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Research Article

Investigation of dual AChE/MAO inhibitory activities of new morpholine and piperazine structured compounds

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ABSTRACT

In this study, a series of new compounds containing piperazine and morpholine rings were synthesized. Characterization studies of the obtained compounds were carried out with the help of HRMS, ^{1}H-NMR and ^{13}C-NMR spectroscopic methods. Acetylcholinesterase (AChE) / Monoamine oxidase B (MAO-B) inhibitory potentials of the compounds were investigated using *in silico* and *in vitro* methods. Compound **3a** was the compound with the highest inhibitory potential against AChE and MAO-B enzymes, with IC_{50}=0.065±0.002 µM and IC_{50}=0.072±0.003 µM values, respectively. Compounds **3a** and **3b** interacted with crucial amino acid residues of the hMAO-B (PDB ID: 2V5Z) and AChE (PDB ID: 4EY7) enzymes in the docking studies. Compounds **3a** and **3b** had the highest affinity for the AChE and MAO-B enzymes.

Keywords: AChE, MAO-B, Piperazine, Morpholine, Docking

1. INTRODUCTION

One of the most serious health issues is Alzheimer's disease (AD). Today, it is one of the leading causes of dementia and directly affects the lives of many people. For this reason, radical and updated treatments are needed for the treatment of AD. There are currently different treatment approaches for AD [1–4].

FDA-approved Acetylcholinesterase (AChE) inhibitors such as donepezil and tacrine are actively used in the treatment of AD. In addition, Monoamine oxidase B (MAO-B) inhibitors are known to degrade reactive oxygen species (ROS) and hydrogen peroxide levels. Compounds that have more effects than only inhibiting the AChE, butyrylcholinesterase (BChE) or MAO-B enzyme have been the subject of numerous investigations [5–9]. In the healthy human brain, AChE activity suppresses BChE activity. The most important feature that distinguishes BChE from AChE is its kinetic responses to ACh concentrations. At low ACh concentrations, BChE is less effective in AChE hydrolysis, but when high ACh concentrations inhibit AChE, BChE begins to show more activity [10]. Numerous benefits can be obtained by using a single chemical to inhibit multiple enzymes, according to studies. Achieving the ideal acetylcholine level, preventing the formation of β amyloid plaque, and other similar activities make

compounds that simultaneously inhibit MAO-B and ChE enzymes important in the search for new compounds to treat neurodegenerative diseases like AD [11–16]. These compounds are also expected to have a neuroprotective effect.

Morpholine and piperazine are 6-membered ring systems containing heteroatoms. It is known that both rings play an active role in both AChE inhibition and MAO-B inhibition. Because of its non-planar, flexible shape, piperazine forms hydrogen bonds with target enzymes. The structure of many pharmacologically active compounds from various indication groups includes piperazine. Since piperazine's hydrophobic nature helps the structure it is a part of across the blood-brain barrier, it is widely employed in studies on the treatment of AD, Parkinson's disease and other neurodegenerative illnesses [12,14,17,18].

Many AChE/MAO-B dual enzyme inhibitors developed today have heterocyclic rings in their structure. And in this direction, five new compounds containing piperazine and morpholine rings were synthesized in this study. The synthesized compounds were subjected to characterization tests. Then, *in silico* and *in vitro* studies of obtained compounds were carried out.

2. MATERIALS AND METHODS

2.1. Chemistry

Every reagent that was acquired from a commercial provider was utilized without any additional purification. The melting points of the compounds were determined with a device (MP90, Mettler-Toledo, OH, USA). The results were given without correction. NMR (nuclear magnetic resonance) spectroscopy was recorded on ^{1}H-NMR Bruker DPX 300 FT-NMR spectrometer; ^{13}C-NMR, Bruker DPX 75 MHz spectrometer (Bruker Bioscience, Billerica, MA, USA). Mass spectra were recorded on a LCMS-IT-TOF (Shimadzu, Kyoto, Japan) using ESI.

2.1.1. Synthesis of 2-chloro-N-(4-morpholinophenyl)acetamide (1)

First, 4-morpholinoaniline (1.78 g, 0.010 mol) was dissolved in 20 mL dimethylformamide (DMF). Then, triethylamine (TEA) (1.02 g, 0.010 mol) was added to the solution and placed in an ice bath. Finally, chloroacetyl chloride (1.12 g, 0.010 mol) was added dropwise to the mixture. After 1 hour of mixing, the precipitated product was filtered and separated from the medium. The obtained product was crystallized from ethanol.

2.1.2. Synthesis of sodium 4-methylpiperazine-1-carbodithioate derivatives (2a-e)

Piperazine derivatives (0.005 mol) and NaOH (0.20 g, 0.005 mol) were dissolved in absolute ethanol. Then, carbon disulfide (0.38 g, 0.005 mol) was added dropwise to the solution placed in an ice bath. After two hours of mixing, the precipitated product was filtered.

2.1.3. Synthesis of target compounds (3a-e)

In acetone, 2-chloro-*N*-(4-morpholinophenyl) acetamide (**1**) (0.38 g, 0.0015 mol) and sodium 4-methylpiperazine-1-carbodithioate derivatives (**2a-e**) (0.0015 mol) were mixed for four hours. Once the reaction was finished, acetone was removed with less pressure. After the precipitated product was dried, it was rinsed with water to remove any remaining salt and recrystallized from EtOH.

*2-((4-Morpholinophenyl)amino)-2-oxoethyl 4-methylpiperazine-1-carbodithioate (**3a**)*

Yield: 81%, M.p.: 166.2-166.6°C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.33 (3H, s), 2.59 (4H, brs), 3.01-3.04 (4H, m), 3.70-3.73 (4H, m), 4.01 (2H, br.s.), 4.21-4.26 (4H, m), 6.88 (2H, d, J= 9.08 Hz), 7.43 (2H, d, J= 9.03 Hz), 10.10 (1H, s). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 41.9, 45.0, 49.3, 54.0, 66.6, 115.9, 120.6, 131.7, 147.7, 164.9, 195.4. HRMS (m/z): $[M+H]^+$ calcd for $C_{18}H_{26}N_4O_2S_2$: 395.1570; found 395.1560.

*2-((4-Morpholinophenyl)amino)-2-oxoethyl 4-ethylpiperazine-1-carbodithioate (**3b**)*

Yield: 78%, M.p.: 160.0-160.3°C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.02 (3H, t, *J*=7.17 Hz), 2.34-2.41 (2H, m), 2.47 (4H, brs), 3.01-3.04 (4H, m), 3.70-3.73 (4H, m), 3.94 (2H, brs), 4.20 (4H, s), 6.88 (2H, d, *J*= 9.09 Hz), 7.43 (2H, d, *J*= 9.04 Hz), 10.07 (1H, s). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 12.3, 41.8, 49.3, 50.1, 51.5, 52.2, 66.6, 115.9, 120.6, 131.8, 147.7, 165.0, 194.9. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{28}N_4O_2S_2$: 409.1726; found 409.1723.

*2-((4-Morpholinophenyl)amino)-2-oxoethyl 4-(4-fluorophenyl)piperazine-1-carbodithioate (**3c**)*

Yield: 79%, M.p.: 187.9-188.3°C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 3.01-3.04 (4H, m), 3.23 (4H, brs), 3.70-3.73 (4H, m), 4.10 (2H, brs), 4.23 (2H, s), 4.35 (2H, brs), 6.89 (2H, d, *J*= 8.98 Hz), 6.94-7.01 (2H, m), 7.04-7.11 (2H, m), 7.44 (2H, d, *J*= 8.93 Hz), 10.09 (1H, s). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 41.8, 49.0, 49.2, 49.3, 49.9, 51.1, 53.2, 66.6, 115.8, 115.9, 116.1, 117.9, 118.0, 120.6, 131.7, 147.4, 147.8, 155.2, 158.3, 165.0, 195.2. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{23}H_{27}N_4O_2FS_2$: 475.1632; found 475.1636.

*2-((4-Morpholinophenyl)amino)-2-oxoethyl 4-(4-(trifluoromethyl)phenyl)piperazine-1-carbodithioate (**3d**)*

Yield: 77%, M.p.: 188.9-190.7°C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 3.01-3.04 (4H, m), 3.51 (4H, br.s.), 3.70-3.73 (4H, m), 4.13 (2H, brs), 4.24 (2H, s), 4.35 (2H, brs), 6.89 (2H, d, *J*= 8.94 Hz), 7.03 (2H, d, *J*= 8.77 Hz), 7.44 (2H, d, *J*= 8.67 Hz), 7.54 (2H, d, *J*= 8.76 Hz), 10.10 (1H, s). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 41.7, 46.2, 49.3, 50.8, 66.6, 114.1, 115.9, 120.6, 126.7, 131.7, 147.8, 152.6, 165.0, 195.2. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{27}N_4O_4F_3S_2$: 525.1600; found 525.1607.

*2-((4-Morpholinophenyl)amino)-2-oxoethyl 4-(4-nitrophenyl)piperazine-1-carbodithioate (**3e**)*

Yield: 79%, M.p.: 104.3-105.3°C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 3.01-3.04 (4H, m), 3.51 (4H, brs), 3.70-3.73 (8H, m), 4.15 (2H, brs), 4.25 (2H, s), 4.35 (2H, brs), 6.89 (2H, d, *J*=8.94 Hz), 6.94 (2H, d, *J*=9.44 Hz), 7.44 (2H, d, *J*=8.85 Hz), 8.10 (2H, d, *J*=9.23 Hz), 10.10 (1H, s). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 41.7, 45.1, 49.3, 50.5, 66.6, 112.3, 115.9, 120.6, 126.3, 131.7, 137.3, 147.8, 154.2, 164.9, 195.2. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{23}H_{27}N_5O_4S_2$: 502.1577; found 502.1572.

2.2. MAO Enzymes Inhibition Assay

Using the available fluorometric method, the *in vitro* MAO inhibition test was carried out and the percentages and IC_{50} values of the compounds obtained were computed in accordance with the previously published research group description [19–22].

2.3. Cholinesterase Enzymes Inhibition Assay

In vitro inhibitory potencies of compounds **3a**-**3e** against to AChE and BChE were investigated as previously published [18,23–28].

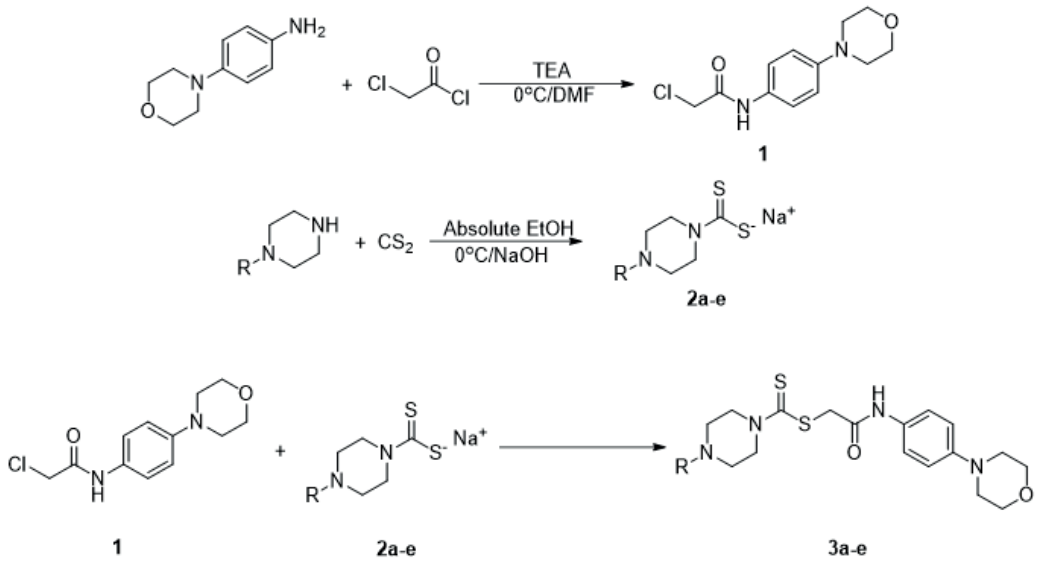
2.4. Molecular Docking Study

Molecular docking investigations were carried out as previously published [9,19,27]. Similar programs were used during the studies [28–31].

3. RESULTS AND DISCUSSION

3.1. Chemistry

As depicted in Scheme 1, the compounds **3a-3e** were synthesized. First, 2-chloro-*N*-(4-morpholinophenyl) acetamide (**1**) was obtained by acetylation of 4-morpholinoaniline. Then, dithiocarbamate salts (**2a-2e**) were obtained from piperazine derivatives with the help of carbon disulfide and NaOH. The target compounds (**3a-3e**) were obtained as a result of the reaction of 2-chloro-*N*-(4-morpholinophenyl) acetamide (**1**) and sodium 4-methylpiperazine-1-carbodithioate derivatives (**2a-2e**).



Compounds	R
3a	$-CH_3$
3b	$-CH_2-C(CH_3)$
3c	4-fluorophenyl
3d	4-trifluoromethylphenyl
3e	4-nitrophenyl

Scheme 1. Synthesis pathway for obtained compounds **(3a-3e)**

Table 1. IC_{50} Values of synthesized compounds, moclobemide and selegiline against MAO enzymes

Compound	MAO-A IC_{50} (µM)	MAO-B IC_{50} (µM)
3a	0.209±0.009	0.072±0.003
3b	0.371±0.017	0.109±0.004
3c	>100	0.167±0.007
3d	>1000	>100
3e	>100	0.212±0.006
Moclobemide	6.0613±0.2625	-
Selegiline	-	0.0374±0.0016

3.2. MAO Enzymes Inhibition Assay

MAO enzyme inhibition test results are presented in Table 1. In order to compare the enzyme inhibition potential of the compounds; moclobemide was chosen as the reference inhibitor molecule for the MAO-A enzyme and selegiline was chosen as the reference inhibitor molecule for the MAO-B enzyme. The compounds show selectivity towards the MAO-B enzyme. Compounds **3a** and **3b** were the compounds with the closest inhibitory potential to selegiline with their IC_{50} values (**3a** IC_{50}=0.072±0.003 µM, **3b** IC_{50}=0.109±0.004 µM) against the MAO-B enzyme.

3.3. Cholinesterase Enzymes Inhibition Assay

Cholinesterase enzyme inhibition test results are presented in Table 2. In order to compare the enzyme inhibition potential of the compounds; donepezil was chosen as the reference inhibitor molecule for the AChE enzyme and tacrine was chosen as the reference inhibitor molecule for the BChE enzyme.

Table 2. IC_{50} Values of synthesized compounds, donepezil and tacrine against AChE/BChE enzymes

Compound	AChE IC_{50} (µM)	BChE IC_{50} (µM)
3a	0.065±0.002	>1000
3b	0.084±0.003	>1000
3c	0.139±0.006	>1000
3d	0.285±0.013	>1000
3e	0.194±0.008	>1000
Donepezil	0.0201±0.0014	-
Tacrine	-	0.0064±0.0002

The compounds show selectivity towards the AChE enzyme. Compounds **3a** and **3b** were the compounds with the closest inhibitory potential to donepezil with their IC_{50} values (**3a** IC_{50}=0.065±0.002 µM, **3b** IC_{50}=0.084±0.003 µM) against the AChE enzyme.

3.4. Molecular Docking Study

The 2D and 3D binding model of compound **3a** with AChE enzyme (PDB ID:4EY7) is presented in Figure 1 and Figure 2, respectively. When the relevant models are examined, it is seen that compound **3a** has pi-pi interactions with Tyr337, His447 and H bonds with Tyr124. Figure 3 and Figure 4 show the 2D and 3D binding models of compound **3b** with the AChE enzyme (PDB ID:4EY7), respectively. Compound **3b** has a salt bridge with Asp74 and pi-pi interactions with Trp286 when the relevant models are looked at. As a result of these observations, it appears that both compounds interact with the catalytic active site of the AChE. While this interaction is provided through the phenyl ring in compound **3a**, it is provided through the piperazine ring in compound **3b**. In addition, the phenyl ring of compound **3b** interacted with the peripheral anionic region of the AChE, just like donepezil.

The 2D and 3D binding model of compound **3a** with hMAO-B enzyme (PDB ID:2V5Z) is presented in Figure 5 and Figure 6, respectively. When the relevant models are examined, it is seen that compound **3a** has pi-cation interactions with Tyr435. The 2D and 3D binding model of compound **3b** with hMAO-B enzyme (PDB ID:2V5Z) is presented in Figure 7 and Figure 8, respectively. When the relevant models are examined, it is seen that compound **3b** has pi-pi interactions with Tyr326. These interactions are provided by the piperazine ring in compound **3a** and the phenyl ring in compound **3b**.

4. CONCLUSION

Within the scope of this study, five piperazine/morpholine derivative compounds were designed and synthesized. Then, characterization studies of the obtained compounds were carried out. The biological activities of the obtained compounds were investigated by *in silico* and *in vitro* methods. The results of *in silico* and *in vitro* studies are in agreement with each other. In the docking studies, compound **3a** and compound **3b** showed interactions against AChE enzyme (PDB ID: 4EY7) and hMAO-B enzyme (PDB ID: 2V5Z) crystals. In *in vitro* activity studies, compound **3a** and compound **3b** have the highest affinity for AChE and MAO-B. When the molecular docking results were examined, interactions were observed with amino acids Asp74, Tyr124, Trp286, Tyr337 and His447, which are known to positively affect AChE activity. Among these interactions, Trp286 can be identified as the key interaction for AChE activity. A similar interaction is observed between the reference drug donepezil and Trp286 [32,33]. It is known that amino acids Tyr435 and Tyr326 are vital in the catalytic activity and selectivity against the MAO-B enzyme [34,35]. It is pleasing in this respect that compound **3a** interacts with Tyr435 and compound **3b** interacts with Tyr326. When the results of *in vitro* and *in silico* activity studies were evaluated, the compounds with the highest inhibitory potential against MAO-B enzyme and AChE enzyme were compound **3a** and compound **3b**. As a result, five new compounds were successfully synthesized, characterization studies were carried out and activity studies were started. *In silico* and *in vitro* activity studies were also successfully completed. And it is seen that all the studies were carried out in harmony and were successfully concluded. In the light of these results, it was observed that piperazine/morpholine derivatives could be potential dual AChE/MAO-B enzyme inhibitors.

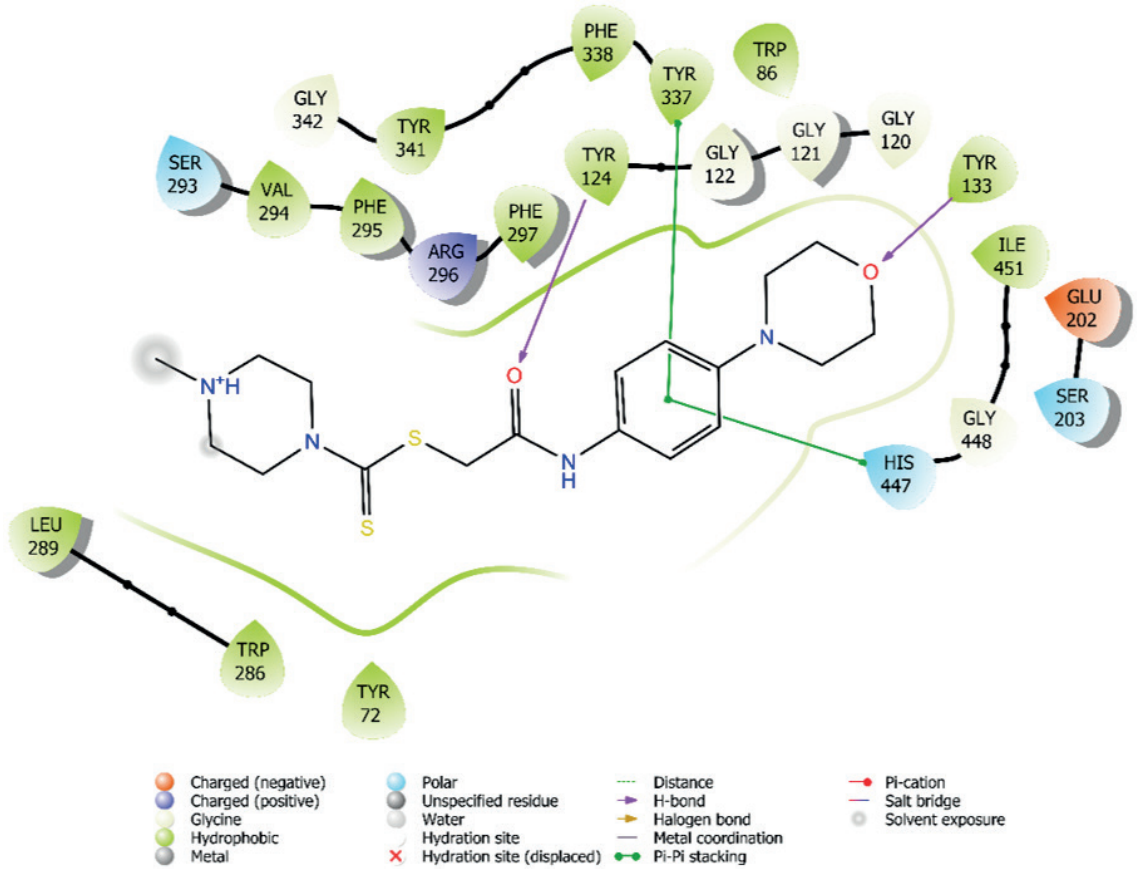


Figure 1. 2D pose of compound **3a** with AChE enzyme (PDB ID: 4EY7)

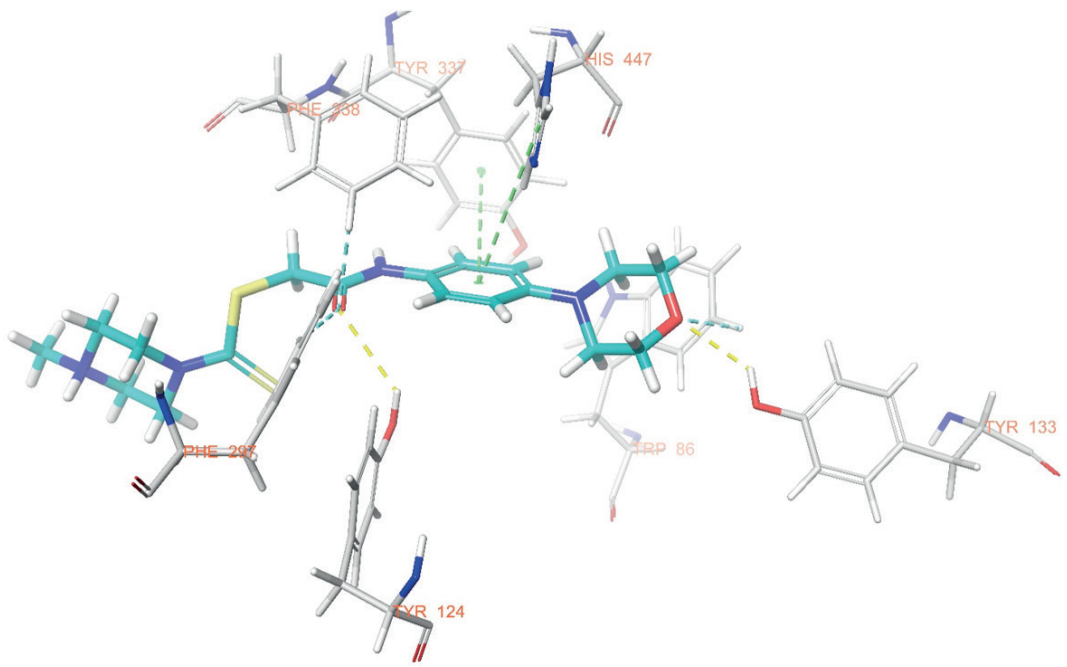


Figure 2. 3D pose of compound **3a** with AChE enzyme (PDB ID: 4EY7)

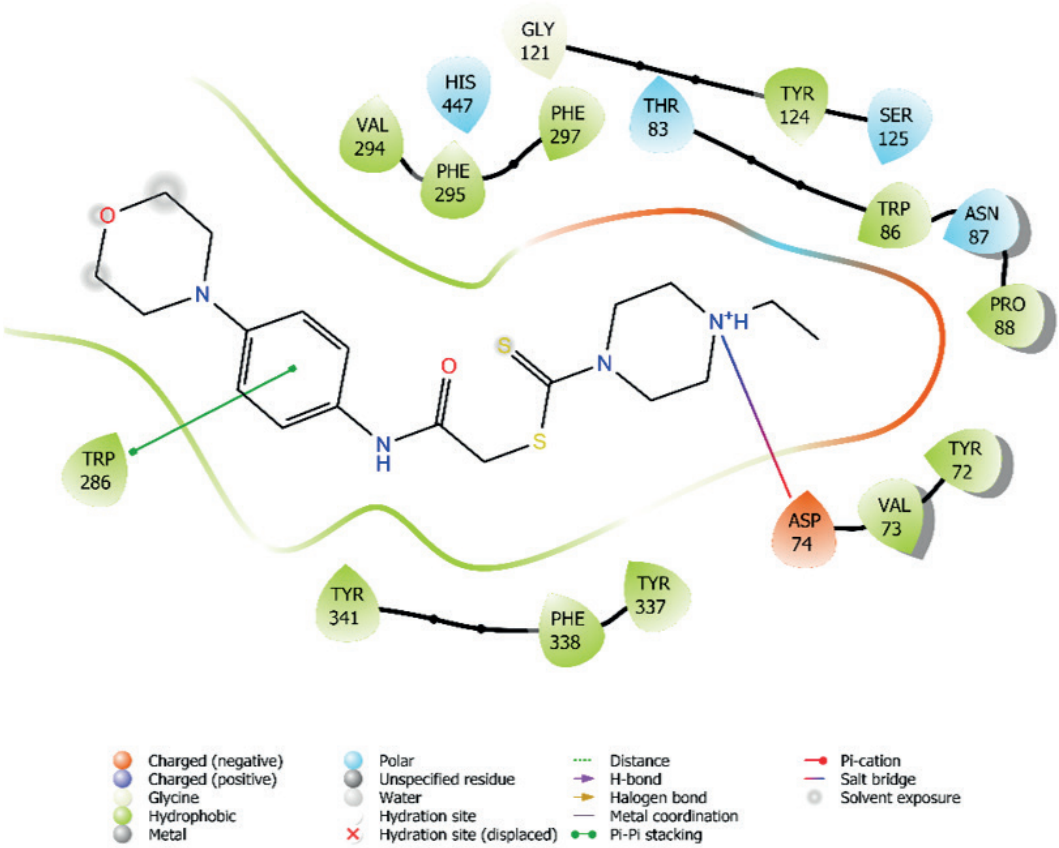


Figure 3. 2D pose of compound **3b** with AChE enzyme (PDB ID: 4EY7)

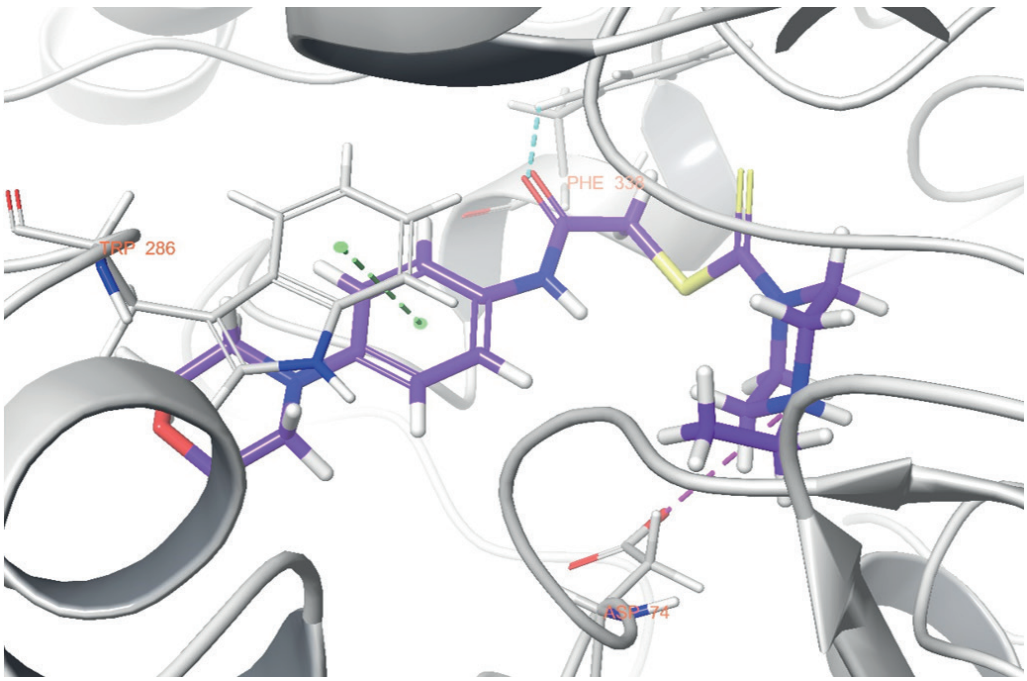


Figure 4. 3D pose of compound **3b** with AChE enzyme (PDB ID: 4EY7)

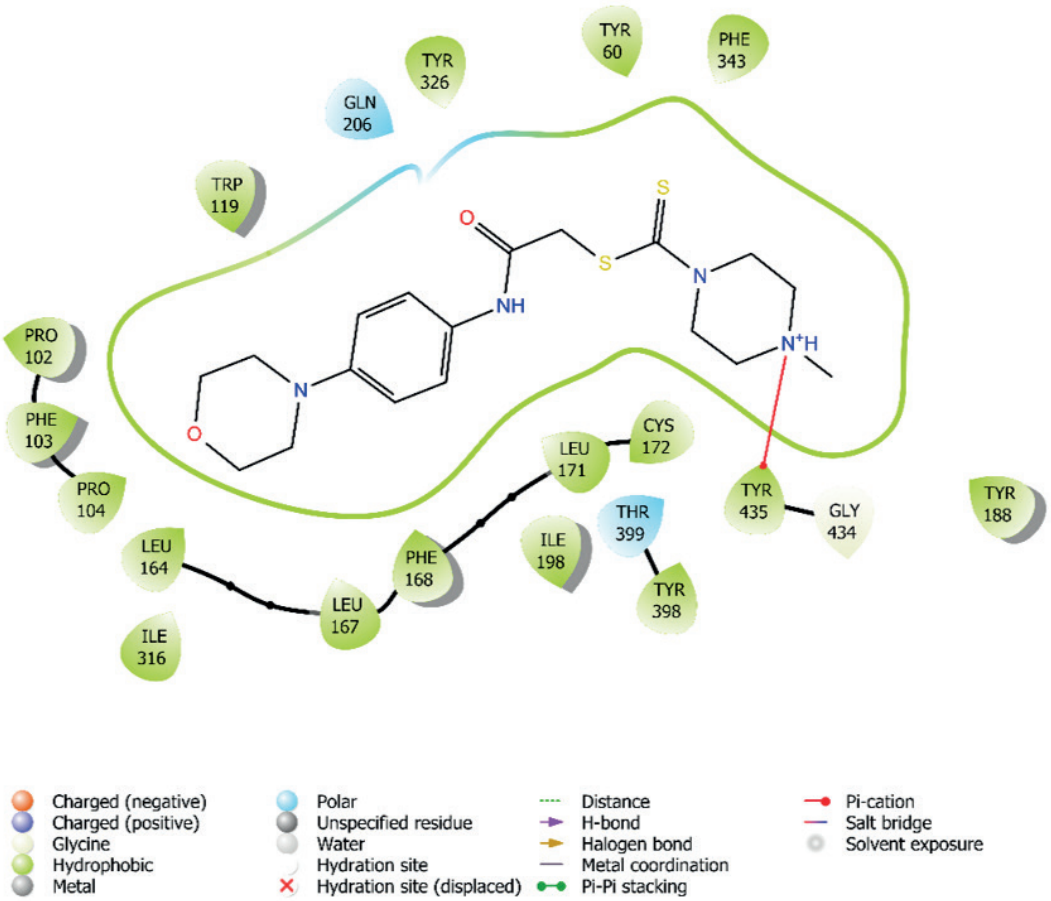


Figure 5. 2D pose of compound **3a** with hMAO-B enzyme (PDB ID: 2V5Z)

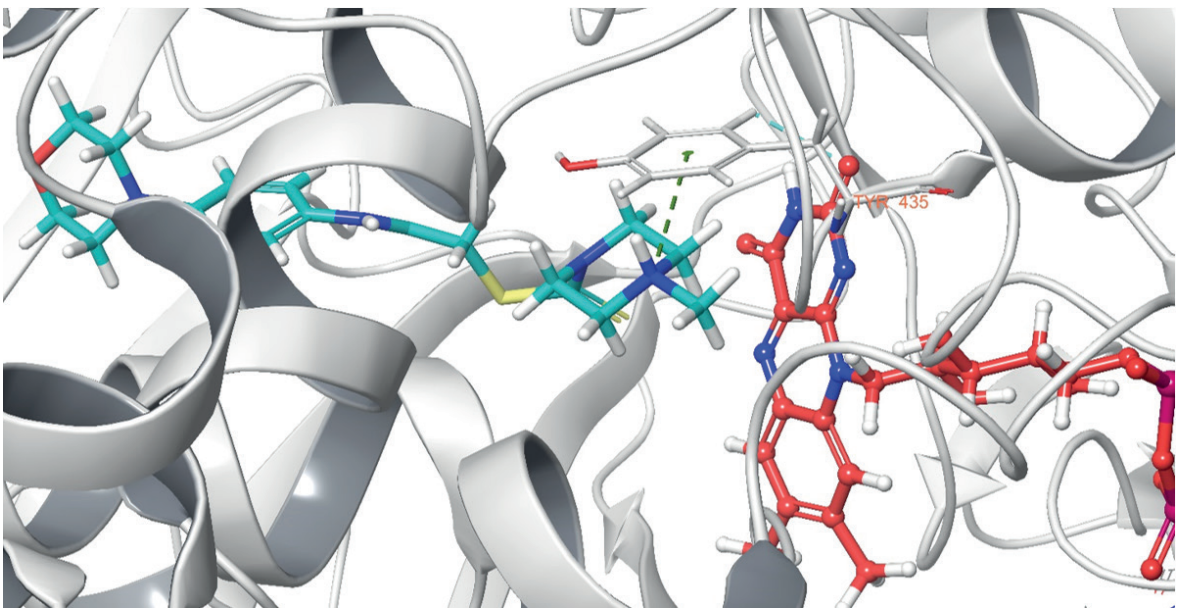


Figure 6. 3D pose of compound **3a** with hMAO-B enzyme (PDB ID: 2V5Z)

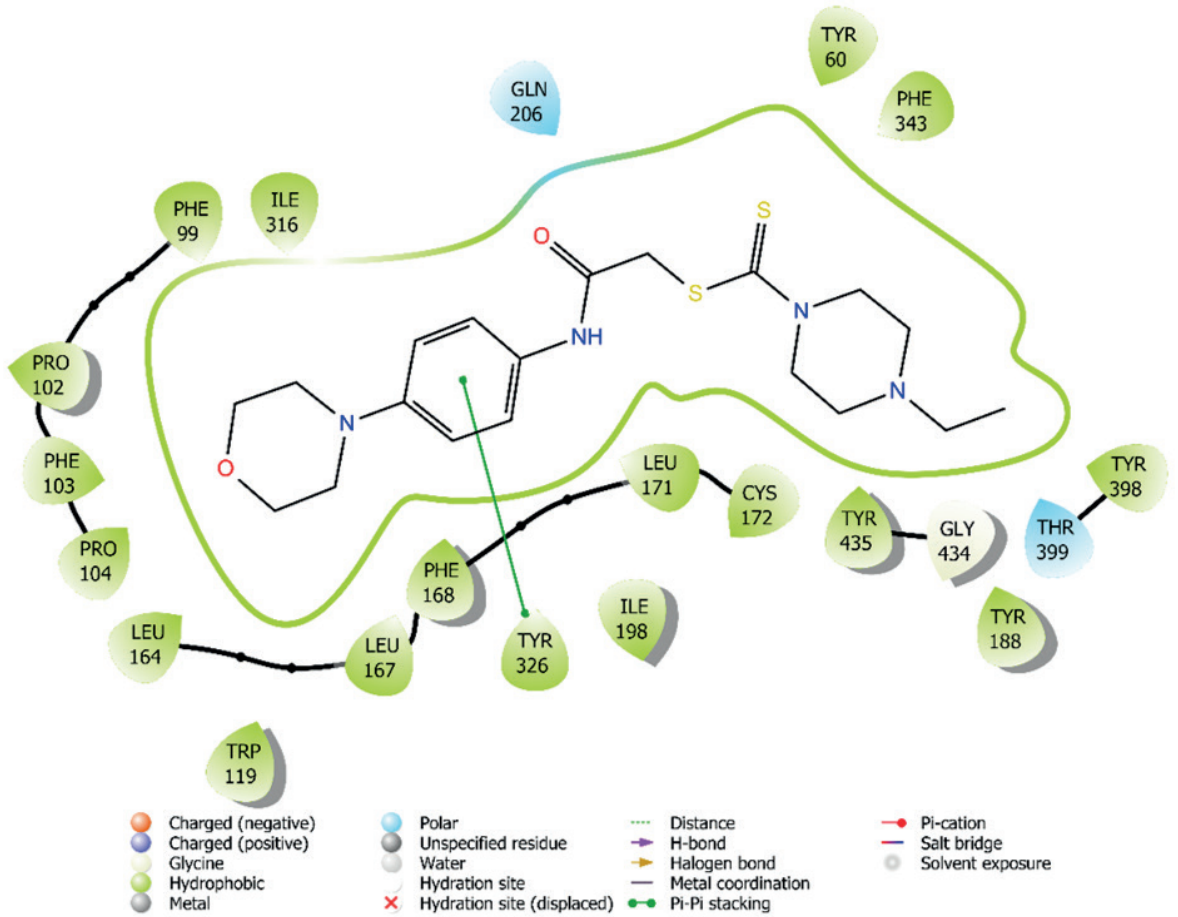


Figure 7. 2D pose of compound **3b** with hMAO-B enzyme (PDB ID: 2V5Z)

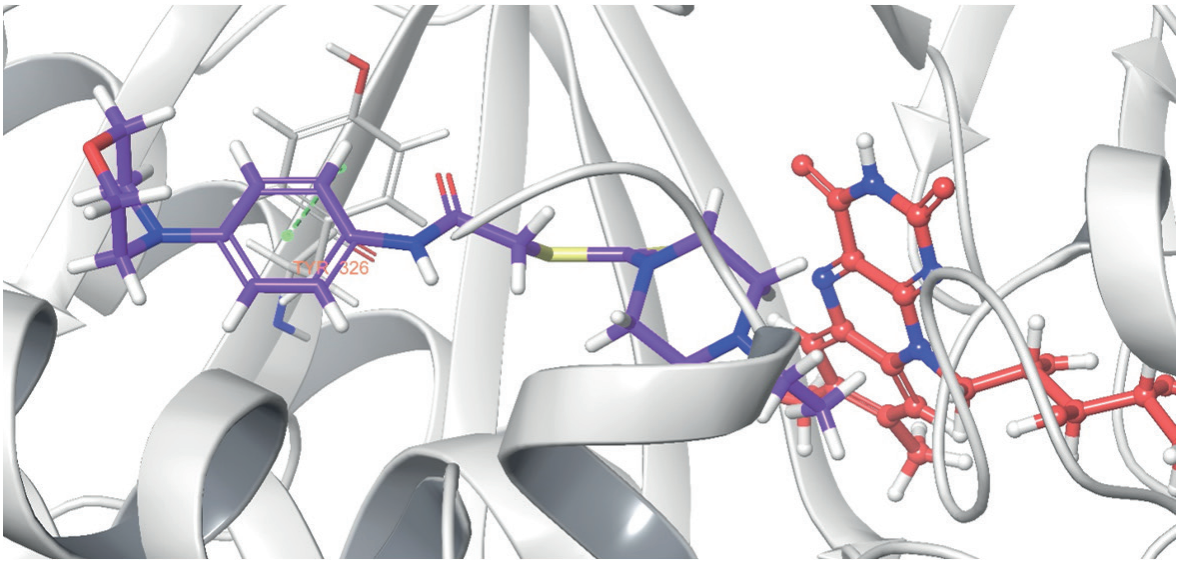


Figure 8. 3D pose of compound **3b** with hMAO-B enzyme (PDB ID: 2V5Z)

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Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Conceptualization, B.K. and Z.A.K.; Methodology, B.K., D.O. and B.N.S.Ö.; Software, D.O.; Validation, Z.A.K.; Formal analysis, B.K.; Investigation, D.O. and B.K.; Resources, B.K., D.O. and B.N.S.Ö.; Data curation, B.K. and Z.A.K.; Writing—original draft preparation, B.K., D.O. and B.N.S.Ö.; Writing—review and editing, B.K. and Z.A.K.; Visualization, D.O.; Supervision, Z.A.K. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. 2024 Alzheimer's disease facts and figures. Alzheimers Dement. (2024); 20:3708–3821. https://doi.org/10.1002/alz.13809
2. Borenstein AR, Copenhaver CI, Mortimer JA. Early-life risk factors for Alzheimer disease. Alzheimer Dis Assoc Disord. (2006); 20(1):63-72. https://doi.org/10.1097/01.wad.0000201854.62116.d7
3. Reitz C, Mayeux R. Alzheimer disease: Epidemiology, diagnostic criteria, risk factors and biomarkers. Biochem Pharmacol. (2014); 88(4):640–651. https://doi.org/10.1016/j.bcp.2013.12.024
4. Scheltens P, De Strooper B, Kivipelto M, Holstege H, Chételat G, Teunissen CE, Cummings J, van der Flier WM. Alzheimer's disease. Lancet. (2021); 397(10284):1577–1590. https://doi.org/10.1016/S0140-6736(20)32205-4
5. Ibach B, Haen E. Acetylcholinesterase Inhibition in Alzheimers Disease. Curr Pharm Des. (2004); 10(3):231-251. https://doi.org/10.2174/1381612043386509
6. Thomas T. Monoamine oxidase-B inhibitors in the treatment of Alzheimers disease. Neurobiol Aging. (2000); 21(2):343-348. https://doi.org/10.1016/S0197-4580(00)00100-7
7. Christen Y. Oxidative stress and Alzheimer disease. Am J Clin Nutr. (2000); 71(2):621-629. https://doi.org/10.1093/ajcn/71.2.621s
8. Cecilia Rodrigues Simoes M, Pereira Dias Viegas F, Soares Moreira M, de Freitas Silva M, Maximo Riquiel M, Mattos da Rosa P, Rosa Castelli M, Henrique dos Santos M, Gomes Soares M, Viegas C. Donepezil: an important prototype to the design of new drug candidates for Alzheimer's disease. Mini Rev Med Chem. (2014); 14(1):2–19. http://dx.doi.org/10.2174/1389557513666131119201353
9. Sağlık BN, Levent S, Osmaniye D, Evren AE, Karaduman AB, Özkay Y, Kaplancıklı ZA. Design, synthesis, and *in vitro* and *in silico* approaches of novel indanone derivatives as multifunctional anti-Alzheimer agents. ACS Omega. (2022); 7(50):47378–47404. http://dx.doi.org/10.1021/acsomega.2c06906
10. Greig NH, Utsuki T, Ingram DK, Wang Y, Pepeu G, Scali C, Yu Q-S, Mamczarz J, Holloway HW, Giordano T, et al. Selective butyrylcholinesterase inhibition elevates brain acetylcholine, augments learning and lowers Alzheimer β-amyloid peptide in rodent. Proc Natl Acad Sci USA. (2005); 102(47):17213–17218. http://dx.doi.org/10.1073/pnas.0508575102
11. Uddin MS, Kabir MT, Rahman MM, Mathew B, Shah MA, Ashraf GM. TV 3326 for Alzheimer's dementia: a novel multimodal ChE and MAO inhibitors to mitigate Alzheimer's-like neuropathology. J Pharm Pharmacol. (2020); 72(8):1001–1012. http://dx.doi.org/10.1111/jphp.13244
12. Mathew B, Baek SC, Thomas Parambi DG, Lee JP, Mathew GE, Jayanthi S, Vinod D, Rapheal C, Devikrishna V, Kondarath SS, et al. Potent and highly selective dual-targeting monoamine oxidase-B inhibitors: Fluorinated chalcones of morpholine versus imidazole. Arch Pharm. (2019); 352(4): http://dx.doi.org/10.1002/ardp.201800309
13. Mathew B, Parambi DGT, Mathew GE, Uddin MdS, Inasu ST, Kim H, Marathakam A, Unnikrishnan MK, Carradori S. Emerging therapeutic potentials of dual-acting MAO and AChE inhibitors in Alzheimer's and Parkinson's diseases. Arch Pharm. (2019); 352(11):1900177. https://doi.org/10.1002/ardp.201900177
14. Sasidharan R, Eom BH, Heo JH, Park JE, Abdelgawad MA, Musa A, Gambacorta N, Nicolotti O, Manju SL, Mathew B, et al. Morpholine-based chalcones as dual-

acting monoamine oxidase-B and acetylcholinesterase inhibitors: synthesis and biochemical investigations. J Enzyme Inhib Med Chem. (2021); 36(1):188–197. https://doi.org/10.1080/14756366.2020.1842390

15. El-Damasy AK, Park JE, Kim HJ, Lee J, Bang EK, Kim H, Keum G. Identification of new *N*-methyl-piperazine chalcones as dual MAO-B/AChE inhibitors. Pharmaceuticals (2023); 16(1):83. https://doi.org/10.3390/ph16010083

16. Osmaniye D, Evren AE, Sağlık BN, Levent S, Özkay Y, Kaplancıklı ZA. Design, synthesis, biological activity, molecular docking, and molecular dynamics of novel benzimidazole derivatives as potential AChE/MAO-B dual inhibitors. Arch Pharm. (2022); 355(3):2100450. https://doi.org/10.1002/ardp.202100450

17. Mathew B, Oh JM, Baty RS, Gaber &, Batiha E-S, Grace D, Parambi T, Gambacorta N, Nicolotti O, Kim H. Piperazine-substituted chalcones: a new class of MAO-B, AChE, and BACE-1 inhibitors for the treatment of neurological disorders. Environ Sci Pollut Res. (2021); 28:38855-38866 https://doi.org/10.1007/s11356-021-13320-y

18. Levent S, Acar Çevik U, Sağlık BN, Özkay Y, Can ÖD, Özkay ÜD, Uçucu Ü. Anticholinesterase activity screening of some novel dithiocarbamate derivatives including piperidine and piperazine moieties. Phosphorus Sulfur Silicon Relat Elem. (2017); 192(4):469–474. https://doi.org/10.1080/10426507.2016.1259228

19. Tok F, Uğraş Z, Sağlık BN, Özkay Y, Kaplancıklı ZA, Koçyiğit-Kaymakçıoğlu B. Novel 2,5-disubstituted-1,3,4-oxadiazole derivatives as MAO-B inhibitors: Synthesis, biological evaluation and molecular modeling studies. Bioorg Chem. (2021); 112:104917. https://doi.org/10.1016/j.bioorg.2021.104917

20. Tok F, Sağlık BN, Özkay Y, Ilgın S, Kaplancıklı ZA, Koçyiğit-Kaymakçıoğlu B. Synthesis of new hydrazone derivatives and evaluation of their monoamine oxidase inhibitory activity. Bioorg Chem. (2021); 114:105038. https://doi.org/10.1016/j.bioorg.2021.105038

21. Can NÖ, Osmaniye D, Levent S, Sağlık BN, Korkut B, Atlı Ö, Özkay Y, Kaplancıklı ZA. Design, synthesis and biological assessment of new thiazolylhydrazine derivatives as selective and reversible hMAO-A inhibitors. Eur J Med Chem. (2018); 144:68–81. https://doi.org/10.1016/j.ejmech.2017.12.013

22. Sağlık BN, Kaya Çavuşoğlu B, Osmaniye D, Levent S, Acar Çevik U, Ilgın S, Özkay Y, Kaplancıklı ZA, Öztürk Y. *In vitro* and *in silico* evaluation of new thiazole compounds as monoamine oxidase inhibitors. Bioorg Chem. (2019); 85:97–108. https://doi.org/10.1016/j.bioorg.2018.12.019

23. Sağlık BN, Ilgın S, Özkay Y. Synthesis of new donepezil analogues and investigation of their effects on cholinesterase enzymes. Eur J Med Chem. (2016); 124:1026–1040. https://doi.org/10.1016/j.ejmech.2016.10.042

24. Demir Özkay Ü, Can ÖD, Sağlık BN, Acar Çevik U, Levent S, Özkay Y, Ilgın S, Atlı Ö. Design, synthesis, and AChE inhibitory activity of new benzothiazole–piperazines. Bioorg Med Chem Lett. (2016); 26(22):5387–5394. https://doi.org/10.1016/j.bmcl.2016.10.041

25. Hussein W, Sağlık BN, Levent S, Korkut B, Ilgın S, Özkay Y, Kaplancıklı ZA. Synthesis and biological evaluation of new cholinesterase inhibitors for Alzheimer's disease. Molecules. (2018); 23(8):2033. https://doi.org/10.3390/molecules23082033

26. Acar Cevik U, Saglik BN, Levent S, Osmaniye D, Kaya Cavuşoglu B, Ozkay Y, Kaplancikli ZA. Synthesis and AChE-inhibitory activity of new benzimidazole derivatives. Molecules. (2019); 24:861. https://doi.org/10.3390/molecules24050861

27. Osmaniye D, Sağlık BN, Acar Çevik U, Levent S, Kaya Çavuşoğlu B, Özkay Y, Kaplancıklı ZA, Turan G. Synthesis and AChE inhibitory activity of novel thiazolylhydrazone derivatives. Molecules. (2019); 24(5):2392. https://doi.org/10.3390/molecules24132392

28. Tok F, Koçyiğit-Kaymakçıoğlu B, Sağlık BN, Levent S, Özkay Y, Kaplancıklı ZA. Synthesis and biological evaluation of new pyrazolone Schiff bases as monoamine oxidase and cholinesterase inhibitors. Bioorg Chem. (2019); 84:41–50. https://doi.org/10.1016/j.bioorg.2018.11.016

29. V. Maestro, 10.6, Schrödinger, LLC: New York, NY, USA, (2016).

30. L. Schrödinger, LigPrep, version 3.8, Schrödinger, LLC, New York, NY, USA, (2016).

31. L. Schrödinger, Glide, version 7.1, Schrödinger, LLC: New York, NY, USA, (2016).

32. Sağlık BN, Levent S, Osmaniye D, Çevik UA, Çavuşoğlu BK, Özkay Y, Koparal AS, Kaplancıklı ZA. Design, synthesis, and biological activity evaluation of new donepezil-like compounds bearing thiazole ring for the treatment of Alzheimer's disease. Crystals. (2020); 10(8):637. https://doi.org/10.3390/cryst10080637

33. Honorio P, Hannongbua S, Saparpakorn P. Roles of hybrid donepezil scaffolds as potent human acetylcholinesterase inhibitors using *in silico* interaction analysis, drug-likeness, and pharmacokinetics prediction. Chem Biol Interact. (2022); 368:110227. https://doi.org/10.1016/j.cbi.2022.110227

34. Pacureanu L, Bora A, Crisan L. New insights on the activity and selectivity of MAO-B inhibitors through *in silico* methods. Int J Mol Sci. (2023); 24(11):9583. https://doi.org/10.3390/ijms24119583

35. Boulaamane Y, Ahmad I, Patel H, Das N, Britel MR, Maurady A. Structural exploration of selected C6 and C7-substituted coumarin isomers as selective MAO-B inhibitors. J Biomol Struct Dyn. (2023); 41(6):2326–2340. https://doi.org/10.1080/07391102.2022.2033643