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RESEARCH ARTICLE

CHEMISTRY & BIODIVERSITY

N-Substituted Arylidene-3-(Methylsulfonyl)-2-Oxoimidazolidine-1-Carbohydrazide as Cholinesterase Inhibitors: Design, Synthesis, and Molecular Docking Study

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The development of new enzyme inhibitors in degenerative brain diseases has gained more attention. Enzyme inhibitors play an effective role in controlling central nervous system diseases. For this purpose, a novel series of hydrazone derivatives containing imidazolidine ring aimed against Alzheimer's disease (AD), have been designed and synthesized. The acetylcholinesterase (AChE) enzyme inhibitory activity of these compounds was investigated. The structures of the compounds were determined by IR, ^{1}H and ^{13}C-NMR and mass spectroscopic methods. Inhibition studies on the cholinesterase (ChE) enzymes and β-amyloid plaque inhibition test of the compounds were performed. Based on the experimental results, compound **3j** bearing dimethoxy substituent on the aromatic ring like donepezil exhibited the most AChE inhibitory activity with the IC_{50} values of 0.023 ± 0.001 μM. Owing to obtained biological activity and molecular docking study results, it is thought that the most active compound **3j** may play a role in both symptomatic and palliative treatment of AD.

Keywords: Alzheimer's disease, β-amyloid plaque, hydrazone, imidazolidine ring, molecular modeling.

Introduction

Alzheimer's disease (AD) is characterized by neuritic plaques and neurofibrillary tangles, nerve cell death, and the leading cause of dementia associated with memory loss and impaired cognitive abilities in aging individuals.[1–3] It was estimated that 35.6 million people were living with AD in 2015. It is expected that this number will increase to about 65.7 million in 2030, and about 115.4 million by 2050.[4] AD can lead to a decline in activities of daily living and interfere with the quality of life.[5] Although many drugs are available for symptomatic treatment, new pharmacological interventions are needed to reduce the production of pathology in the brain.[6,7] It is well-known as the cholinergic hypothesis that the lower levels of acetylcholine and increased acetylcholinesterase (AChE) enzyme activity in the Alzheimer's brain.[8] Therefore AChE inhibitors become an important option for the treatment of Alzheimer's disease.

Hydrazones are an important intermediate in many enzymatic reactions involving the interaction of the substrate of enzymes with an amino or a carbonyl group. They play a critical role in the enzymatic or non-enzymatic transamination reactions of carbonyl compounds with amino acids.[9] Hydrazones contain unique structures as N–N and C=N bonds in their molecular structures and these chemical groups exhibit an extensive range of chemical, therapeutic, biological, and industrial properties.[10,11] They have a remarkable role in the mechanism of the transformation and racemization reaction in the biological system

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due to their flexibility and structural similarity with various natural substances of biological importance.[12]

Nitrogen-containing heterocycles such as imidazole (or imidazolidine) have attracted special attention.[13] Many imidazolidine-based drugs such as mephenytoin (anticonvulsant), imidapril (antihypertensive), azlocillin (antibiotic) and emicerfont (anxiety disorders) have been widely used for the treatment of various types of diseases due to their high therapeutic potential. The literature revealed that the combination of the hydrazide-hydrazone moiety with hetero-aromatic rings provided compounds with an extensive range of biological properties such as anti-inflammatory,[14] anticancer,[15,16] antimicrobial,[17] antidiabetic activity[18] and, more importantly, anti-cholinesterase.[19] The α-hydrogen atom of the hydrazone is more acidic than ketones. Hence, the carbon atom has both electrophilic and nucleophilic character. In addition, the hydrazones may increase absorption and reduce cytotoxicity compared to parent amides due to their higher lipophilicity.[20] It was reported that many compounds among the great variety of hydrazone derivatives behave as AChE inhibitors.[21–23]

We aim to incorporate these two independent biologically active moieties into a single molecule to enhance the biological availability of the designed compounds. Given the above fact, we focused on the synthesis of hybrid compounds bearing imidazolidine ring and hydrazone moiety and evaluated them for their anti-acetylcholinesterase activities. For this purpose, the refence agents donepezil and tacrine which are selective inhibitors for AChE and BChE, respectively, and curcumin which has inhibitor potential to β-amyloid plaque aggregation were used in the experiments.

Results and Discussion

Synthesis

3-(Methylsulfonyl)-2-oxoimidazolidine-1-carbonyl chloride (**1**) was reacted with hydrazine hydrate to afford 3-(methylsulfonyl)-2-oxoimidazolidine-1-carbohydrazide (**2**). Treatment of compound **2** with aromatic aldehydes in an ethanolic acidic medium gave the hydrazone derivatives (**3a**–**3z**) (*Scheme S1*). The structure elucidation of the hydrazone derivatives was performed by IR, ^{1}H-NMR, ^{13}C-NMR, and HR-MS results.

The infrared spectrum of hydrazone derivatives displayed the stretching vibrations of NH at 3192–3362 cm^{-1}. The C=N stretching bands were characteristic for hydrazones observed at 1595–1622 cm^{-1}. The C=O bands belonging to the imidazolidine ring and hydrazone were seen at 1703–1732 and 1687–1714 cm^{-1}, respectively. In the ^{1}H-NMR spectrum, the NH protons of hydrazone derivatives appeared as a singlet between 10.81 and 11.37 ppm. The N=CH protons resonated as a singlet at 8.17–8.81 ppm. The methylene protons of the imidazolidine ring were observed at 3.73–3.92 ppm. The methylsulfonyl protons were detected at 3.32–3.43 ppm. The ^{13}C-NMR spectrum of hydrazones displayed the imine carbon (C=N) between 152.78–153.23 ppm. High-resolution mass spectrometry (HR-MS) of all compounds was also in agreement with theoretical *m/z*.

Evaluation of Biological Activity Studies

ChE Enzymes Inhibition

The results of BChE enzyme activity revealed that all derivatives excluding compounds **3b**, **3e**, **3i**, **3p**, and **3z** had activity higher than 50% at 10^{-3} M concentration. But, when the results of the activity at 10^{-4} M concentration were analyzed none of the synthesized compounds showed remarkable inhibition rates. When it was compared with the results obtained in the AChE enzyme inhibition test at these concentrations, almost all of the compounds in the series showed a more selective inhibition against the AChE enzyme. The reference agent tacrine had inhibitory activity at the rate of 99.827 ± 1.378% and 98.651 ± 1.402% at 10^{-3} and 10^{-4} M concentrations (*Table 1*).

It was observed from AChE enzyme inhibition results that all of the obtained compounds demonstrated a high rate of activity (more than 50%) at 10^{-3} M concentrations. Among the synthesized compounds, derivatives **3g**, **3h**, **3i**, **3j**, **3k**, **3l**, **3m**, **3n**, **3q**, **3v** and **3w** displayed a quite strong AChE enzyme inhibition profile by generating at least 90% activity. Compounds **3g**, **3h**, **3i**, **3j**, **3k**, **3l**, **3m**, **3n**, **3q**, **3v**, **3w** and **3x** were selected for the second stage of the enzyme activity test by having higher than 50% inhibitory activity at 10^{-4} M concentration. Meanwhile, the reference drug donepezil had inhibitory activity at the rates of 99.254 ± 2.104% and 97.426 ± 1.890%, respectively, at 10^{-3} and 10^{-4} concentrations. The further concentrations of the selected derivatives were prepared with serial dilutions for the second stage of the AChE enzyme inhibition assay (*Table 1*).

The IC_{50} value of donepezil was found at 0.021 ± 0.001 μM on the AChE enzyme; while the IC_{50} values of compounds **3g**, **3h**, **3i**, **3j**, **3k**, **3l**, **3m**, **3n**, **3q**, **3v**, **3w** and **3x** were found in the range of 0.023 ± 0.001–

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Table 1. % Inhibition and IC_{50} values of the synthesized compounds, donepezil and tacrine against AChE and BChE.

Compd.	AChE% Inhibition		AChE IC_{50} (μM)	BChE % Inhibition		BChE IC_{50} (μM)
	10^{-3} M	10^{-4} M		10^{-3} M	10^{-4} M	
3a	**85.056 ± 1.265**	45.465 ± 0.995	> 100	**55.420 ± 0.932**	30.559 ± 0.861	> 100
3b	**80.861 ± 1.321**	40.620 ± 0.847	> 100	48.991 ± 0.815	28.164 ± 0.724	> 1000
3c	**76.647 ± 1.048**	47.047 ± 0.962	> 100	**59.748 ± 1.067**	35.311 ± 0.967	> 100
3d	**83.185 ± 1.195**	48.299 ± 0.802	> 100	**57.167 ± 0.949**	33.727 ± 0.759	> 100
3e	**79.490 ± 1.402**	46.751 ± 0.754	> 100	47.050 ± 0.781	36.489 ± 0.628	> 1000
3f	**75.223 ± 1.256**	43.925 ± 0.966	> 100	**60.627 ± 1.102**	31.934 ± 0.731	> 100
3g	96.957 ± 1.065	92.208 ± 1.311	**0.058 ± 0.002**	**53.815 ± 1.054**	37.865 ± 0.805	> 100
3h	93.122 ± 1.162	90.437 ± 1.348	**0.181 ± 0.008**	**54.564 ± 0.989**	40.008 ± 0.764	> 100
3i	95.657 ± 1.248	90.033 ± 1.699	**0.064 ± 0.003**	45.228 ± 0.977	27.624 ± 0.618	> 1000
3j	98.571 ± 1.846	96.137 ± 1.420	0.023 ± 0.001	**57.433 ± 0.861**	29.265 ± 0.897	> 100
3k	92.459 ± 1.359	89.254 ± 0.989	**0.105 ± 0.004**	**62.319 ± 1.003**	33.118 ± 0.755	> 100
3l	93.614 ± 1.047	88.716 ± 1.095	**0.128 ± 0.005**	**60.458 ± 1.154**	37.449 ± 0.620	> 100
3m	90.162 ± .1.122	86.327 ± 1.265	**0.149 ± 0.006**	**58.714 ± 0.967**	31.875 ± 0.889	> 100
3n	96.188 ± 1.308	93.475 ± 1.678	**0.028 ± 0.001**	**55.098 ± 0.881**	30.068 ± 0.946	> 100
3o	**86.747 ± 1.344**	40.189 ± 0.847	> 100	**51.567 ± 1.002**	29.624 ± 0.637	> 100
3p	**83.995 ± 1.085**	42.074 ± 0.995	> 100	49.951 ± 0.869	25.460 ± 0.749	> 1000
3q	97.590 ± 1.495	93.067 ± 1.802	**0.032 ± 0.001**	**62.402 ± 1.101**	36.733 ± 0.982	> 100
3r	**80.120 ± 1.495**	45.648 ± 0.714	> 100	**57.731 ± 0.964**	34.954 ± 0.810	> 100
3s	**82.446 ± 1.220**	47.765 ± 1.062	> 100	**52.279 ± 0.977**	30.895 ± 0.765	> 100
3t	**74.651 ± 1.114**	48.924 ± 0.955	> 100	**54.884 ± 0.859**	27.227 ± 0.648	> 100
3u	**76.874 ± 1.262**	46.507 ± 1.048	> 100	**51.944 ± 0.782**	32.008 ± 0.722	> 100
3v	90.116 ± 1.067	85.649 ± 0.962	**0.193 ± 0.007**	**56.165 ± 1.130**	37.697 ± 0.897	> 100
3w	92.622 ± 1.248	84.466 ± 1.237	**0.207 ± 0.009**	**59.002 ± 0.914**	29.462 ± 0.664	> 100
3x	89.497 ± 0.902	83.318 ± 1.318	**0.216 ± 0.008**	**63.691 ± 1.159**	39.818 ± 0.911	> 100
3y	**78.288 ± 1.355**	42.448 ± 0.990	> 100	**60.348 ± 0.837**	30.387 ± 0.847	> 100
3z	**81.095 ± 1.547**	40.669 ± 0.847	> 100	45.132 ± 0.960	31.285 ± 0.728	> 1000
Donepezil	99.254 ± 2.104	97.426 ± 1.890	**0.021 ± 0.001**	–	–	–
Tacrine	–	–	–	99.827 ± 1.378	98.651 ± 1.402	0.0064 ± 0.0002

*The bold style means that higher than 50% inhibition at 10^{-3} M concentration. The red style means that higher than 50% inhibition at both 10^{-3} M and 10^{-4} M concentration.

0.216 ± 0.008 μM. Among these compounds, compounds **3j** and **3n** were found to have the strongest AChE inhibition with the lowest IC_{50} value. The IC_{50} values of compounds **3j** and **3n** were calculated as 0.023 ± 0.001 μM and 0.028 ± 0.001 μM, respectively (*Table 1*). These compounds demonstrated an inhibition profile with very close IC_{50} values to that of donepezil.

β-Amyloid 1-42 (Aβ42) Inhibitor Screening Study

In addition to the decrease in cholinergic transmission in Alzheimer's patients, amyloid plaque accumulation consisting of beta-amyloid (Aβ) peptides can be considered among the important causes. In this study β-amyloid aggregation inhibition activities of compounds **3g**, **3h**, **3i**, **3j**, **3k**, **3l**, **3m**, **3n**, **3q**, **3v**, **3w** and **3x** showed high inhibitory activity against the AChE enzyme were carried out using the 'β-Amyloid 1-42 (Aβ42) Ligand Screening Assay (BioVision, Milpitas, CA, USA)' kit based on the fluorometric method.[24,25] The test kit, based on the fluorometric method, was used following the recommended procedure. If an Aβ42 ligand is present, this reaction is inhibited/destroyed, thereby reducing or eliminating fluorescence. Curcumin and donepezil were used as reference drugs. β-Amyloid plaque inhibition profiles were examined by preparing reference drugs and compounds **3g**, **3h**, **3i**, **3j**, **3k**, **3l**, **3m**, **3n**, **3q**, **3v**, **3w** and **3x** at 10 μM, 1 μM, 0.1 μM, and 0.01 μM concentrations. All test compound concentrations were applied in quadruplicate on the plates. When the kit protocol was completed, the % inhibition ratios of curcumin, donepezil, and test compounds were calculated based on the control group. The percent inhibitions of β-amyloid 1-42 (Aβ42) peptide aggregation were given in *Figure 1*.

Curcumin displayed inhibition of 92.895 ± 2.310%, 85.798 ± 2.107%, 44.740 ± 1.050% and 32.897 ±

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0.970%; while donepezil had inhibition 95.467±2.025%, 88.340±2.237%, 69.741±1.033% and 52.488±0.895% at 10 μM, 1 μM, 0.1 μM and 0.01 μM concentrations, respectively. More than 50% inhibition was seen in all tested compounds at concentrations of 10 μM and 1 μM. For 0.1 μM concentration, all obtained derivatives except for compounds **3h** and **3m** showed more than 50% inhibitor activity. Also, only compounds **3j** and **3k** were found to display more than 50% inhibitor activity at 0.01 μM concentration. Corresponding to *Figure 1*, compound **3j** was determined to have the highest inhibition rate among the tested derivatives. This compound showed inhibition at related concentrations as follows: 93.245±2.145%, 85.167±1.856%, 65.323±1.689%, and 50.477±1.502%, respectively. Also, it was seen that compound **3j** displayed higher inhibition than curcumin; whereas it displayed an inhibition profile at a similar rate to donepezil.

These findings indicate that the related compounds can inhibit β-amyloid plaque aggregation in varying proportions in addition to their AChE enzyme inhibitory potentials. Preventing the accumulation of β-amyloid plaque, which is one of the important causes of AD, will be accompanied by an increase in cholinergic activity with enzyme inhibition, and may show a stronger approach in the treatment of AD.

Evaluation of Molecular Docking Studies

To determine the possible interactions of compound **3j** with the enzyme active site, which had high AChE enzyme inhibition activity, docking studies were carried out on the crystal structure of the AChE enzyme (PDB Code: 4EY7)[26] The rendered docking poses of compound **3j** were given in *Figures 2* and *3*.

When the AChE enzyme structure is examined, it is striking that it has two different binding sites: the catalytic site (CAS) and the peripheral anionic site (PAS). It has been reported that amino acids Ser203, Glu334, His447, Trp86, Tyr130, Tyr133, Tyr337, Phe338 are effective in binding to the CAS region, while Tyr72, Asp74, Tyr124, Tyr341, Trp286 amino acids are required to bind to the PAS region.[26] It was determined in many modeling studies that donepezil interacts with both regions of the AChE enzyme and settles very well in the gorge with its dual binding side-DBS feature.[24–27]

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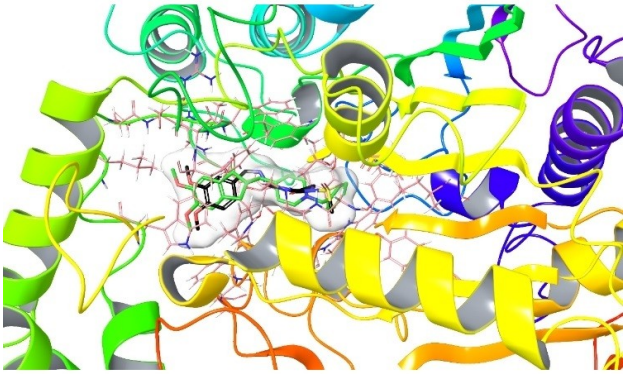

Figure 2. The superimposition pose of donepezil (green) and compound **3j** (black) in the enzyme active site of the AChE enzyme (PDB Code: 4EY7).

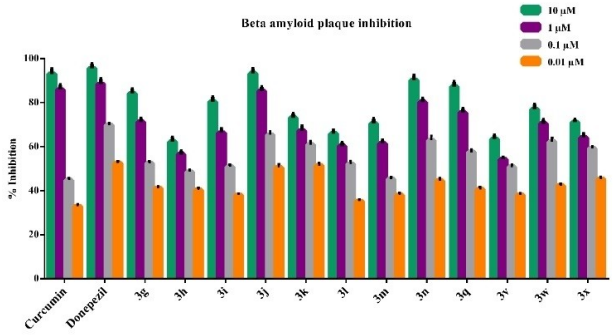

Figure 1. β-Amyloid plaque inhibition (%) of curcumin, donepezil, and compounds **3g**, **3h**, **3i**, **3j**, **3k**, **3l**, **3m**, **3n**, **3q**, **3v**, **3w** and **3x**.

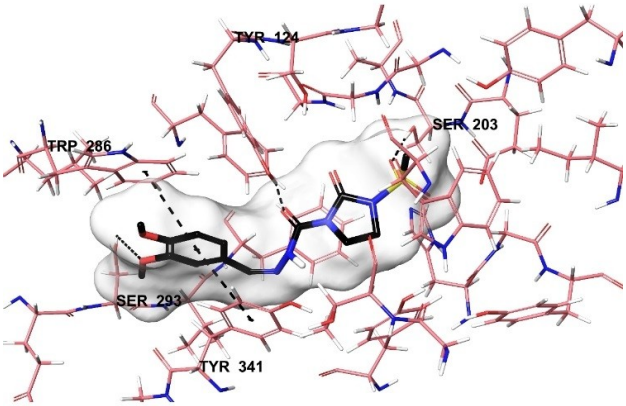

Figure 3. The 3D interacting mode of compound **3j** in the active region of the AChE enzyme (PDB Code: 4EY7). Compound **3j** was shown in a tube model and black colored.

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dual binding site (DBS) feature.[26] Cheung et al. (2012) examined the binding sites of donepezil to enzyme active sites and explained the interactions of donepezil with active sites. In the CAS region, the benzyl ring interacts π-π with the indole ring of Trp86 amino acid in the active site. The carbonyl oxygen in the structure has an important interaction. Hydrogen bonding is established between the carbonyl oxygen of indanone and the amino group of Phe395. In the PAS region, the indanone structure binds with the indole in Trp286 by π-π interaction. In addition, the piperidine ring is in van der Waals interaction with both the CAS and PAS domains.[26] When the docking study of the compound **3j**, which has the highest activity among the synthesized compounds, is examined with the AChE enzyme, it is seen that the compound **3j** is very strongly bound to the enzyme active site by forming many and various interactions. As with donepezil, it is seen that the compound **3j** binds to both the CAS and PAS regions of the enzyme and settles in the gate, and thus, thanks to its DBS properties, it can fully interact with the enzyme.

Figure 2 showed the localization of the most active compound **3j** and donepezil to the enzyme active site. According to this figure, derivative **3j** was localized to the enzyme active site in a similar position to donepezil. It was determined by looking at *Figure 3* that compound **3j** was settled down at the enzyme active site by having various interactions. *Figure 3* showed that the oxygen atom of methylsulfonyl moiety formed a hydrogen bond with the hydroxy of Ser203. Another hydrogen bond was seen between the carbonyl near to 2-oxoimidazolidine ring and the hydroxy of Tyr124. The phenyl ring in the structure exhibited two π-π interactions with the phenyl rings of Trp286 and Tyr341. Especially the interaction with Trp286 was the strongest interaction of the donepezil molecule in the peripheral anionic region.[24–28] The observation of this interaction in the most effective derivative **3j** was a very important finding in terms of the explanation of binding this compound to the AChE enzyme. Finally, the last interaction was detected as a hydrogen bond belonging to the substituted methoxy group in the phenyl ring. This methoxy group at the 3rd position of the phenyl ring created a hydrogen bond with the hydroxy of Ser293. This finding also supported the difference in activity between the synthesized compounds. Compound **3j** had a 3,4-dimethoxy substituent differently from the other derivatives and it additionally formed the last-mentioned interaction. Also, this interaction differed from donepezil. When comparing the donepezil and compound **3j** chemically, the presence of the dimethoxy moiety was the common feature. But, as mentioned above donepezil molecule showed any interaction about this group. The observation of this hydrogen bond at the related to compound **3j** was very valuable in terms of stronger binding. The contribution of this substituent to the activity was clearly seen.

Conclusions

Enzyme inhibition studies have been a hot area driving research into the development of beneficial treatments against various diseases in medicinal chemistry. Alzheimer's disease is closely related to cholinergic disorder. Therefore acetylcholinesterase inhibitors have attracted more attention in this area. For this purpose, new hydrazone derivatives bearing imidazolidine rings were synthesized and evaluated their acetylcholinesterase inhibitory activity. The structures of the obtained compounds were confirmed with IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopic methods. The biological activities of the final compounds were studied along with reference materials, and molecular modeling was performed. Compounds **3j** and **3n** were the most potent ones in the series. Especially, compound **3j** bearing 3,4-dimethoxy substituent on the phenyl showed significant inhibition of AChE with the IC_{50} values of 0.023 ± 0.001 μM. A β-amyloid 1-42 (Aβ42) ligand screening assay kit was used to investigate the effects of selected compounds, which strongly inhibited AChE, on amyloid plaques. The results suggested that compound **3j** displayed higher inhibition than curcumin; whereas it displayed an inhibition profile at a similar rate to donepezil. Molecular docking studies performed with the most active derivative **3j** showed that this molecule had similar binding properties with donepezil and bound very strongly to the active site of the AChE enzyme. Consequently, about the results of our activity and molecular modeling studies, future studies should aim to design, synthesize and investigate the activities of new and more potent compounds with similar chemical structures.

Experimental Section

Chemistry

All the chemicals were obtained from Merck (Darmstadt, Germany) or Sigma–Aldrich (St. Louis, MO, USA).

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SMP II melting point apparatus (Schorpp Geaetetechnik Gehrden, Germany) was used to determine the melting points of the target compounds. IR spectra were recorded on a Shimadzu FT-IR-8400S spectrophotometer (Shimadzu, Tokyo, Japan). ^{1}H-NMR and ^{13}C-NMR spectra were recorded by a Bruker 300–400 MHz and 75 MHz digital FTNMR spectrometer (Bruker Bioscience, Billerica, MA, USA) in (D_6)DMSO, respectively. Mass spectra were obtained on an LCMS-IT-TOF (Shimadzu, Kyoto, Japan).

General Procedure for the Synthesis of the Compounds **3a**–**3z**

The synthesis of 3-(methylsulfonyl)-2-oxoimidazolidine-1-carbohydrazide (**2**) has been reported in our previous study.[29] In this study, 3-(methylsulfonyl)-2-oxoimidazolidine-1-carbohydrazide (0.001 mol) and substituted aromatic aldehydes (0.002 mol) were taken in a bottom flask and dissolved in 15 mL ethanol. A catalytic amount of glacial acetic acid was added to the mixture. The reaction was stirred on a magnetic stirrer for 30 min. Then the mixture was refluxed for 12–16 h.[30] After completion of the reaction, the solvent was evaporated through a rotary evaporator and the residue was taken by washing with water. The products were recrystallized from methanol. All the spectroscopic data can be found in *Supporting Information*.

Biological Activity Studies

In Vitro *ChE Enzymes Inhibition Assay*

AChE and BChE inhibitory activities of the synthesized compounds were assessed using the modified Ellman method, as described in the previous studies published by our team.[24,25,27,28,31,32]

β-Amyloid 1-42 (Aβ42) Inhibitor Screening Assays

The experimental procedure was based on the 'β-Amyloid 1-42 (Aβ42) Ligand Screening Assay' kit (BioVision, Milpitas, CA, USA) protocol based on the fluorometric method.[24,25]

Molecular Docking Studies

The structure-based *in silico* docking method was applied to determine possible binding and interaction points of the most active compound **3j** in the series with the active site of the AChE enzyme. For this purpose, protein-ligand interaction analysis was performed using the crystal structures AChE (PDB: 4EY7).[26]

The crystal structure was first prepared for docking studies with the *Protein Preparation Wizard* protocol in *Schrödinger Suite 2015 Update* 2,[33] the bond lengths were adjusted using the OPLS 2005 force field, and the possible charges of the atoms on the charged amino acids under the specified ambient conditions were determined automatically. The compounds were prepared for molecular docking studies using *Ligprep 2.3* module.[34] Grids were generated with *Glide 7.1*[35] and docking runs were performed with the single-precision (SP) docking mode of the same module.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Author Contribution Statement

F.T. and B.N.S. performed the experiments, analyzed the data, and wrote the article. F.T., B.N.S., Y.Ö., Z.A.K. and B.K.K. contributed samples/reagents/materials/analysis tools and analyzed the data. F.T. and B.N.S. conceived and designed the experiments.

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