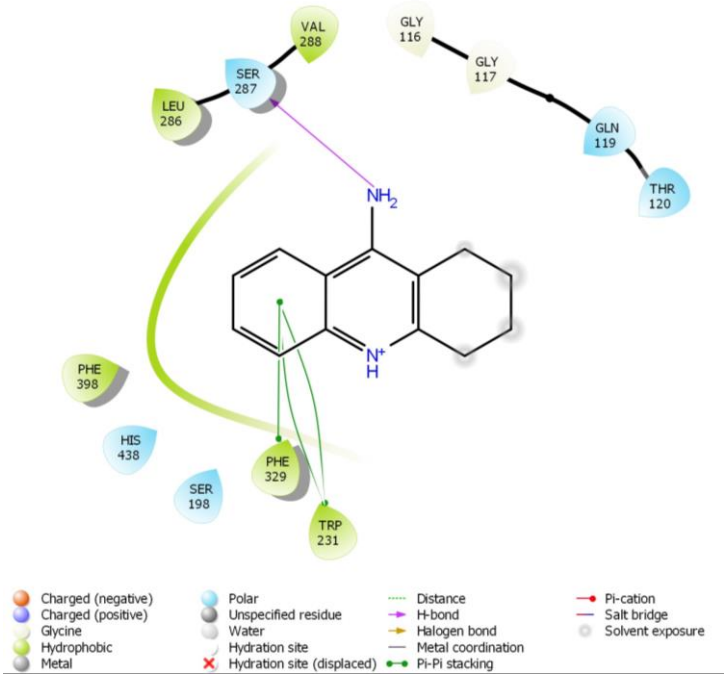

Figure 7. Two-dimensional (2D) ligand interactions of tacrine with BChE enzyme

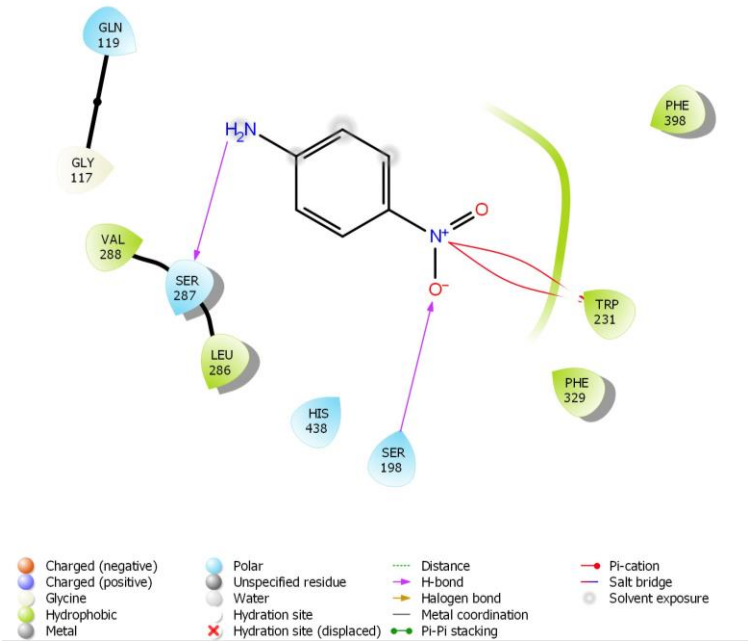

Figure 8. Two-dimensional (2D) ligand interactions of p-nitroaniline with BChE enzyme

References

[1] Tugrak, M., Gul, H. İ., & Gulcin, İ. "Acetylcholinesterase inhibitory potencies of new pyrazoline derivatives," Journal of Research in Pharmacy, (**2020**) 24(4).

[2] Gulcin, İ., Petrova, O. V., Taslimi, P., Malysheva, S. F., Schmidt, E. Y., Sobenina, L. N., ... & Sujayev, A. R. "Synthesis, Characterization, Molecular Docking, Acetylcholinesterase and α-Glycosidase Inhibition Profiles of Nitrogen-Based Novel Heterocyclic Compounds," ChemistrySelect, (**2022**), 7(19), e202200370.

[3] Bilginer, S., Gul, H. I., Anil, B., Demir, Y., & Gulcin, I. "Synthesis and in silico studies of triazene-substituted sulfamerazine derivatives as acetylcholinesterase and carbonic anhydrases inhibitors," Archiv der Pharmazie, (**2021**) 354(1), 2000243.

[4] Craig, L. A., Hong, N. S., & McDonald, R. J. "Revisiting the cholinergic hypothesis in the development of Alzheimer's disease." Neuroscience & Biobehavioral Reviews, (**2011**) 35(6), 1397-1409.

[5] Gülçin, İ., Bingöl, Z., Taslimi, P., Gören, A. C., Alwasel, S. H., & Tel, A. Z. "Polyphenol contents, potential antioxidant, anticholinergic and antidiabetic properties of mountain mint (Cyclotrichium leucotrichum)." Chemistry & Biodiversity, (**2022**) 19(3), e202100775.

[6] Burmaoglu, S., Yilmaz, A. O., Polat, M. F., Kaya, R., Gulcin, İ., & Algul, O. "Synthesis and biological evaluation of novel tris-chalcones as potent carbonic anhydrase, acetylcholinesterase, butyrylcholinesterase, and α-glycosidase inhibitors." Bioorganic chemistry, (**2019**) 85, 191-197.

[7] Aksu, K., Akıncıoğlu, H., Akıncıoğlu, A., Goksu, S., Tuemer, F., & Gulcin, I. "Synthesis of novel sulfonamides incorporating phenethylamines and determination of their inhibition profiles against some metabolic enzymes." Archiv der pharmazie, (**2018**) 351(9), 1800150.

[8] [8] Turkan, F., Cetin, A., Taslimi, P., & Gulçin, İ. "Some pyrazole derivatives: Potent carbonic anhydrase, α-glycosidase, and cholinesterase enzyme inhibitors." Archiv der pharmazie, (**2018**) 351(10), 1800200.

[9] Vitaku, E., Smith, D. T., & Njardarson, J. T. "Analysis of the structural diversity, substitution patterns, and frequency of nitrogen heterocycles among US FDA approved pharmaceuticals: mini perspective." Journal of medicinal chemistry, (**2014**). 57(24), 10257-10274.

[10] Kerru, N., Gummidi, L., Maddila, S., Gangu, K. K., & Jonnalagadda, S. B. "A review of recent advances in nitrogen-containing molecules and their biological applications." Molecules, (**2020**) 25(8), 1909.

[11] Ellman, G. L., Courtney, K. D., Andres Jr, V., & Featherstone, R. M."A new and rapid colorimetric determination of acetylcholinesterase activity." Biochemical pharmacology, (**1961**) 7(2), 88-95.

[12] Garibov, E., Taslimi, P., Sujayev, A., Bingol, Z., Cetinkaya, S., Gulcin, İ., ... & Supuran, C. T. "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." Journal of enzyme inhibition and medicinal chemistry, (**2016**) 31(sup3), 1-9.

[13] Mao, F., Li, J., Wei, H., Huang, L., & Li, X. "Tacrine–propargyl amine derivatives with improved acetylcholinesterase inhibitory activity and lower hepatotoxicity as a potential lead compound for the treatment of Alzheimer's disease." Journal of Enzyme Inhibition and Medicinal Chemistry, (**2015**) 30(6), 995-1001.

[14] Taslimi, P., Erden, Y., Mamedov, S., Zeynalova, L., Ladokhina, N., Tas, R., ... & Gulcin, I. "The biological activities, molecular docking studies, and anticancer effects of 1-arylsuphonylpyrazole derivatives." Journal of biomolecular structure and dynamics, (**2021**) 39(9), 3336-3346.

[15] Schrödinger Release Glide; Schrödinger, LLC: New York, NY, USA. (**2020-3**)

[16] Nepovimova, E., Uliassi, E., Korabecny, J., Pena-Altamira, L. E., Samez, S., Pesaresi, A., ... & Bolognesi, M. L. "Multitarget drug design strategy: quinone–tacrine hybrids designed to block amyloid-β aggregation and to exert anticholinesterase and antioxidant effects." Journal of medicinal chemistry, (**2014**) 57(20), 8576-8589.

[17] Brus, B., Kosak, U., Turk, S., Pislar, A., Coquelle, N., Kos, J., ... & Gobec, S. "Discovery, biological evaluation, and crystal structure of a novel nanomolar selective butyrylcholinesterase inhibitor." Journal of medicinal chemistry, (**2014**) 57(19), 8167-8179.

[18] Işık, M. "The binding mechanisms and inhibitory effect of intravenous anesthetics on AChE in vitro and in vivo: kinetic analysis and molecular docking." Neurochemical research, (**2019**) 44(9), 2147-2155.

[19] Ozgun, D. O., Yamali, C., Gul, H. I., Taslimi, P., Gulcin, I., Yanik, T., & Supuran, C. T. "Inhibitory effects of isatin Mannich bases on carbonic anhydrases, acetylcholinesterase, and butyrylcholinesterase." Journal of enzyme inhibition and medicinal chemistry, (**2016**) 31(6), 1498-1501.

[20] Aksu, K., Topal, F., Gulcin, I., Tümer, F., & Göksu, S. "Acetylcholinesterase inhibitory and antioxidant activities of novel symmetric sulfamides derived from phenethylamines." Archiv der Pharmazie, (**2015**) 348(6), 446-455.

[21] Ökten, S., Ekiz, M., Koçyiğit, Ü. M., Tutar, A., Çelik, İ., Akkurt, M., ... & Gülçin, İ. "Synthesis, characterization, crystal structures, theoretical calculations and biological evaluations of novel substituted tacrine derivatives as cholinesterase and carbonic anhydrase enzymes inhibitors." Journal of Molecular Structure, (**2019**) 1175, 906-915.