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Research Article

Synthesis of some benzothiazole-piperazine derivatives, investigation by *in vitro* and molecular modelling for hMAO inhibitory activities

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ABSTRACT

Monoamine oxidase (MAO) is an enzyme that helps regulate the functions of intracellular amines, as well as chemicals such as dopamine, serotonin and norepinephrine, in the brain and its tissues. Active substances that are inhibitors of monoamine oxidases (MAOs) are used in the treatment of anxiety, depression and Alzheimer's disease. Previous studies have shown that compounds containing piperazine rings show MAO-A inhibitory activity. Based on these studies, 4 compounds containing piperazine and benzothiazole rings were designed, and the structures of the compounds were elucidated using spectroscopic methods such as HRMS and ^{1}H-NMR. hMAO-A and hMAO-B inhibitory activity was examined by *in vitro* methods. An *in silico* procedure was applied to investigate the residues and binding modes that interact with the docking of compounds **3a-d** to the active site of the hMAO-A (PDB ID: 2Z5X) enzyme identified in the previous study. Compound **3b** was found to be the most effective agent among the synthesized compounds with an IC_{50} value of 0.104±0.004 µM against the MAO-A enzyme.

Keywords: Enzyme Inhibition, MAO-A, Molecular Docking, Piperazine

1. INTRODUCTION

Monoamine oxidase (MAO) is an enzyme that plays a role in the oxidative deamination of intracellular amines as well as neurotransmitters such as dopamine, serotonin and norepinephrine, and helps regulate the concentrations of these chemicals in the brain and tissues outside the brain [1,2]. MAOs (MAO-A, MAO-B), which have 2 different isoforms with 70% homology, are located in the outer mitochondrial membranes of cells. The most common places in the body are the brain and liver [3].

While MAO-A is involved in the metabolization of neurotransmitters such as serotonin and adrenaline, MAO-B is involved in the metabolization of neuromodulatory neurotransmitters such as phenylethylamine [4]. MAOs have had different therapeutic uses due to their affinity for different substrates. While Monoamine oxidase-A inhibitors are used as antidepressants in the treatment of depression, Monoamine oxidase-B inhibitors are mostly used in Parkinson's and Alzheimer's disease [5-7].

The piperazine ring is a heterocyclic compound that exhibits a wide range of biological activities. It is found in the structure of compounds used in the treatment of anxiety disorders, such as the active ingredient buspirone [8-10]. When previous studies were examined, MAO inhibition activity was observed in many compounds containing phenylpiperazine and benzothiazole rings [11-13]. Although MAO-A inhibitors such as iproniazid, isocarboxazid, moclobemide and transylpromine have effective results in the treatment of depression, their clinical use has been limited due to side effects such as food-drug interactions or drug-drug interactions. Therefore, the emergence and discovery of new pharmacological groups have become important [14]. It is known that the diseases mentioned in the above text are not simpler diseases such as flu and cold, which have side effects and can be solved easily. Side effects that make life functions and quality of life unbearable and emotional states that can lead to suicide and end of life have led to more studies and efforts on these critical diseases.

In this study, four compounds containing piperazine and benzothiazole rings were synthesized, and their molecular structures were elucidated by various methods. Molecular docking studies and biological evaluation of their human MAO-A and MAO-B inhibition were carried out.

2. MATERIALS AND METHODS

2.1. Chemistry

While carrying out this study, all chemicals used and planned to be used during the reaction and pathways were supplied from Sigma-Aldrich (Sigma-Aldrich Corp., USA) or Merck (Merck KGaA, Germany). ^{1}H-NMR spectra were recorded in DMSO-d_6 by a Bruker digital FT-NMR spectrometer (Bruker Bioscience, USA) at 300 MHz. MS experiments were planned and carried out on the LCMS-IT-TOF device (Shimadzu, Japan). Termination checks between reaction steps were checked with classical TLC applications on silica gel 60 F254 (Merck KGaA, Germany). Melting degree determination was determined with the Mettler Toledo-MP90 (Greifensee, Switzerland).

2.1.1. Synthesis of 2-chloro-N-(6-substitutedbenzo[d]thiazol-2-yl)acetamide derivaties (1a-d)

Chloroacetyl chloride was added slowly to a mixture of 6-substituted benzothiazole-2-amine (0.9 g, 0.006 mol) and triethylamine (TEA) (0.894 mL) in tetrahydrofuran (THF) (12 mL) in ice bath. After it was determined that the reaction was over, THF was removed and the compound was washed with water to remove the salt [15-16].

2.1.2. Synthesis of sodium 4-(4-methoxyphenyl) piperazine-1-carbodithioate (2a)

NaOH and carbon disulfide (0.05 mol) were added to the mixture of 1-(4-methoxyphenyl) piperazine (0.05 mol) dissolved in ethanol and stirred in a mixture of ice and water for 4 hours. When the reaction was monitored by TLC and determined to have ended, the precipitated substances were filtered, washed with diethyl ether and left to dry [15-16].

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

2-chloro-*N*-(6-substitutedbenzothiazol-2-yl)acetamide (**1a-d**) (0.0011 mol), sodium 4-(4-substituted phenyl) piperazine-1 carbodithioate (**2a**) (0.0011 mol) were stirred for 6 hours in acetone. After detection of disruption of the reaction, acetone was removed with a rotary evaporator. It was cleaned with water to remove salts from the substances synthesized as a result of the reactions and allowed to dry. Then, recrystallization was performed with ethanol [15-16].

*2-(Benzo[d]thiazol-2-ylamino)-2-oxoethyl-4-(4-methoxyphenyl)piperazine-1-carbodithioate (**3a**)*

Yield: 79%, M.p: 225-227°C, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 3.16 (4H, m, CH_2-CH_2), 3.69 (3H, s, -OCH_3), 4.12 (2H, brs, CH_2), 4.34 (2H, brs, CH_2), 4.45 (2H, s, -CH_2), 6.85 (2H, d, *J*=6.8 Hz), 6.94 (2H, d, *J*=7.1 Hz), 7.30 (1H, t, *J*=15.3 Hz, benzothiazole), 7.44 (1H, t, *J*=15.4 Hz, benzothiazole), 7.75 (1H, d, *J*=8.0 Hz, benzothiazole), 7.96 (1H, d, *J*=8.1 Hz, benzothiazole), 12.66 (1H, s, -NH). HRMS (ESI) (*m/z*): $[M+2H]^{2+}$ calculated for $C_{21}H_{22}N_4O_2S_3$: 230.0511; found 230.0525.

*2-((6-Methylbenzo[d]thiazol-2-yl)amino)-2-oxoethyl-4-(4-methoxybenzyl)piperazine-1-carbodithioate (**3b**)*

Yield: 84%, M.p: 237-239°C, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.40 (3H, s, -CH_3), 3.16 (4H, m, CH_2-CH_2), 3.69 (3H, s, -OCH_3), 4.11 (2H, brs, CH_2), 4.34 (broad s, 2H, CH_2), 4.44 (2H, s, -CH_2), 6.84 (2H, d, *J*=9.0 Hz), 6.94 (2H, d, *J*=9.1 Hz), 7.25 (1H, d, *J*=8.5 Hz, benzothiazole), 7.63 (1H, d, *J*=8.2 Hz, benzothiazole), 7.76 (1H, s, benzothiazole), 12.56 (1H, s, -NH). HRMS (ESI) (*m/z*): $[M+H]^+$ calculated for $C_{22}H_{24}N_4O_2S_3$: 473.1127; found 473.1134.

*2-((6-Methoxybenzo[d]thiazol-2-yl)amino)-2-oxoethyl-4-(4-methoxybenzyl)piperazine-1-carbodithioate (**3c**)*

Yield: 81%, M.p: 242-244°C, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 3.16 (4H, m, CH_2-CH_2), 3.69 (3H, s, -OCH_3), 3.80 (3H, s, -OCH_3, benzothiazole), 4.11 (2H, brs, CH_2), 4.34 (2H, brs, CH_2), 4.43 (2H, s, -CH_2), 6.84 (2H, d, *J*=8.8 Hz), 6.94 (2H, d, *J*=9.1 Hz), 7.03 (1H, d, *J*=8.8 Hz, benzothiazole), 7.57 (1H, s, benzothiazole), 7.64 (1H, d, *J*=8.8 Hz, benzothiazole), 12.53 (1H, s, -NH). HRMS (ESI) (*m/z*): $[M+H]^+$ calculated for $C_{22}H_{24}N_4O_3S_3$: 489.1072; found 489.1083.

*2-((6-Nitrobenzo[d]thiazol-2-yl)amino)-2-oxoethyl-4-(4-methoxybenzyl)piperazine-1-carbodithioate (**3d**)*

Yield: 73%, M.p: 259-261°C, ^{1}H-NMR (300 MHz, DMSO-d_6): δ =3.16 (4H, m, CH_2-CH_2), 3.69 (3H, s, -OCH_3), 4.11 (2H, brs, CH_2), 4.33 (broad s, 2H, CH_2), 4.49 (2H, s, -CH_2), 6.85 (2H, d, *J*=9.1 Hz), 6.94 (2H, d, *J*=9.1 Hz), 7.91 (1H, d, *J*=9.0 Hz, benzothiazole), 8.29 (1H, d, *J*=8.9 Hz, benzothiazole), 9.06 (1H, s, benzothiazole), 13.11 (1H, s, -NH). HRMS (ESI) (*m/z*): $[M+H]^+$ calculated for $C_{21}H_{21}N_5O_4S_3$: 504.0792; found 504.0828.

Table 1. IC_{50} (μM) values of the obtained compounds against MAO-A and MAO-B enzyme

Compounds	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)
3a	0.198±0.008	>100
3b	0.104±0.004	0.120±0.005
3c	0.167±0.007	0.280±0.013
3d	>100	>100
Moclobemide	6.0613±0.2625	-
Selegiline	-	0.0374±0.0016

2.2. *In vitro* MAO inhibition assay

In vitro fluorometric enzymatic analysis, which allows accurate and accurate detection and observation of monoamine oxidase activities, was applied to investigate the inhibitory potential of **3a-d** coded compounds on hMAO-A and hMAO-B. Compounds were used at concentrations of 10^{-5} M-10^{-9} M to calculate their IC_{50} inhibition values of the obtained compounds were calculated as described in previous studies [17-20] (Table 1).

2.3. Prediction of ADME Parameters

The online SwissADME program was used to estimate ADME parameters. [21] (Table 2).

Table 2. Predicted ADME parameters of compounds **3a-d**

Comp	Physicochemical Properties							Lipo.	Druglikeness					Water Solubility		Pharmacokinetics	
	MW	Fsp3	RB	HBA	HBD	MR	TPSA	cLogP	Lipinski	Ghose	Veber	Egan	Muegge	LogS	Class	GI abs.	F
3a	458.62	0.29	8	3	1	136.39	143.33	3.43	+	-	-	-	+	-5.37	Moderately	Low	0.55
3b	472.65	0.32	8	3	1	141.36	143.33	3.76	+	-	-	-	+	-5.68	Moderately	Low	0.55
3c	488.65	0.32	9	4	1	142.89	152.56	3.57	+	-	-	-	-	-5.46	Moderately	Low	0.55
3d	503.62	0.29	9	5	1	145.22	189.15	2.71	+	-	-	-	-	-5.45	Moderately	Low	0.55

Comp: Compounds, MW: Molecular weight, Fsp3: Fraction Fsp3, RB: Number of rotatable bonds, HBA: Number of hydrogen bond acceptors, HBD: Number of hydrogen bond donors, MR: Molar refractivity, TPSA: Total polar surface area, Lipo: Lipophilicity, GI abs: Gastrointestinal absorption, F: Bioavailability score.

Table 3. Molecular docking scores, interaction types and estimated inhibition constants of synthesized compounds **(3a-d)** and MAO-A (PDB ID: 2Z5X)

Comp.	Autodock Results				Vina Results
	Interacting Residues	Interaction Types	Estimated Inhibition Constant, K_i	Best Docking Score	Best Docking Score
3a	-	-	16.31 mM	-6.53	-8.2
3b	PHE112	Pi-Pi Stacking	106.25 nM	-9.51	-8.8
	TYR124	Pi-Pi Stacking			
	TRP128	Pi-Pi Stacking			
3c	HIS488	Pi-Pi Stacking	144.15 nM	-9.33	-8.7
	ASP132	H-Bond			
	HİS488	Pi-Pi Stacking			
3d	ASP132	H-Bond	7.13 nM	-11.11	-9.4
	LYS136	Salt Bridge			

Table 4. Molecular docking scores, interaction types and estimated inhibition constants of synthesized compounds **(3a-d)** and MAO-B (PDB ID:2V5Z)

Comp.	Autodock Results				Vina Results
	Interacting Residues	Interaction Types	Estimated Inhibition Constant, K_i	Best Docking Score	Best Docking Score
3a	CYS172	H-Bond	36.35 nM	-10.15	-8.2
3b	PHE343	Pi-Pi Stacking	34.84 nM	-10.17	-8.8
3c	-	-	31.24 nM	-10.24	-8.6
3d	ILE199	H-Bond	12.81 nM	-10.77	-8.3

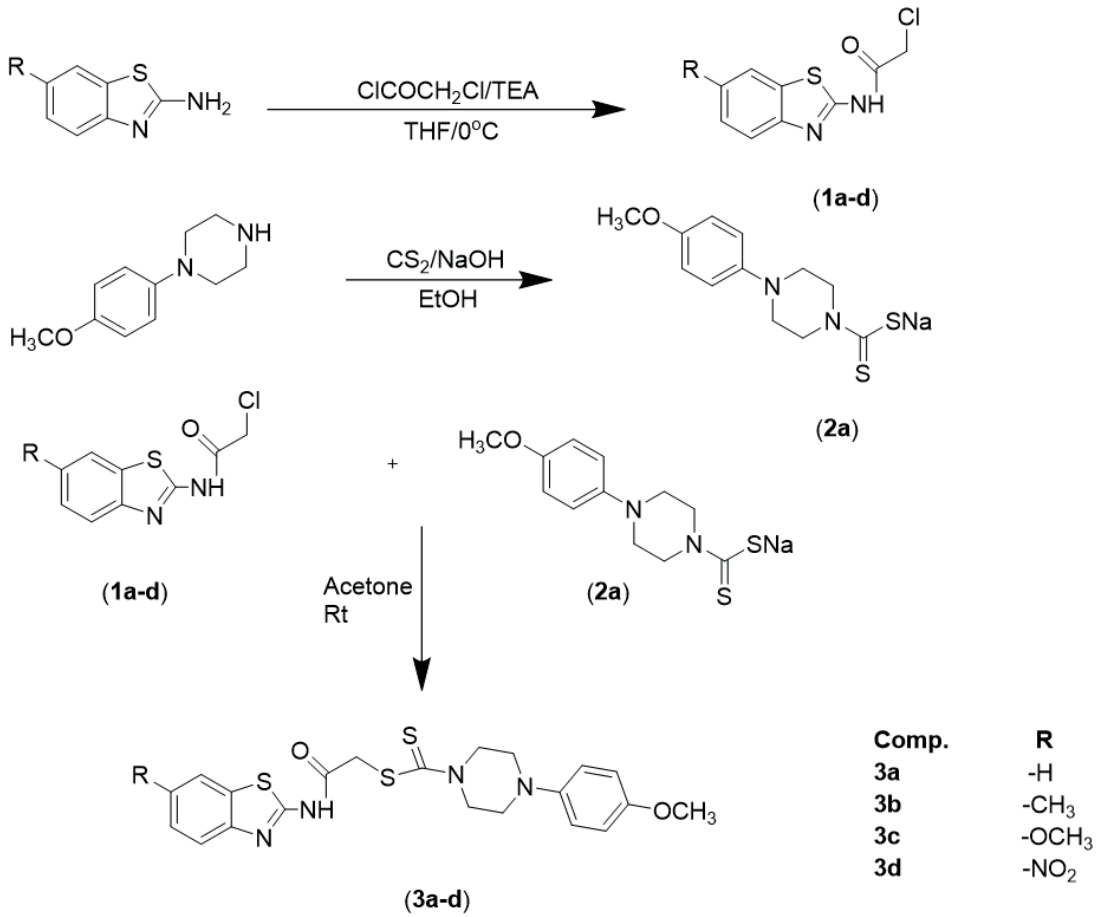
2.4. Molecular Docking Study

An *in silico* procedure was applied to investigate the residues and binding modes that interact with the docking of compounds **3a-d** to the active site of the hMAO-A (PDB ID: 2Z5X) [22], the hMAO-B (PDB ID: 2V5Z) [23] enzymes identified in the previous study. The macromolecular structure of hMAO-A crystallized with harmine was obtained from the Protein Data Bank and the molecular docking procedure was performed by researchers in our research group, as done in previous docking studies [24-26]. The Pdb file of the macromolecule was optimized using Maestro Version 6.4.135, Release 2023-4 [27]. In both receptors (MAO-A: 2Z5X and MAO-B: 2V5Z), the (6Å) waters around the previously determined active site (MAO-A: HRM700 and MAO-B: SAG601) were left and all other water molecules were removed. Preprocessing and H-Bond optimization for both receptors was done using Maestro. Then, the obtained pdb formatted macromolecules were edited with Autodock and saved in pdbqt format. The regular space of the Grid boxs are determined as 0.375 Å, centered on SAG601 (40*40*40 $Å^3$) and HRM700 (50*50*50 $Å^3$). Lamarckian Genetic Algorithm was preferred in all studies, detailed results such as docking scores were obtained using both AutoDock 4.2 [28] and AutoDock Vina programs [29] and results are presented in Table 3 and Table 4. To validate the molecular docking studies, redocking studies were performed with both HRM700 on MAO-A and SAG601 on MAO-B, and RMSD values were found to 0.92 and 1.08 respectively.

3. RESULTS AND DISCUSSION

3.1. Chemistry

Compounds **3a-d** were obtained as shown in Scheme 1. In this study, a synthesis involving dithiocarbamate salt and benzothiazole rings was carried out. The planned and realized synthesis consists of 3 steps.



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

As the first step, 2-chloro-*N*-(6-substitutedbenzo[*d*] thiazol-2-yl)acetamide derivatives were obtained by acetylation (**1a-d**). As a second step, dithiocarbamate salt was obtained by the reaction of carbon disulfide with secondary amines (**2a**). In the third and last step, the two products obtained were dissolved in acetone, boiled under reflux, filtered and dried, and substances (**3a-d**) were obtained (Scheme 1). The structures of the compounds **3a-d** were confirmed by using spectroscopic methods (HRMS and ^{1}H-NMR).

When the NMR results of the synthesized compounds were examined, it was observed that the proton peaks of the piperazine ring appeared in 3 different forms (2H, 2H and 4H) between 3.16 ppm and 4.30 ppm. The proton peaks of the acetyl group attached to the piperazine ring were detected as singlets between 4.43 ppm and 4.49 ppm. It was observed that the protein belonging to the amine group was between 12.53-13.11 ppm and protons belonging to disubstituted benzene were observed between 6.84 ppm and 6.94 ppm. Proton peaks of benzothiazole are also observed between 7.03 ppm and 9.06 ppm. While the CH_3 group in compound **3b** was observed as a singlet at 2.40 ppm, the OCH_3 group of compound **3c** was observed to peak as a singlet at 3.80 ppm. Mass spectra were performed using high-resolution liquid chromatography. In the mass spectra taken using the electron sputtering method, all compounds were recorded as having excess molecular weights.

3.2. *In vitro* MAO inhibition assay

In vitro fluorometric enzymatic analysis, which allows accurate and accurate detection and observation of monoamine oxidase activities, was applied to investigate the inhibitory potential of **3a-d** coded compounds on hMAO-A and hMAO-B.

3a-d coded compounds were used at concentrations of 10^{-5} M-10^{-9} M to calculate their IC_{50} inhibition values of the obtained compounds were calculated [30]. The inhibition of MAO-A and MAO-B at the initial concentrations of the resulting compound and moclobemide and selegiline are shown in Table 1. When the results obtained were examined, compounds **3a**, **3b** and **3c** showed IC_{50} values of 0.198±0.008 µM, 0.104±0.004 µM and 0.167±0.007 µM on MAO-A, respectively. The reference drug moclobemide showed a value of 6.0613±0.2625 µM. On the other hand, compounds **3b** and **3c** showed values of 0.120±0.005 µM and 0.280±0.013 µM on MAO-B, respectively. The IC_{50} value of selegiline used as the reference drug was measured as 0.0374±0.0016 µM. Based on these results, it was observed that the synthesized compounds gave approximately 50 times better results on MAO-A than the reference drug, and were approximately 9 times less active on MAO-B than the reference drug. *In vitro* results mostly overlapped with the MAO-A enzyme site interactions examined *in silico* results.

3.3. Prediction of ADME Parameters

The online SwissADME was used and the estimated ADME parameters of the obtained compounds were calculated [21]. Looking at Table 2 showing the results, it is observed that none of the synthesized compounds violate the Lipinski rule [31]. Gastrointestinal absorption provides a preliminary result as to whether the obtained compounds can be used orally. When the table was examined, it was seen that the compounds had low absorption. Log S values of the compounds are between -5.37 and -5.68, and their solubility is estimated to be moderate. The F value, which shows the oral bioavailability of the compounds, is 0.55, which is the ideal value [32], in contrast to the result in gastrointestinal absorption.

3.4. Molecular Docking Studies

As stated in the *in vitro* MAO-A inhibition results, compounds **3b** and **3d** were found to be the 2 compounds with the highest inhibition activity on MAO-A enzyme among the 4 compounds synthesized. Among the synthesized and obtained compounds, compound **3b** with an IC_{50} value of 0.104±0.004 µM was found to be the best compound. By using X-ray crystal structure of MAO-A (PDB ID: 2z5x) docking studies were performed, and binding modes of compound **3b** were assigned (Figures 1 and 2). Molecular docking poses of all synthesized and obtained compounds are presented in 2D and 3D images in supp. mat. file.

The interaction domain of MAO-A and its cocrystal ligand Harmine (PDB ID: HRM700) has been previously revealed, TYR69, ILE180, ASN181, PHE208, GLN215, ILE335, LEU337, PHE352, TYR407 and TYR444 were emphasis to be important for the interaction (https://www.ebi.ac.uk/pdbe/entry/pdb/2z5x/bound/HRM#700A).

The interaction domain of MAO-B and its cocrystal ligand Safınamide (PDB ID: SAG601) has been previously revealed, PRO102, TRP119, LEU164, PHE168, ILE171, CYS172, ILE199, GLN206, ILE316, TYR326, PHE343, TYR398, TYR435, FAD600, HOH798, HOH808 and HOH839 were emphasis to be important for the interaction (https://www.ebi.ac.uk/pdbe/entry/pdb/2v5z/bound/SAG#601A).

Compound **3a** was sufficiently bound to the amino acid residues in the macromolecule and was also observed in a very close position to the FAD enzyme. Compounds **3b**, **3c**, and **3d** bind sufficiently to amino acid residues spanning the gap and are located close to DCX1 and DCX2 (Figure 2). When the docking poses of all compounds were examined, it was clearly seen that it had many interactions such as salt bride, pi-pi stacking and H-bond. The pi-pi stacking was detected in the benzothiazole ring of compounds **3b, 3c** and **3d**. Also, there was a H-bond interaction between the nitrogen atom of amide functional group and ASP132 (Figures 1 and 2). Moreover, there was a salt bridge interaction between the nitro group of benzothiazole and LYS136 (Table 3). In this study, it was determined that compound **3b** interacted with these residues of MAO-A in a similar way. When the docking poses of MAO-B was examined, compounds **3a** and CYS172 was observed to make hydrogen bonds. Compound **3b** and PHE343 were observed to exhibit pi-pi stacking. Additionally, compounds **3d** and ILE199 were observed to form hydrogen bonds.

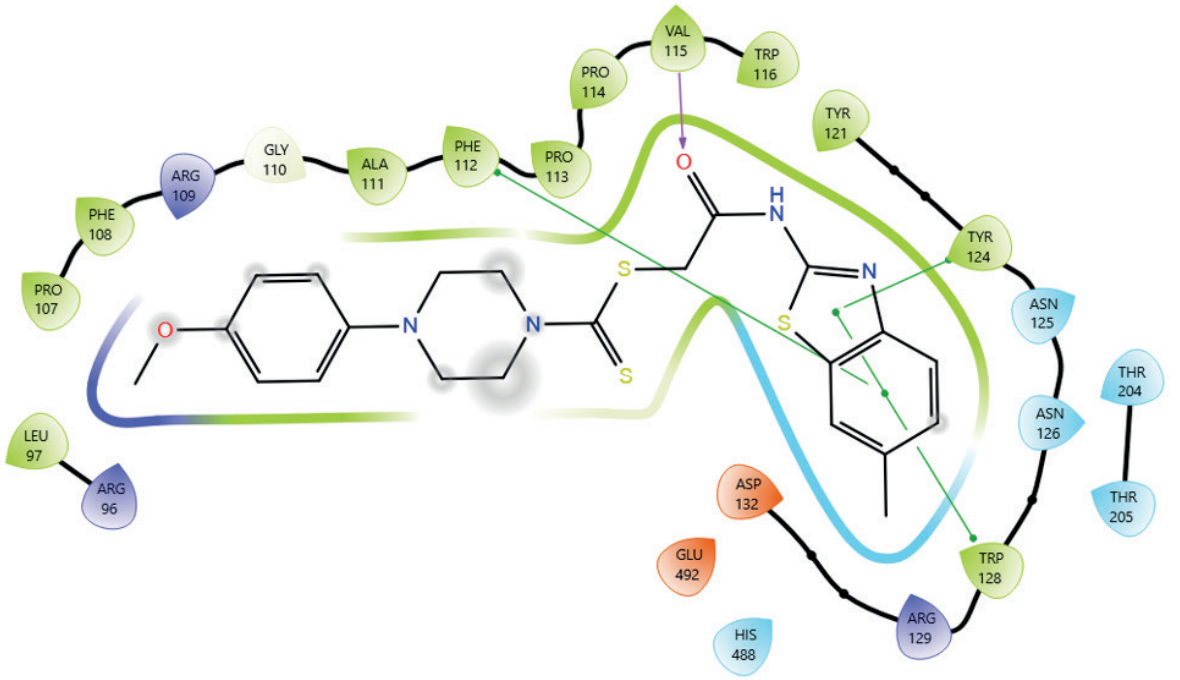


Figure 1. 2D interaction diagram with 2Z5X for compound **3b**

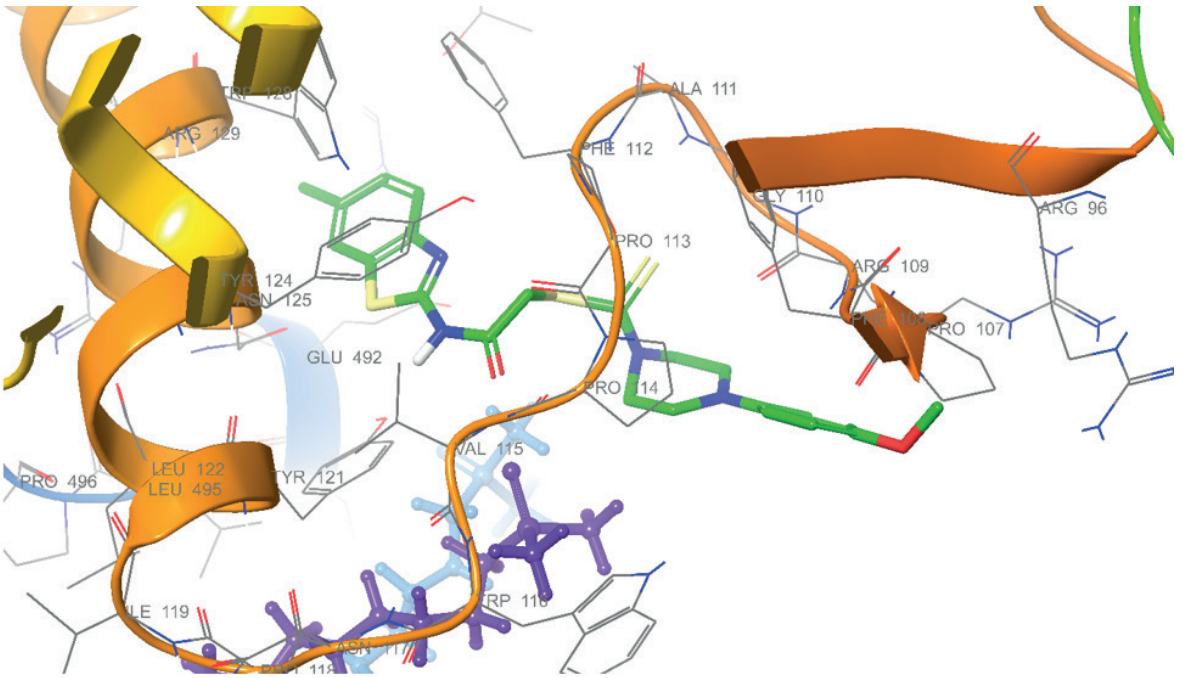


Figure 2. 3D interaction diagram with 2Z5X for compound **3b**

4. CONCLUSION

Compounds containing piperazine rings have previously been proven to be effective, have been synthesized and are still used today. In this study, compounds were synthesized with reference to a previous study. hMAO inhibition activities were investigated. ADME results showed that the synthesized compounds were moderate to good in terms of pharmacokinetics. When molecular docking studies were examined, it was observed that the compounds interacted with the residues in the active site. *In vitro* results show that the MAO-A inhibition of our compounds is better than the MAO-B inhibition. When the *in vitro* activity results were examined, compound **3b** showed the best inhibition value with an IC_{50} of 0.104±0.004 µM compared to the moclobemide reference drug. Moclobemide showed an IC_{50} value of 6.0613±0.2625 µM. Our compounds coded **3a**, **3b** and **3c** showed approximately 50 times better activity than the reference drug. The data obtained in this study can be used as a source for subsequent compound synthesis studies that can be used in the treatment of anxiety and depression.

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Ethical approval

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

Author contribution

Conceptualization, H.U., D.O. and Y.Ö.; Methodology, B.G., S.L. and B.N.S.Ö.; Software, H.U., D.O. and B.N.S.Ö.; Formal analysis, B.G., D.O. and S.L.; Investigation, B.G.; Resources, S.P.G.; Writing—original draft preparation, B.G., H.U., D.O. and S.P.G.; Writing—review and editing, Y.Ö.; Supervision, Y.Ö. 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.

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