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Research Article

Synthesis, Biological Activity Studies and Molecular Modeling Studies of Chalcone Compounds with Methyl Group

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ABSTRACT

A series of new chalcone derivatives (**1-5**) were synthesized as a result of the Claisen-Schmidt condensation of different substituted methyl aldehydes of 4′-Piperazinoacetophenone. Anticholinesterase (AChE and BChE) inhibitory activity and antidiabetic (α-glucosidase and α-amylase inhibitory) activities of the synthesized compounds were examined. While compound 1 is the most active molecule in AChE (IC_{50}= 16.29±0.44 µM), BChE (IC_{50} = 10.19±0.04 µM) and α-amylase (IC_{50}= 105.51±0.24 µM) inhibitor activities; Compound **5** was found to be the most active molecule in α-glucosidase inhibitor activity. *In silico* and molecular docking studies of compounds **1-5** were performed. According to molecular docking results, all molecules were found to be more active than the reference compounds.

Keywords: Chalcone, anticholinesterase inhibitory, α-amylase inhibitory, α-glycosidase inhibitory, molecular docking

1. INTRODUCTION

They have the α, β- unsaturated carbonyl structure (-C=O-CH=CH-). Since chalcones are easy to use orally against various diseases; It has a rich range of biological activities that have the potential to be used clinically. Chalcones have important biological activities such antidiabetic[1], anti-alzeihmer[2], alzantiprotozoal[3], antiobesity[4], antioxidant[5], antimalarial[6], hypolipidemic[7], antihistamine[8], antiretroviral[9], anti-inflammatory[10], angiogenic[11], antigout[12], antibacterial[13], antineoplastic[14], hypnotic[15], antituberculosis[16] and anxiolytic[17] has activities. Diabetes is a disease that causes hyperglycemia due to impaired insulin function or secretion. Diabetes is generally divided into two groups: Type 1 and Type 2. The most common type is Type 2 diabetes. (90-95 percent of diabetic patients). Insulin resistance has become an important problem with the increase in obesity in recent years. Insulin resistance is the main cause of 90% of Type 2 diabetes. According to the International Diabetes Federation, it is estimated that the number of patients with diabetes may increase to 643 million in 2030 and 783 million in 2045. Since diabetes reduces a person's quality of life and damages other organs in the body, its treatment is of great importance. New drugs are needed due to the side effects of the drugs used in the current clinic and the body's resistance when used continuously.

Alzheimer's disease is the most common type of dementia and is a degenerative brain disease. In the majority of patients diagnosed with Alzheimer's; symptoms of progressive memory loss, difficulties in self-control, behavioral differences, hallucinations, difficulty speaking, impairment in cognitive function, and deterioration of the integrity of the individual are observed. These symptoms show how great a challenge the individual and those caring for the patient face. Therefore, just like in diabetes, the treatment of Alzheimer's disease is of great importance. Recently, the number of diabetic and Alzheimer's patients has been increasing rapidly all over the world. After major disasters such as epidemics (such as Covid 19) and earthquakes; It is predicted that there will be an increase in the number of diabetic and Alzheimer's patients due to stress, fear and irregular nutrition. Therefore, in this study, the antidiabetic activities (α-glucosidase and α-amylase enzymes) and Alzheimer activities (acetyl acetylcholinesterase and butrylcholinesterase) of chalcones will be examined.

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2.EXPERIMENTAL

2.1. General

Chemicals and solvents were in analytical grade and purchased from Merck and Sigma-Aldrich. All chemical reactions were monitored with thin layer chromatography (TLC) using Merck silica gel 60 F254 plates. Melting points: 18 Taken automatically with Stuart SMP20 instrument. FTIR spectra: were determined with a Perkin Elmer 1620 model FTIR spectrophotometer. Elemental analyses (CHNS): were performed on a VarioMICRO elemental analyzer. ^{1}H and ^{13}C NMR spectra: were recorded on Agilent 600 MHz spectrometer. All biological activity measurements were using a 96-well microplate reader (SpectraMax 340PC^{384}, Molecular Devices, USA).

2.2. General Procedure for Chalcone Compounds with Methyl Group (1-5)

4′-piperazine acetophenone (0.001 mol) was dissolved in 20 mL of methanol and of substituted aldehyde (0.001 mol) dissolved in methanol and solid NaOH was added and mixed at room temperature in a magnetic stirrer for 12 hours. After the completion of the reaction was determined by TLC, and extracted dichloromethane/water. The liquid organic phase was evaporated in the evaporator and the solid material was crystallized with dichloromethane/hexane solvent to obtain pure substance.[18]

2.2.1. (E)-1-(4-(piperazin-1-yl) phenyl)-3-(p-tolyl) prop-2-en-1-one (1) [19]

Yellow solid, yield:53 %, m.p: 263-264 °C.

2.2.2. (E)-3-(2,4-dimethylphenyl)-1-(4-(piperazin-1-yl) phenyl) prop-2-en-1-one (2)

Brown solid, yield: 41 %, m.p: 258-259 °C.

2.2.3. E-3-(2,6-dimethylphenyl)-1-(4-(piperazin-1-yl) phenyl) prop-2-en-1-one (3)

Yellow solid, yield: 45 %, m.p: 282-283 °C.

2.2.4. E-1-(4-(piperazin-1-yl) phenyl)-3-(2,4,5-trimethylphenyl) prop-2-en-1-one (4)

Yellow solid, yield: 42 %, m.p: 275-276 °C.

2.2.5. E-3-mesityl-1-(4-(piperazin-1-yl) phenyl) prop-2-en-1-one (5)

Yellow solid, yield: 46 %, m.p: 277-278°C.

2.3. *In vitro* Enzyme Inhibitory Activities

Galantamine for anticholinesterase and acarbose for α-amylase and α-glucosidase were used as positive standard to compare the inhibitory activity.

2.3.1. Determination of Anticholinesterase Activity of the 1-5 Derivatives

The electric eel acetylcholinesterase (AChE, Type-VI-S, EC 3.1.1.7, 425.84 U/mg) and horse reddish butyrylcholinesterase (BChE, EC 3.1.1.8, 11.4 U/mg) were used to determine the anticholinesterase activity at DMSO of **1-5** derivatives, where acetylthiocholine iodide and butyryl-thiocholine chloride were employed as substrates using spectroscopic method.[20] Briefly, 130 µL sodium phosphate buffer (100 mM, pH 8.0), 10 µL **1-5** derivatives at different concentrations, and 20 µL AChE or BChE enzymes in buffer were mixed. After incubation for 15 min at 25 °C, 20 µL 0.5 mM DTNB (5,50-dithiobis (2-nitrobenzoic acid) and 20 µL acetylthiocholine iodide (0.71 mM) or butyryl-thiocholine chloride (0.2 mM) were added. Then the absorbance was measured at 412 nm.

2.3.2. Determination of α-amylase Inhibitory Activity of the 1-5 Derivatives

α-Amylase inhibitory activity of the 1-5 derivatives was tested by using the spectroscopic method with slight changes21. Briefly, 25 µL sample solution in different concentrations and 50 µL α-amylase solution (0.1 U/mL) in phosphate buffer (20 mM pH=6.9 phosphate buffer prepared with 6 mM NaCl) were mixed in a 96-well microplate. The mixture was pre-incubated for 10 min at 37 °C. After pre-incubation, 50 µL starch solution (0.05 %) was added and incubated for more 10 min at 37 °C. The reaction was stopped by addition of 25 µL HCl (0.1 M) and then 100 µL Lugol solutions were added for monitoring. 96-well microplate reader was used to measure absorbance at 565 nm.

2.3.3. Determination of α-glucosidase Inhibitory Activity of the 1-5 Derivatives

α-Glucosidase inhibitory activity of the **1-5** derivatives was determined using the spectroscopic method with slight modifications.[22] Briefly, 50 µL phosphate buffer (10 mM pH=6.9), 25 µL PNPG (*p*-nitrophenyl-*α*-*D*-glucopyranoside) in phosphate buffer (10 mM pH=6.9), 10 µL sample solution and 25 µL α-glucosidase (0.1 U/mL) in phosphate buffer (10 mM pH=6.0) were mixed in a 96-well microplate. After 20 min incubation at 37 °C, 90 µL sodium carbonate (100 mM) was added into the each well to stop the enzymatic reaction. Absorbance of the 96-well microplate reader was recorded at 400 nm.

2.4. Absorption, Distribution, Metabolism and Excretion Properties and Toxicity Parameters

Basic parameters affecting drug metabolism, such as the Absorption, Distribution, Metabolism and Elimination (ADME) properties of chalcones **1-5** in the body, were performed with the web-based SwissSimilarty (SwissADME) program according to Lipinski and Veber rules. While the similarity model score features of chalcone compounds **1-5** were performed with the Molsoft software program; Toxicity parameters were performed with the Organic Chemistry Portal program.[23-25]

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2.5. Molecular Docking

This study aimed to investigate the binding affinities and molecular interaction mechanisms of compounds 1-5 towards AChE, BChE, α-amylase, and α-glucosidase by molecular docking method. To conduct this investigation, the 3D crystal structures of the target enzymes (AChE, BChE, α-amylase, and α-glucosidase) were retrieved from the Protein Data Bank website (http://www.rcsb.org/pdb) with the respective PDB IDs: 4EY6, 6QAA, 4W93, and 5NN4, respectively. The docking grid box was set according to the binding site location of the crystallized ligands. Employing a rigid protein and a flexible ligand, the standard docking procedure was carried out through a 100-generation run using the Lamarckian Genetic algorithm with AutoDock 4.2.[26] Throughout this analysis, binding free energy (ΔG) and inhibition constant (K) values were predicted for each compound, identifying those that best fit the 3D structure of the target enzymes.

3.RESULTS AND DISCUSSION

3.1. Chemistry

Chalcones with methyl group were synthesized via Claisen–Schmidt condensation of acetophenone to 4′-piperazine and various aldehydes with methyl group in the presence of sodium hydroxide in methanol (Figure 1). Compound **1-5** were synthesized in 41-53% yield. The FT-IR spectra of chalcones (**1-5**) showed the NH stretching band at 3360-3111, aromatic CH stretching band at 3047-2723 cm-1, C=O stretching band at 1646-1660 cm-1.

While H_1 protons of the piperazine ring were detected in the range of 3.16-3.18 ppm (s, 4H); H_2 protons of compound **1-4** of the piperazine ring are in the range of 3.60-3.61 ppm (t, 4H, J_1=5.40 Hz, J_2=5.40 Hz); The compound **5** was detected as 3.58 (s, 4H). The biggest evidence of the synthesis of chalcone compounds; AB spin system has been observed. H_α and H_β protons 7.34-7.73 ppm (J=15.00-18.00), respectively; There is resonance at 7.74-7.87 ppm (J=13.20-16.20). NH protons of the piperazine ring resonate in the range of 9.38-9.64 ppm. ^{13}C NMR spectrum was examined, the C=O peaks of all compounds (**1-5**) resonated in the range of 187.16-187.17 ppm (Table 1). All peaks were observed within the expected range. [27-29]

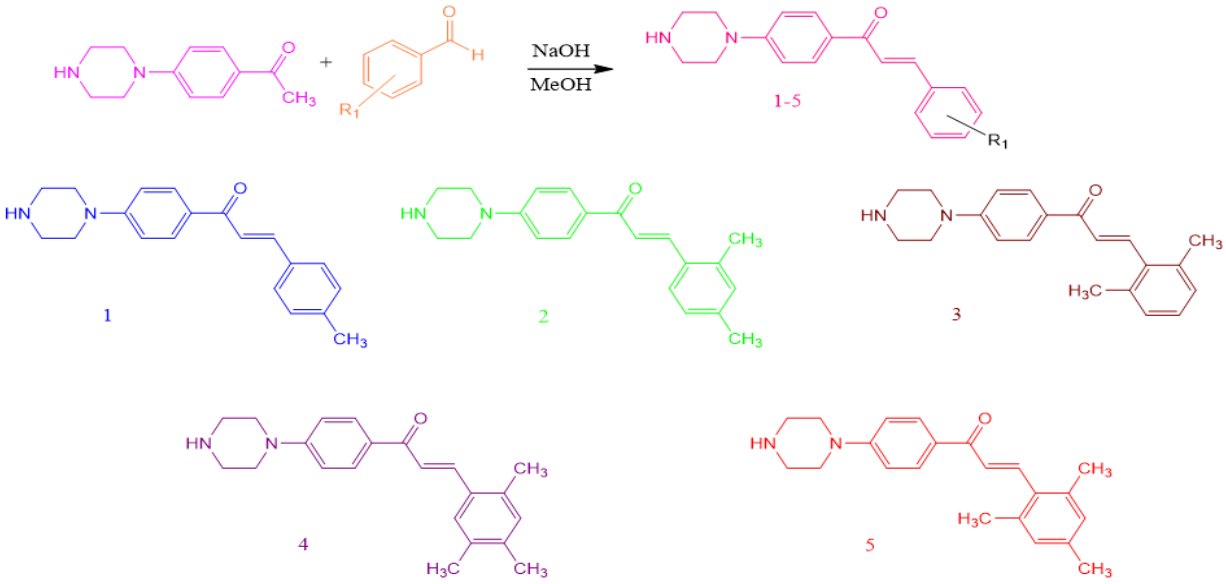

Figure 1. Synthetic route of target compounds (1-5).

3.2. Biological Activity (Anticholinesterase inhibitory activity and Antidiabetic inhibitory activities)

When the anticholinesterase inhibitor activities of molecules **1-5** were examined, it was determined that they showed selectivity against BChE. Except for the second molecule, all molecules have higher inhibitory activities than galantamine, which is used as standard. It was determined that the activity order in AChE inhibitory activity was **1**>**5**>**4**>**3**>**2** and in BChE inhibitory activity the activity order was **1**>**5**>**4**>**3**>**2**. When the antidiabetic inhibitor activities of molecules **1-5** are examined; It was determined that α-glucosidase inhibitor activities were higher than acarbose used as standard. It was determined that α-amylase inhibitory activity was **1**>**5**>**4**>**3**>**2** while α-glucosidase inhibitory activity was **5**>**1**>**4**>**3**>**2** (Table 2).

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Table 1. FTIR, ^{1}HNMR and ^{13}C NMR spectral data of chalcone 1-5.

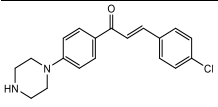
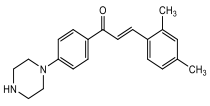
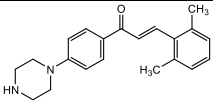
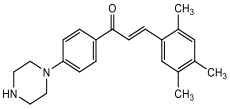
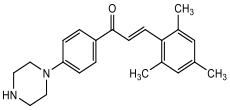
	Compound				
	1	**2**	**3**	**4**	**5**
					
FT-IR	(Ar-CH)=2723 (C=O)=1647 (C=C)=1601,1587, 1566, 1448 (N-H)=3301	(Ar-CH)=2968 (C=O)=1660 (C=C)=1584,1448, 1407 (N-H)=3360	(Ar-CH)=2957 (C=O)=1647 (C=C)=1578,1516, 1391, 1358 (N-H)=3172	(Ar-CH)=3047, 2964 (C=O)=1648 (C=C)=1601,1584, 1498, 1448 (N-H)=3176	(Ar-CH)=2999, 2951 (C=O)=1646 (C=C)=1598,1581,1547, 1519 (N-H)=3111
^{1}H NMR	2.32 (s, 3H, C<u>H</u>$_3$), 3.18 (s, 4H), 3.60 (t, 4H, J_1=5.40, J_2=5.40), 7.05 (d, 2H, J=9.00), 7.23 (d, 2H, J=8.40), 7.62 (d, 1H, J=15.60), 7.73 (d, 2H, J=8.40), 7.85 (d, 1H, J=15.60), 8.06 (d, 2H, J=9.00), 9.47 (s, 1H, N<u>H</u>).	2.26 (s, 3H, C<u>H</u>$_3$), 2.36 (s, 3H, C<u>H</u>$_3$), 3.16 (s, 4H), 3.61(t, 4H, J_1=5.40, J_2=5.40), 7.04-7.06 (m, 4H), 7.73 (d, 1H, J=15.00), 7.86 (d, 1H, 8.40), 7.87 (d, 1H, J=15.60), 8.05 (d, 2H, J=9.00), 9.64 (s, 1H, NH).	2.33 (s, 6H, C<u>H</u>$_3$), 3.17 (s, 4H), 3.60 (t, 4H, J_1=5.40, J_2=5.40), 7.05 (d, 2H, J=9.60), 7.09 (d, 2H, J=7.80), 7.12-7.15 (dd, 1H, J_1=6.60, J_2=6.60), 7.37 (d, 1H, J=16.20), 7.74 (d, 1H, J=16.20), 7.99 (d, 2H, J=9.00), 9.48 (s, 1H, NH).	2.18 (s, 3H, C<u>H</u>$_3$), 2.22 (s, 3H, C<u>H</u>$_3$), 2.32 (s, 3H, C<u>H</u>$_3$), 3.18 (s, 4H), 3.60 (t, 4H, J_1=5.40, J_2=5.40), 7.01 (s, 1H), 7.06 (d, 2H, J=9.00), 7.73 (d, 1H, J=15.60), 7.76 (s, 1H), 7.86 (d, 1H, J=15.60), 8.06 (d, 2H, J=9.00), 9.51 (s, 1H, NH).	2.22 (s, 3H, C<u>H</u>$_3$), 2.31 (s, 6H, C<u>H</u>$_3$), 3.17 (s, 4H), 3.58 (s, 4H), 6.92 (s, 2H), 7.05 (d, 2H, J=7.20), 7.34 (d, 1H, J=18.00), 7.74 (d, 1H, J=13.20), 7.98 (d, 2H, J=9.60), 9.38 (s, 1H, NH).
^{13}C NMR	21.51, 42.66, 44.25, 114.35, 121.49, 128.67, 129.18, 129.94, 130.99, 132.65, 140.72, 143.02, 153.42, 187.16.	19.72, 21.37, 42.59, 44.20, 114.36, 122.25, 127.21,127.51, 128.65, 130.98,131.14, 131.84, 138.16,139.98, 140.36, 153.42, 187.17.	21.22, 42.61, 44.21, 114.42, 128.28, 128.32, 128.60, 131.04, 134.95, 136.91, 140.90, 153.49, 187.17.	19.15, 19.27, 19.70, 42.65, 44.25, 114.36, 121.84, 128.01, 128.74, 130.96, 131.20, 132.38, 134.60, 135.61, 139.31, 140.15, 153.43, 187.16.	19.71, 21.36, 42.57, 44.19, 114.36, 122.25, 127.21, 127.51, 128.64, 130.98, 131.13, 131.83, 138.15, 139.97, 140.36, 153.43, 187.17.

Table 2. Enzyme inhibition activities of synthesized 1-5a.

Compounds/standarts	**Anticholinesterase inhibitory activity**		**Antidiabetic inhibitory activities**	
	AChE assay (IC_{50} µM)	BChE assay (IC_{50} µM)	α-Amylase assay (IC_{50} µM)	α-Glucosidase assay (IC_{50} µM)
1	16.29±0.44	10.19±0.04	50.01±0.76	121.18±0.47
2	53.19±0.37	49.77±0.51	86.75±0.21	188.20±0.76
3	41.44±0.82	40.16±0.37	80.13±0.90	168.60±0.08
4	35.72±0.21	23.47±0.78	66.48±0.81	135.62±0.29
5	21.18±0.53	13.40±0.17	55.19±0.35	105.51±0.24
Galantamin[b]	4.73±0.60	48.27±0.64	NT	NT
Acarbose[b]	NT	NT	71.20±0.54	191.44±0.32

[*a] Values expressed herein are mean ± SEM of three parallel measurements. p<0.05. [NT]: not tested. [NA]: not active. [b]Reference compound.

3.3. Molecular Properties, Lipinski Rule, ADME and Toxicity Risks

The molecular weight of all compounds (**1**-**5**) was between 306.40-334.45 g (150 g/mol <MW<500 g/mol). All compounds, crossed the brain barrier. **1**-**5** compounds had a high gastrointestinal absorption value. The TPSA value of 1-5 compounds was determined to be 32.34 Å² (20 Å²<TPSA<130 Å²). The iLOGP values were in the range that should be between 3.09-3.52. GI (gastrointestinal absorption) is high for all compounds. **1**, **5, 6** cross the blood brain barrier. All compounds damage the central nervous system because they cross the blood brain barrier; and as a result causes depression and lethargy (Table 3).[30,31]

When the mutagenic effect, tumorigenic effect, irritant effect and reproductive effect of all methylated chalcone compounds (**1**-**5**) were examined, the risk levels varied depending on the positions of the methyls in the phenyl ring (Table 4). There is no risk in compounds **1** and **5**. While compound **3** were found to have a high risk of irritant properties; It was determined that there was no risk in other parameters. While compound **2** have no risk in terms of mutagenic tumorigenic, Irritant has a medium risk, and reproductive effective has a high risk. While the 4th compound has medium risk in terms of

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mutagenic and tumorigenic; It has been determined that there is no risk in terms of irritant and reproductive effectiveness.

3.4. Molecular Docking Studies

Based on docking analysis, compounds **1**–**5** showed a range of −11.71 kcal/mol to −10.03 kcal/mol against AChE and −10.30 kcal/mol to −9.62 kcal/mol against BChE showed higher activity than positive control galantamine with binding energies (Table 5). It was also observed that compound **1** exhibited a more effective inhibitory activity against AChE than other compounds, with an inhibitory concentration of IC_{50} = 16.29±0.44 µM as given in Table 2.

Table 3. Drug-likeness properties and bioavailability radar of chalcone compounds with methyl group (1-5).

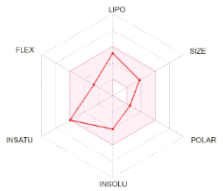
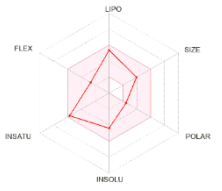
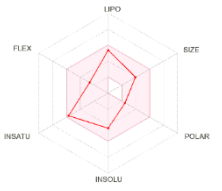
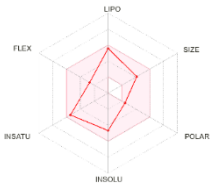
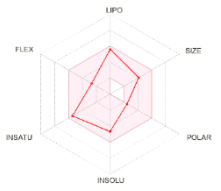
Compound	**1**	**2**	**3**	**4**	**5**
Formula	$C_{20}H_{22}N_2O$	$C_{21}H_{24}N_2O$	$C_{21}H_{24}N_2O$	$C_{22}H_{26}N_2O$	$C_{22}H_{26}N_2O$
SMILES	CC1=CC=C(\C=C\C(=O)C2=CC=C(C=C2)N2CCNCC2)C=C1	CC1=CC=C(\C=C\C(=O)C2=CC=C(C=C2)N2CCNCC2)C(C)=C1	CC1=CC=CC(C)=C1\C=C\C(=O)C1=CC=C(C=C1)N1CCNCC1	CC1=CC(C)=C(C)C=C1\C=C\C(=O)C1=CC=C(C=C1)N1CCNCC1	CC1=CC(C)=C(\C=C\C(=O)C2=CC=C(C=C2)N2CCNCC2)C(C)=C1
RADAR					
Molecular weight	306.40 g/mol	320.43 g/mol	320.43 g/mol	334.45 g/mol	334.45 g/mol
Num. heavy atoms	23	24	24	25	25
Num. arom. heavy atoms	12	12	12	12	12
TPSA	32.34 Å²	32.34 Å²	32.34 Å²	32.34 Å²	32.34 Å²
Lipinski	Yes	Yes	Yes	Yes	Yes
Log Po/w (iLOGP	3.09	3.25	3.16	3.52	3.48
GI absorption	High	High	High	High	High
BBB permeant	Yes	Yes	Yes	Yes	Yes

Table 4. Toxicity risks of chalcone compounds with methyl group (1-5).

Compound	**Toxicity Risks**			
	Mutagenic	**Tumorigenic**	**Irritant**	**Reproductive Effective**
1	No Risk	No Risk	No Risk	No Risk
2	No Risk	No Risk	Medium Risk	High Risk
3	No Risk	No Risk	High Risk	No Risk
4	Medium Risk	Medium Risk	No Risk	No Risk
5	No Risk	No Risk	No Risk	No Risk

The essential catalytic functional site of human AChE includes the catalytic triad containing Glu334, His447, and Ser203, alongside the anionic subsite composed of Trp86, Tyr337, and Phe338. It also involves a peripheral anionic site containing Tyr72, Trp286, and Tyr341.32,33 Compound **1**, the most active compound against the AChE in in vitro and in silico studies, formed non-covalent binding interactions with these residues that play an important role in the catalytic functional domain of human AChE. Moreover, this compound exhibited hydrogen bond interactions with Glu202 and His447 residues, pi-pi- T-shaped interactions involving Trp86, Tyr337, Phe297, and Tyr341, and van der Waals interactions with Gly121, Asp74, Tyr124, Trp286, Ser293, Phe295, Phe338, Gly448, Ile451, and Ser203 within the AChE enzyme, as illustrated in Figure 2. Furthermore, compound **5** showed a strong binding affinity with the lowest binding energy of -10.06 kcal/mol against BChE among all synthesiscompounds.

Table 5. The lowest binding energy values of the compound **1-5** and reference compounds from each docking analysis in the active site of AChE, BChE α-Amylase, and α-Glucosidase.

	AChE	**BChE**	**α-Amylase**	**α-Glucosidase**
Compound Number	**Binding Energy (kcal/mol)**	**Binding Energy (kcal/mol)**	**Binding Energy (kcal/mol)**	**Binding Energy (kcal/mol)**
1	-11.62	-9.93	-8.43	-7.98

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2	-11.31	-9.62	-7.90	-7.52
3	-11.15	-10.06	-8.72	-7.90
4	-11.71	-9.73	-8.33	-7.36
5	-10.03	-10.30	-8.88	-7.12
Reference Compounds	-9.13[a]	-7.61[a]	-6.30[b]	-4.66[b]

* [a]Galantamine; [b]Acarbose.

It also exhibited highly effective BChE enzyme inhibitory activity with a concentration of 13.40±0.17 µM than the reference compound galantamine (IC_{50} :48.27±0.64 µM). The catalytic triad in BChE includes Ser198, His438, and Glu325. Through site-directed mutagenesis and affinity studies, the importance of Trp82 in ligand binding has been established 34,35. This compound exhibited a pi-pi stacked bond with Trp82, a pi-alkyl bond with Tyr332, and a hydrogen bond with Glu197. Additionally, it engaged in van der Waals interactions with Ser198, Ile442, Gly116, Tyr128, Gly121, Pro84, Thr120, Asn83, Gln67, Ile69, Asn68, and Ser72, as shown in the Figure 2. As a result, the observed binding modes of these compounds within the BChE active site were considered appropriate, highlighting the crucial role of these interactions in enhancing inhibitor binding affinity.

Additionally, molecular docking analyses on α-amylase and α-glucosidase enzymes showed that the reference compound (acarbose) exhibited a binding energy of -6.30 kcal/mol and -4.66 kcal/mol towards these enzymes. The range of binding energies of all synthesized compounds towards α-amylase and α-glucosidase is between -8.88 kcal/mol to -7.90 kcal/mol and -7.98 kcal/mol to -7.12 kcal/mol, respectively, and these compounds showed better binding affinities compared to the positive compound acarbose. Likewise, in vitro analysis revealed that all compounds showed higher activity against α-amylase and α-glucosidase than positive control acarbose. Also, compounds **1** and **5**, which were the most active among the tested compounds, exhibited inhibitory activity against α-glucosidase at concentrations of 105.51±0.24 µM and 121.18±0.47 µM, respectively.

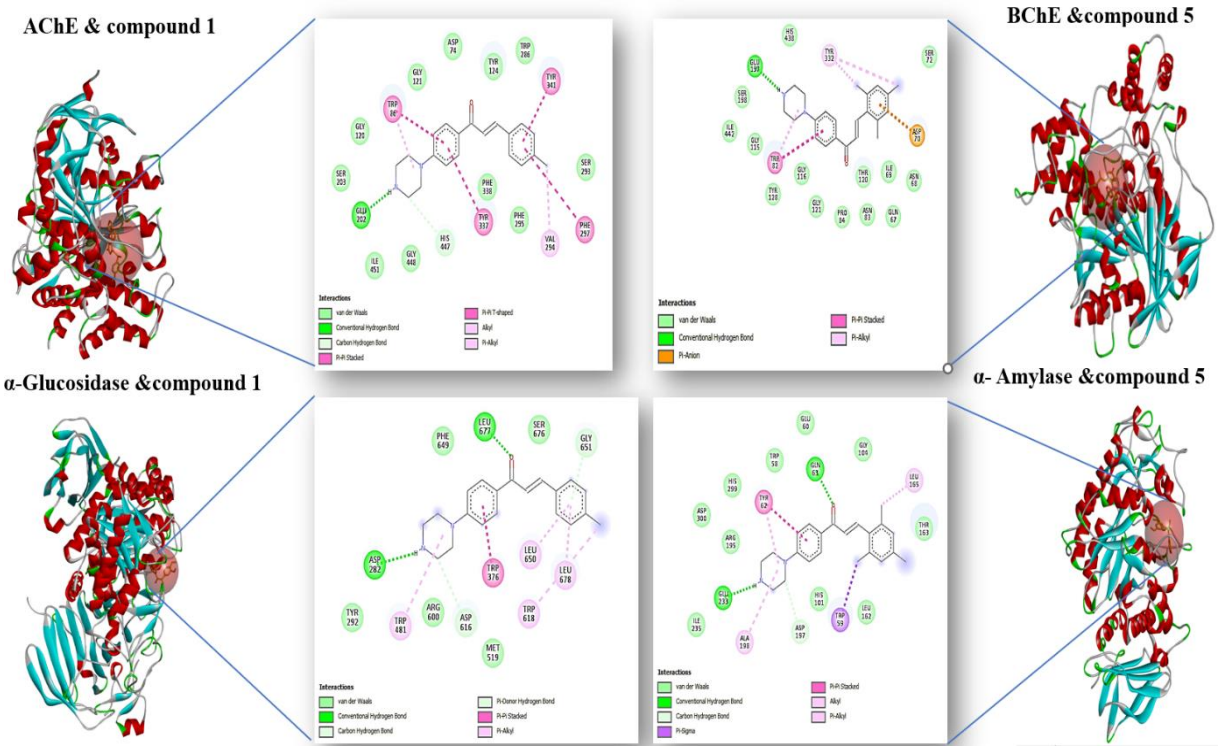

Figure 2. The 2D analysis of the most active compounds against AChE, BChE, α-amylase and α-glucosidase enzyme.

Through in silico molecular docking studies, it was observed that the active compound 1 formed hydrogen bonds with Leu677 and Asp282 while interacting with Trp481, Leu650, Leu618, and Leu678 through pi-alkyl, Trp376 pi-pi stacked bonds and Tyr292, Arg600, Met519, Ser676 van der Waals bond (Figure 2). Besides, compounds 1 and 5, recognized as the most active among the compounds tested, displayed inhibitory activity against α-amylase at concentrations of 50.01±0.76 µM and 55.19±0.35 µM, respectively. These compounds exhibited binding affinities towards α-amylase with binding energies of -8.43 kcal/mol and -

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8.88 kcal/mol, respectively. Compound **5**, proven to be the most effective in both in silico and in vitro investigations, established hydrogen bond interactions with Glu233 residues, π-π stacked interactions with Tyr62, and pi-alkyl bond interaction with Ala198 and Leu165 within α-amylase (Figure 2). It's noteworthy that the amino acids accountable for these interactions during the molecular docking process correspond to previously reported interaction sites in the literature.[36,37,38]

4.CONCLUSION

All compounds were synthesized in 41-53% yield. All compounds except compound **1** are original. Anticholinesterase and antidiabetic activities of all compounds were examined. Compound 1 is the most active molecule in anticholinesterase inhibitor activity. In antidiabetic activity, the most active molecule in α-amylase inhibitor activity is compound **1** (50.01±0.76 IC_{50} µM), and the most active molecule in α-glucosidase activity is compound **5** (105.51±0.24 IC_{50} µM). In silico and molecular docking studies of all molecules were performed. According to molecular docking results, all molecules were found to be more active than the reference compounds.

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Conflict of Interest

I declare that there is no conflict of interest with any person, institute, company, etc.

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