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Synthesis, *in vitro* acetylcholinesterase inhibitory activity and molecular modeling studies of imidazo[2,1-*b*]thiazole derivatives bearing thiosemicarbazide moiety

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ABSTRACT: Herein, we report the *in vitro* Acetylcholinesterase (AChE) inhibitory activities of imidazo[2,1-*b*]thiazole derivatives bearing thiosemicarbazide moiety. The compounds were characterized by diverse spectral methods and reported by our research team previously. As a novelty we conducted *in vitro* AChE inhibitory activities of the title compounds and performed *in silico* studies to provide insight related to activity mechanism of the compounds. Compound **4f** displayed the best biological activity within our compounds with 69.92% enzyme inhibition value (at a final concentration of 0.08 mg/mL) and 0.0245 mg/mL IC_{50} value.

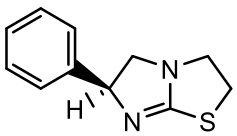
KEYWORDS: Synthesis; acetylcholinesterase; biological evaluation; molecular modeling.

1. INTRODUCTION

Alzheimer's disease is the most common neurodegenerative disease of this century and is the leading cause of dementia in the elderly. The diagnosis of Alzheimer's disease requires clinical evidence of impairment in at least one other cognitive domain, in addition to a decline in social or occupational functioning and memory loss [1]. One of the most significant biochemical changes in Alzheimer's patients is the reduction in acetylcholine levels in the cortex and hippocampus of the brain [2]. Acetylcholinesterase (AChE) is enzyme catalyzing the hydrolysis of acetylcholine into choline and acetic acid, a necessary process for the regeneration of cholinergic neurotransmission. AChE inhibitors such as donepezil, rivastigmine, and galantamine are used in the symptomatic treatment of Alzheimer's disease due to their effects in increasing synaptic levels of neurotransmitters by blocking acetylcholine hydrolysis [3].

Lately, synthesis and biological activity evaluation of novel fused heterocyclic systems gained the interest of researchers because of their broad spectrum of pharmacological activities. Among this heterocyclic groups, imidazo[2,1-b]thiazole derivatives possess specific importance since their wide range of pharmacological activitiy [4].

The imidazo[2,1-*b*]thiazole moiety bearing compound Levamisole, is a drug that has immunomodulatory and anthelmintic activities [5,6] (Figure 1).



Levamisole

Figure 1. Chemical structure of the antihelminthic compound Levamisole

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Compounds containing hydrazinecarbothioamide group have been considered as pharmacologically significant because of their wide range of reported biological activities [4]. Antitubercular thioacetazone, anticancer triapine, and methisazone are drug molecules that are used in treatment currently and containing thiosemicarbazide/thiosemicarbazone moiety (Figure 2).

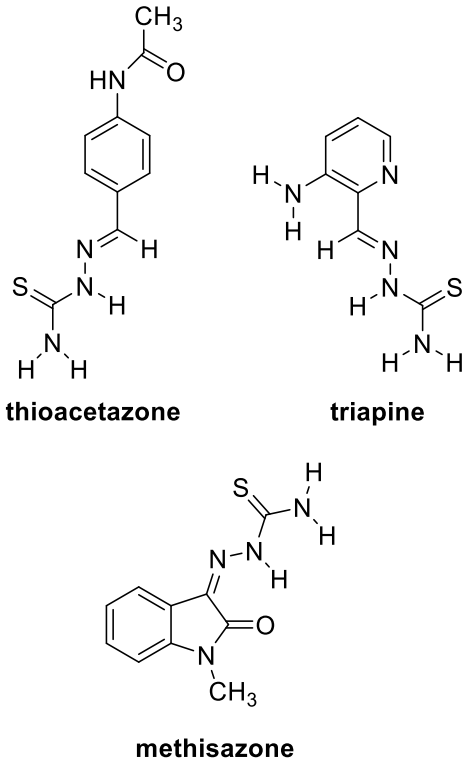

Figure 2. Chemical structures of some drugs bearing thiosemicarbazide/thiosemicarbazone moiety.

Herein, we report the *in vitro* AChE inhibitory activity of imidazo[2,1-*b*]thiazole derivatives containing hydrazinecarbothioamide group. Moreover, we performed *in silico* studies to provide insight related to activity mechanism of the compounds.

2. RESULTS AND DISCUSSION

2.1. Chemistry

2-amino-3-[(4-fluorobenzoyl)methyl]-4-(ethoxycarbonylmethyl)thiazolium bromide (**1**) was refluxed in ethanol and with the ring closure of compound **1**, ethyl [6-(4-fluorophenyl)imidazo[2,1-*b*]thiazole-3-yl] acetate hydrobromide (**2**) was yielded. By refluxing compound **2** and hydrazine hydrate in EtOH, 2-[6-(4-fluorophenyl)imidazo[2,1-*b*]thiazole-3-yl] acetohydrazide (**3**) was obtained. Compound **3** and diverse isothiocyanates were refluxed in EtOH to yield corresponding **4a-f**. The spectral data was discussed in our previous studies [4,7].

2.2. Enzyme inhibition studies

The percent AChE inhibition values of the title compounds were determined between 48.48±0.73 (**4b**) and 69.92±1.78 (**4f**). Phenethyl substituted **4f** displayed the best AChE inhibitory activity within our compounds. After the determination of that, IC_{50} value of **4f** was evaluated and described as 0.0245±0.0028 mg/mL. Overall compounds displayed AChE inhibitory activity (Table 1).

Table 1. AChE inhibitory activities of the title compounds

Compound	IC_{50} (mg/mL)*	% inhibition**
4a	N.D.	50.06±1.95
4b	N.D.	48.48±0.73
4c	N.D.	50.90±12.60
4d	N.D.	54.73±2.12
4e	N.D.	54.50±0.98
4f	0.0245±0.0028	69.92±1.78
Galantamine	0.00088±0.00005	71.25±1.60

Values are given as the mean of 3 replicates ± SD. IC_{50}: The concentration of compound or standard required to inhibit the activity of the enzyme by 50%. IC_{50} values were calculated by creating dose-response curves. N.D. : Not determined.
* IC_{50} values (mg/mL) indicated the final sample and standard concentration in the mixture.
** Percent inhibition (%), at a final concentration of 0.08 mg/mL for the synthesized compounds, at a final concentration of 0.002 mg/mL for Galantamine.

2.3. Molecular docking studies

Considering the obtained *in vitro* AChE inhibitory activity results, some profound docking studies were performed to consider the possible binding modes of the most active anti-cholinesterase compound **4f**, inside the active site of 1DX6 [8]. Firstly, to validate the Glide, we modelled the interaction between co-crystalized ligand galantamine with 1DX6. Glide successfully reproduced the experimental binding conformations of galantamine with a good root-mean-square deviation (RMSD) of 0.160 Å [9].

The docking score of galantamine was determined as -13.558 kcal/mol. Herein, galantamine formed hydrogen bond interactions with GLH 199 and SER 200. Moreover, it formed a pi-pi stacked interaction with PHE 331, and a pi-cation interaction with PHE 330. It also formed a salt bridge with ASP 72. Furthermore, it formed hydrophobic contacts with TRP 84, TYR 130, ILE 444, TRP 233, PHE 290, PHE 288, TYR 334, TYR 121, PHE 331 and PHE 330.

The docking score of **4f** was determined as -8.688 kcal/mol. Herein, it formed pi-pi stacking interactions with PHE 330, TYR 70, TYR 121 and TYR 334. It also formed hydrophobic contacts with PHE 290, TRP 84, PHE 330, PHE 331, TYR 334, TYR 121 and TYR 70 (Figure 3).

On the other hand, the binding site of AChE is characterized by five subunits. The peripheral anionic site (PAS) includes ASP 72, TRP 279, TYR 70, TYR 121, and TYR 334 residues. The amino acids PHE 288 and PHE 290 from acyl pocket while TRP 84 and PHE 330 make up the anionic site. The oxyanion hole contains GLY 118, GLY 119, and Ala 201. The catalytic triad involving SER 200, HIS 440, and GLU327 is another important subsite in the enzyme. In contrast to pharmacologically important ligands that are known to bind especially at the deep bottom of the narrow active-site gorge, a number of inhibitors have been shown to interact with other subunits suggesting to inhibit AChE in a novel manner [10]. Herein **4f** formed pi-pi stacking interactions with anionic site (PHE 330) and peripheral anionic site (TYR 70, TYR 121 and TYR 334).

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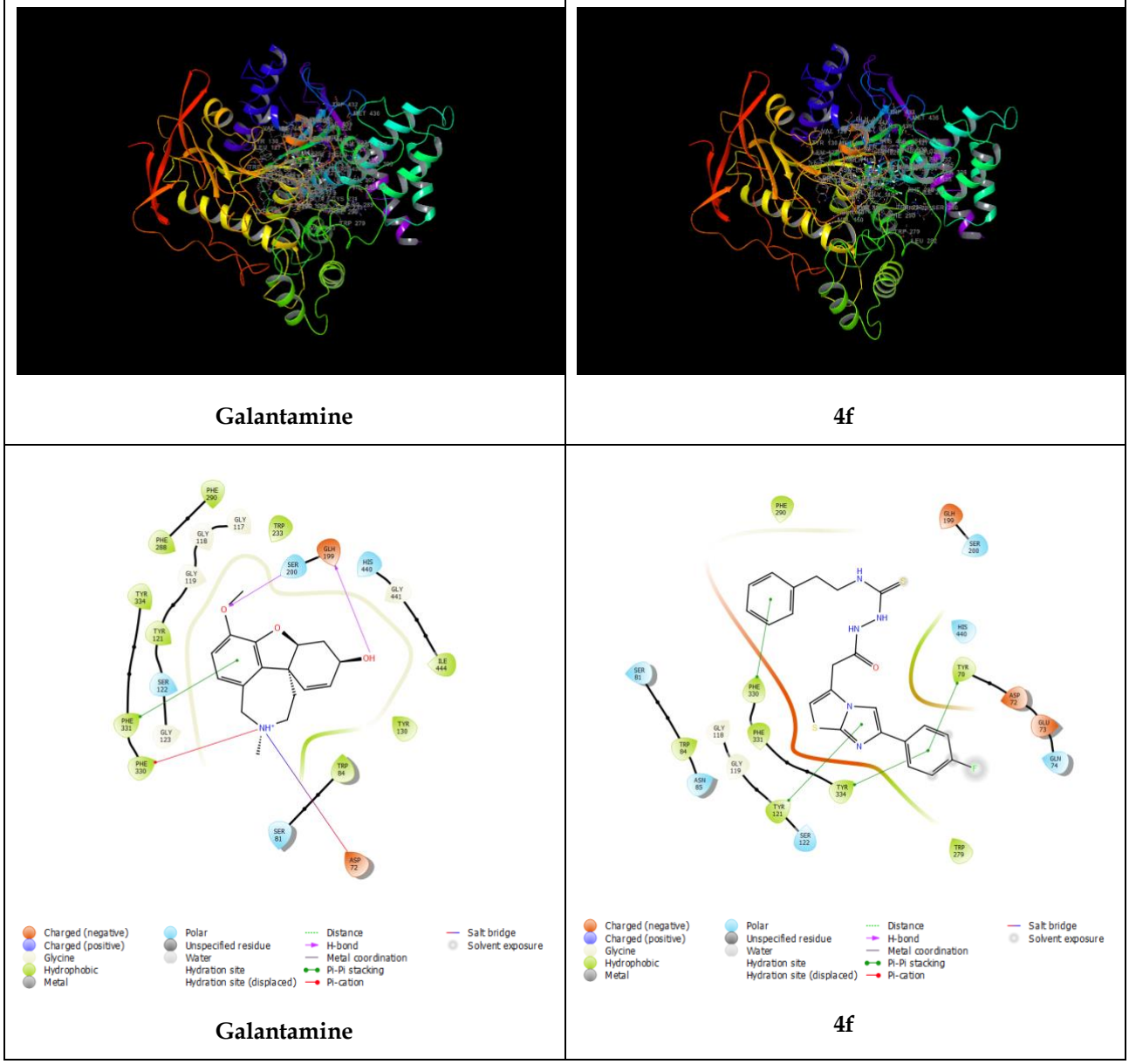


Figure 3. Compound **2f**, **Galantamine** and their 3D interactions with the active site of AChE enzyme crystal structure (PDB ID: 1DX6).

2.4. *In silico* ADME studies

Six descriptors related to the ADME properties of the synthesized compounds were calculated using the QikProp module of Schrödinger Software Suite. Herein, QPPMDCK (Permeability Maden Darby Canine Kidney) reflects the ability of the drug to pass through the blood/brain barrier. The higher the value of MDCK cells, the higher is the cell permeability (Table 2) [9].

Table 2. The analyzed descriptors related to ADME properties of the title compounds [4].

No.	Descriptor	Optimal ranges of the values
1	Molecular Weight (MW)	150-200
2	Octanol/water partition coefficient (QPLog Po/w)	-2 to 5
3	Aqueous solubility (QPLog S)	−6.5 to 0.5
4	Apparent MDCK cell permeability (QPPMDCK)	< 25 poor, > 500 great
5	Brain/blood partition coefficient (QPLog BB)	−3 to 1.2
6	Percent of human oral absorption (HOA%)	≥ 80% is high, ≤ 25 is poor

The MW values of the synthesized compounds were between 389.465 (**4b**) and 453.551 (**4f**). The MW values of **4a-f** and **galantamine** were <500 and matched Lipinski's rule of five. The QPLog Po/w values of the compounds were between 4.158 (**4b**) and 5.665 (**4f**). Excluding **4e** and **4f** the overall compounds followed Lipinski's rule of five. However QPLog Po/w values of **4e** (Log Po/w: 5.149) and **4f** (Log Po/w: 5.665) were not so high and less than 6. Log Po/w value of **galantamine** was determined as 2.077 and matched Lipinski's rule of five too. QPLog S values of the synthesized compounds were between -6.393 (**4b**) and -8.324 (**4f**) and did not follow drug likeness criteria. This problem may be solved with drug formulation techniques. The QPPMDCK values of the title compounds were between 2045.347 (**4d**) and 2793.281 (**4f**) and followed drug likeness criteria. The QPLog BB values of the title compounds were between -0.639 (**4b**) and -0.785 (**4f**) and followed the drug likeness criteria. HOA% values of the title compounds were determined as 100% except for **4e** of which HOA% was 96.305. The HOA% values of overall compounds were determined as better than the reference compound **galantamine** (Table 3).

According to Lipinski's rule of five, the number of hydrogen bond donors must be less than or equal to 5, and the number of H-bond acceptors must be less than or equal to 10 [11]. **4a-d** and **galantamine** complied with each rule belonging to Lipinski's rule of five. Since the QPLog Po/w values of **4e** and **4f**, they displayed 1 violation. However, this could be passed with drug formulation techniques.

Table 3. Prediction of ADME properties of the title compounds using QikProp module of Schrödinger

Compound	MW	QPLog Po/w	QPLog S	QPPMDCK	QPLog BB	HOA%	Rule of Five Violation
4a	391.480	4.306	-6.947	2709.456	-0.683	100.000	0
4b	389.465	4.158	-6.393	2390.757	-0.639	100.000	0
4c	405.507	4.589	-7.083	2460.743	-0.758	100.000	0
4d	425.498	4.669	-7.059	2045.347	-0.647	100.000	0
4e	439.524	5.149	-7.552	2426.115	-0.674	96.305	1
4f	453.551	5.665	-8.324	2793.281	-0.785	100.000	1
Galantamine	287.358	2.077	-1.974	543.318	0.506	92.746	0

ADME Absorption, distribution, metabolism, and excretion, QPPMDCK permeability Maden-Darby canine kidney, *HOA* Human oral absorption

3. CONCLUSION

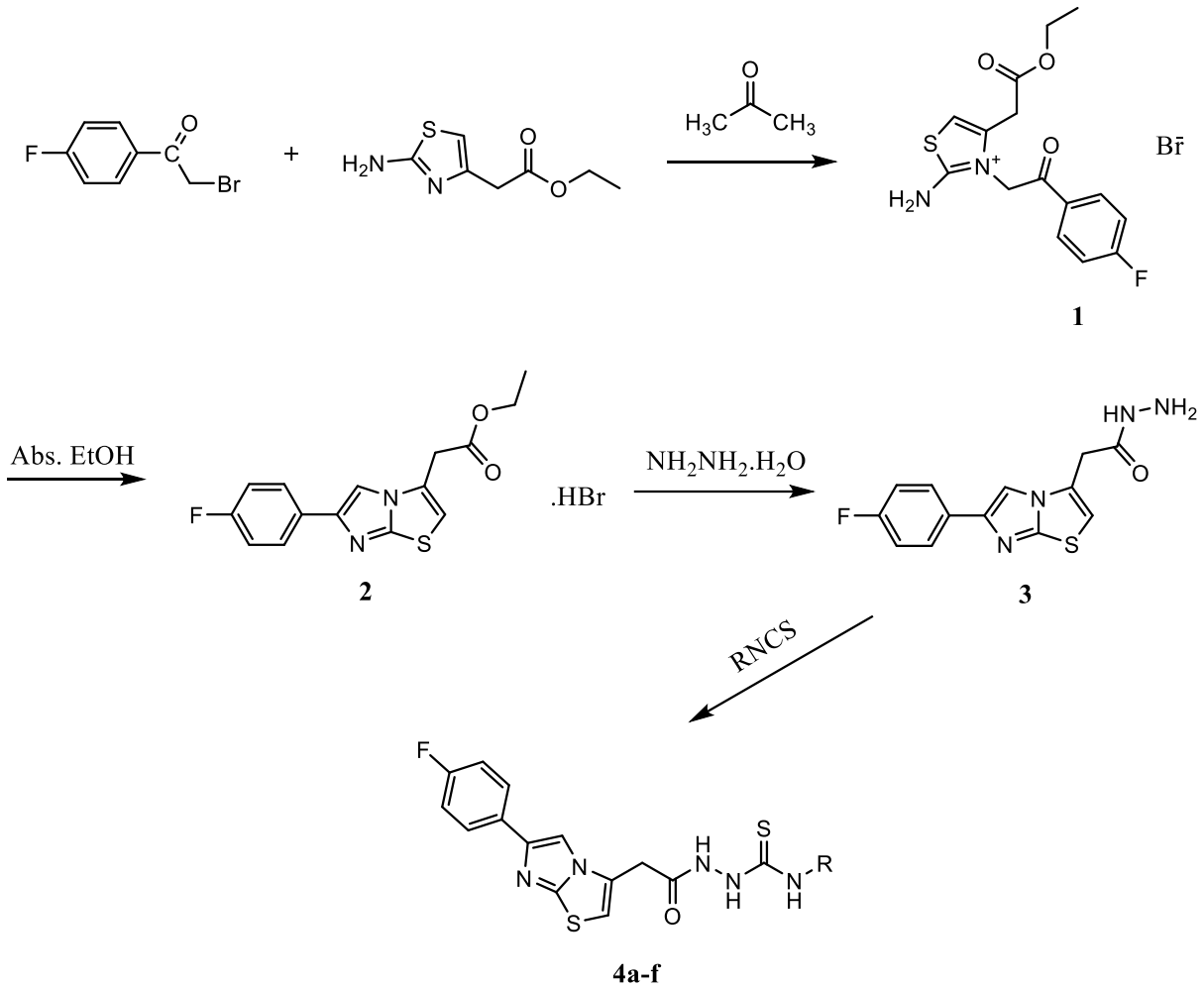
Herein, we reported the AChE inhibitory activities of a series of imidazo[2,1-*b*]thiazole based thiosemicarbazide derivatives. Moreover, molecular docking studies were performed to illuminate the drug-receptor interactions. The results indicated the AChE inhibitory activities potential of imidazo[2,1-*b*]thiazole based thiosemicarbazide derivatives. *N*-phenethyl substitution was especially determined as significant for the mentioned biological activity in this project. *In silico* ADME studies were performed to explain the pharmacokinetic properties of the synthesized compounds. This study will provide insight for researchers in their drug-discovery processes targeting AChE.

4. MATERIALS AND METHODS

4.1. Chemical synthesis

The procedure for the synthesis of 2-amino-3-[(4-fluorobenzoyl)methyl]-4-(ethoxycarbonylmethyl)thiazolium bromide (**1**)
Compound **1** was obtained according to the procedure described by Robert and Panouse [12].
The procedure for the synthesis of ethyl [6-(4-fluorophenyl)imidazo[2,1-*b*]thiazole-3-yl] acetate hydrobromide (**2**)
Compound **2** was obtained according to the procedure described by Robert and Panouse [12].
The procedure for the synthesis of 2-[6-(4-fluorophenyl)imidazo[2,1-*b*]thiazole-3-yl] acetohydrazide (**3**)
Compound **3** was obtained according to the procedure described by Harraga et al [13].
General procedure for the synthesis of 2-[2-(6-(4-fluorophenyl)imidazo[2,1-*b*]thiazole-3-yl)acetyl]-*N*-alkyl/aralkylhydrazindecarbothioamide (**4a-f**)
0.005 mol various substituted isothiocyanates were added to a solution of 0.005 mol 2-[6-(4-fluorophenyl)imidazo[2,1-*b*]thiazole-3-yl] acetohydrazide (**3**) in 30 mL of absolute ethanol and heated in water bath for 3 h under reflux. After cooling the precipitate was separated and purified either by washing with hot EtOH or recrystallization from EtOH (Figure 4).
2-(2-(6-(4-fluorophenyl)imidazo[2,1-*b*]thiazol-3-yl)acetyl)-*N*-propylhydrazine-1-carbothioamide (**4a**)
The spectral data was reported in our previous study [4,7].
2-(2-(6-(4-fluorophenyl)imidazo[2,1-*b*]thiazol-3-yl)acetyl)-*N*-allylhydrazine-1-carbothioamide (**4b**)
The spectral data was reported in our previous study [4,7].
2-(2-(6-(4-fluorophenyl)imidazo[2,1-*b*]thiazol-3-yl)acetyl)-*N*-butylhydrazine-1-carbothioamide (**4c**)

The spectral data was reported in our previous study [4,7].
2-(2-(6-(4-fluorophenyl)imidazo[2,1-*b*]thiazol-3-yl)acetyl)-*N*-cyclohexylhydrazine-1-carbothioamide (**4d**)
The spectral data was reported in our previous study [4,7].
2-(2-(6-(4-fluorophenyl)imidazo[2,1-*b*]thiazol-3-yl)acetyl)-*N*-benzylhydrazine-1-carbothioamide (**4e**)
The spectral data was reported in our previous study [4,7].
2-(2-(6-(4-fluorophenyl)imidazo[2,1-*b*]thiazol-3-yl)acetyl)-*N*-phenethylhydrazine-1-carbothioamide (**4f**)
The spectral data was reported in our previous study [4,7].



Compound	R	Compound	R
4a	$CH_3CH_2CH_2$-	**4d**	C_6H_{11}-
4b	CH_2=CH-CH_2-	**4e**	C_6H_5-CH_2-
4c	$CH_3CH_2CH_2CH_2$-	**4f**	C_6H_5-CH_2-CH_2-

Figure 4. Synthesis pathway of the title compounds **(4a-f)**

4.2. Enzyme inhibition studies

4.2.1. Acetylcholinesterase (AChE) inhibitory activity

The AChE inhibitory activities of the compounds were determined with some modifications to the method developed by Ellman et al. (1961) [14]. The Ellman reagent consisted of phosphate buffer (pH 7.5) with

318 µM DTNB and 955 µM ATChI was prepared. 20 µL of the sample was mixed with 220 µL of the Ellman reagent. Subsequently, 10 µL of AChE solution (0.5 U/mL) was added, and the increase in absorbance at 412 nm was measured against a blank for 10 minutes. The control was prepared using the same amount of solvent instead of the sample. **galantamine** was used as a standard AChE inhibitor. The AChE inhibitory activity of the samples was calculated according to the following formula.

$$\text{Inhibition } \% = \left(1 - \frac{\text{Absorbance change of sample at 412 nm}}{\text{Absorbance change of control at 412 nm}}\right) \times 100$$

4.3. Molecular docking studies

The crystal structure of acetylcholinesterase (PDB ID: 1DX6) was retrieved from RCSB Protein Data Bank and used for docking studies. Schrödinger Software Suite (Schrödinger Release 2020-3) was used for *in silico* studies. The crystal structure was prepared by Protein Preparation Wizard Module and optimized by removing water molecules, heteroatoms, and co-factors. The hydrogens, missing atoms, bonds, and charges were computed through Maestro. The ligands were prepared and optimized using the LigPrep module of Schrödinger Software Suite. Besides, to define the binding site, a receptor grid was generated around the co-crystalized ligand of the enzyme by Grid Generation implemented in Glide. The docking was performed at Extra Precision (XP) mode.

4.4. *In silico* ADME studies

The pharmacokinetic properties of all compounds were predicted by using Qikprop module of Schrödinger Software Suite (Schrödinger Release 2020-3).

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