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Synthesis of (*p*-tolyl)-3(2*H*)pyridazinone Derivatives as Novel Acetylcholinesterase Inhibitors

İrem Bozbey Merde,*[a] Gülce Taşkor Önel,[b] Burçin Türkmenoğlu,[b] Şule Gürsoy,[c] Esra Dilek,[c] Azime Berna Özçelik,[d] and Mehtap Uysal[a]

In this study, a series of *N*-substituted-(*p*-tolyl)pyridazin-3(2*H*)-one derivatives were synthesized and evaluated for their AChE inhibitory activity. The chemical structures of novel compounds **5**(**a**–**m**) were confirmed by ^{1}H-NMR, ^{13}C-NMR, IR and HRMS analysis. In order to eliminate the symptomatic effects of Alzheimer's disease, the proposed compounds were evaluated by acetylcholinesterase inhibition activity study in accordance with the cholinergic hypothesis. The results revealed that the *N*-substituted-(*p*-tolyl)pyridazin-3(2*H*)-one derivatives inhibited the enzymes significantly. K_i values for acetylcholinesterase in the range of 0.56 ± 0.15–4.12 ± 1.42 μM. Compound **5h** demonstrated the greatest in AChE activity compared with tacrine (0.56 ± 0.15 μM). Molecular docking studies were performed for all compounds that compared tacrine in AChE activity *in-vitro*. As a result of molecular docking studies (ΔG_{Bind}, docking score, XP Gscore, Glide energy, Glide emodel), **5f**, **5g** and **5h** compounds showed good inhibitory properties in the AChE active site as *in silico*.

Introduction

Pyridazinone derivatives like 2,6-disubstituted-pyridazinones,[1] 3(2*H*)pyridazinones[2] have appeared as potent Alzheimer's disease (AD) agents. Research on acetylcholinesterase of many structures for example liquiritigenin, deoxyvasicinone hybrids etc. different from pyridazinones against Alzheimer's is ongoing.[3] In addition, pyradizinones have antihypertensive activity,[4] anticancer activity,[5] sortase A inhibitors effect[6] and selective cyclooxygenase-2 inhibitor effect.[7] Due to these positive bioactive effects, it has become important to conduct research on pyridazinones.

Despite significant advances in understanding the pathogenesis of AD, the exact cause of AD is still unknown. That's why AD has now been recognized as an international public health priority by the World Health Organization.[8] AD is the leading cause of dementia in older people, and about 50–75% of people with dementia have Alzheimer's. As a result of studies conducted worldwide, women are more prone to AD than men.[9] The population aged 65 and over is expected to increase from 9.3% in 2020 to 16.0% in 2050.[10] This situation has a heavy burden on families and society in the rapidly aging society. Finding new treatments for AD has become important.[11]

Although the exact cause of AD is not known yet, it is known that this disease increases as ACh deficiency increases. When brain tissues of AD patients were examined, it was shown that there was a decrease in acetylcholine transferase activity, and a decrease in ACh synthesis, uptake and release.[12] Making ACh stay longer in the synaptic gap is one of the methods used in AD treatment. For this purpose, cholinesterase enzyme inhibitors are used.[13] Acetylcholinesterase inhibitors (AChEIs) prevent the breakdown of acetylcholine as it reduces AChE's activity. Thus, it increases the amount and duration of action of the neurotransmitter acetylcholine. AChEIs act on this event reversibly, semi-reversibly and irreversibly.[14]

In our previous studies, AChE inhibitor compound derivatives were investigated using aldehydes and sulfonyl chlorides, with the 3(2*H*)-pyridazinone ring as the main ring structure.[2b,c,15] In this study, we studied the compounds that we expect to have a stronger inhibitory effect by modifying the structure. Newly designed *N*-substituted-(*p*-tolyl)pyridazin-3(2*H*)-ones with triazole ring-substituted were synthesized (Figure 1) and *in-vitro* and *in silico* studies were performed.

Recently, modern drug design has been used to recommend and support lower cost and more economical drug design.[16] Developmental strategies for drug design can also be validated by molecular docking study.[16] In addition, in this study, experimental results of new compounds that can be potent on AChE were supported by molecular docking method.

[a] *Dr. İ. B. Merde, Prof. M. Uysal*
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Erzincan Binali Yıldırım University, Yalnızbağ 24002 Erzincan, Turkey
E-mail: irem.bozbey@erzincan.edu.tr
[b] *Dr. G. T. Önel, Dr. B. Türkmenoğlu*
Department of Analytical Chemistry, Faculty of Pharmacy, Erzincan Binali Yıldırım University, Yalnızbağ 24002 Erzincan, Turkey
[c] *Dr. Ş. Gürsoy, Dr. E. Dilek*
Department of Biochemistry, Faculty of Pharmacy, Erzincan Binali Yıldırım University, Yalnızbağ 24002 Erzincan, Turkey
[d] *Dr. A. B. Özçelik*
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey

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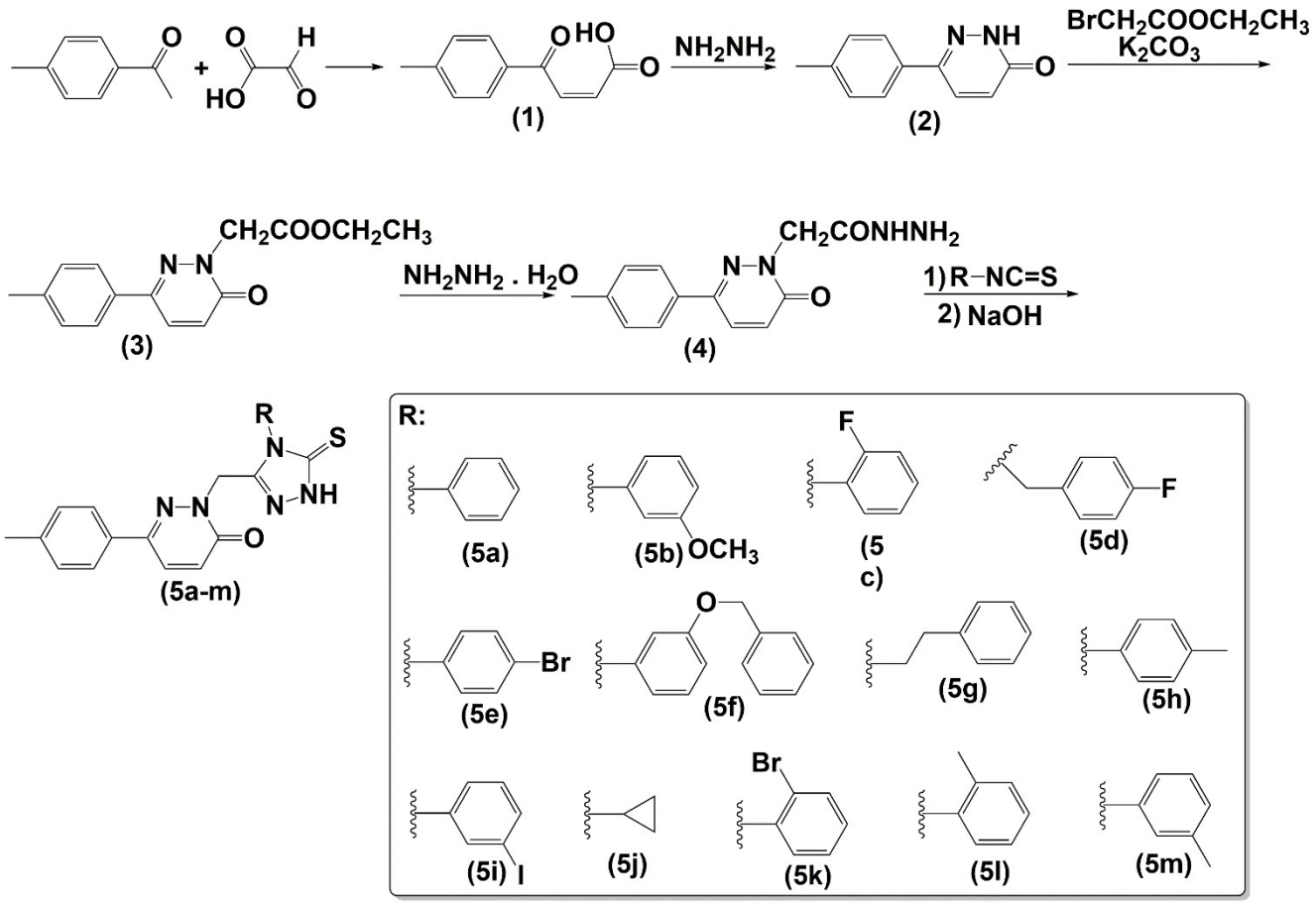

Figure 1. Synthesis of the N-substituted-(p-tolyl)-3(2H)pyridazinone derivatives.

Table 1. The IC_{50} values, K_i constants and inhibition types determined for synthesized compounds **5**(**a**–**m**) and Tacrine (TAC) drugs having inhibitory effects on AChE.

Compounds	AChE IC_{50} (μM)	R^2	Ki (μM)	Inhibition Type
5a	4.50	0.9917	1.09 ± 0.15	uncompetitive
5b	3.63	0.9903	4.12 ± 1.42	noncompetitive
5c	2.79	0.9959	2.50 ± 0.31	noncompetitive
5d	2.69	0.9931	3.90 ± 1.18	noncompetitive
5e	2.48	0.9945	2.38 ± 0.19	noncompetitive
5f	2.13	0.9693	1.35 ± 0.33	noncompetitive
5g	1.77	0.9769	1.22 ± 0.18	noncompetitive
5h	2.63	0.9958	0.56 ± 0.15	uncompetitive
5i	2.10	0.9740	2.53 ± 0.31	noncompetitive
5j	2.83	0.9917	1.98 ± 0.25	competitive
5k	3.40	0.9951	1.04 ± 0.12	uncompetitive
5l	4.23	0.9964	1.14 ± 0.40	Uncompetitive
5m	3.59	0.9870	3.28 ± 0.36	noncompetitive
Tacrine (**TAC**)	4.18	0.9915	3.34 ± 0.82	competitive

Mean from at least three determinations. Errors in the range of 3–5 % of the reported value (data not shown)
The values of Tacrine are used to compare with our results.

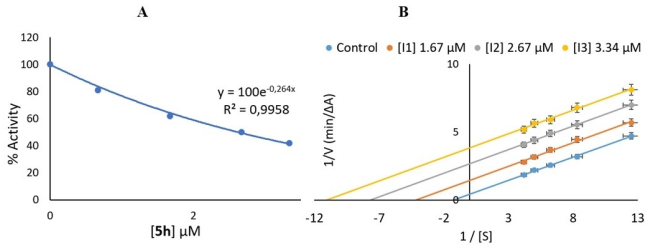

Figure 2. IC_{50} graph (A) and Lineweaver-Burk graph (B) of excellent inhibitor (**5h**) for AChE.

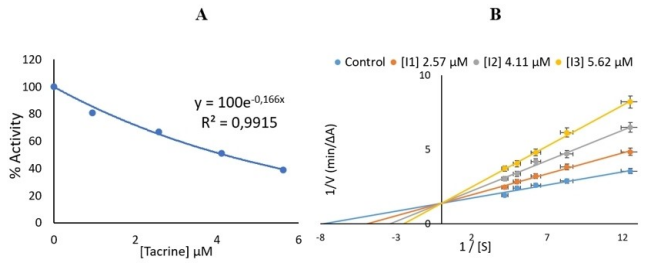

Figure 3. IC_{50} graph (A) and Lineweaver-Burk graph (B) of **Tacrine** (**TAC**) for AChE.

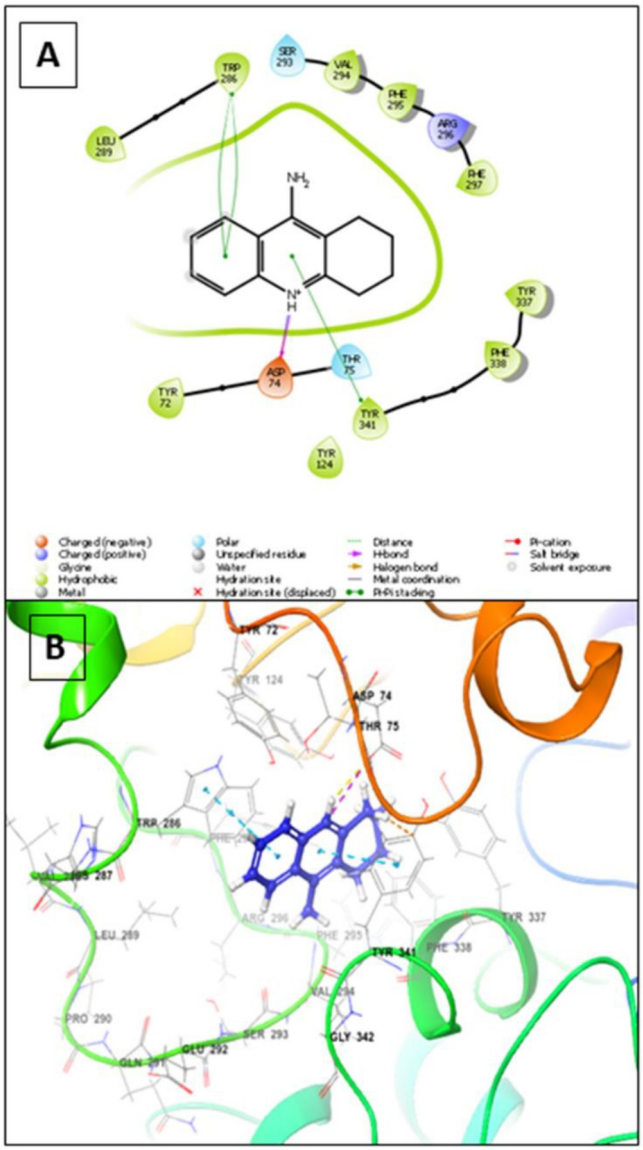

Figure 4. Tacrine-AChE (PDB ID:4M0E) complex (**A**) 2D interaction diagram. (**B**) 3D representation.

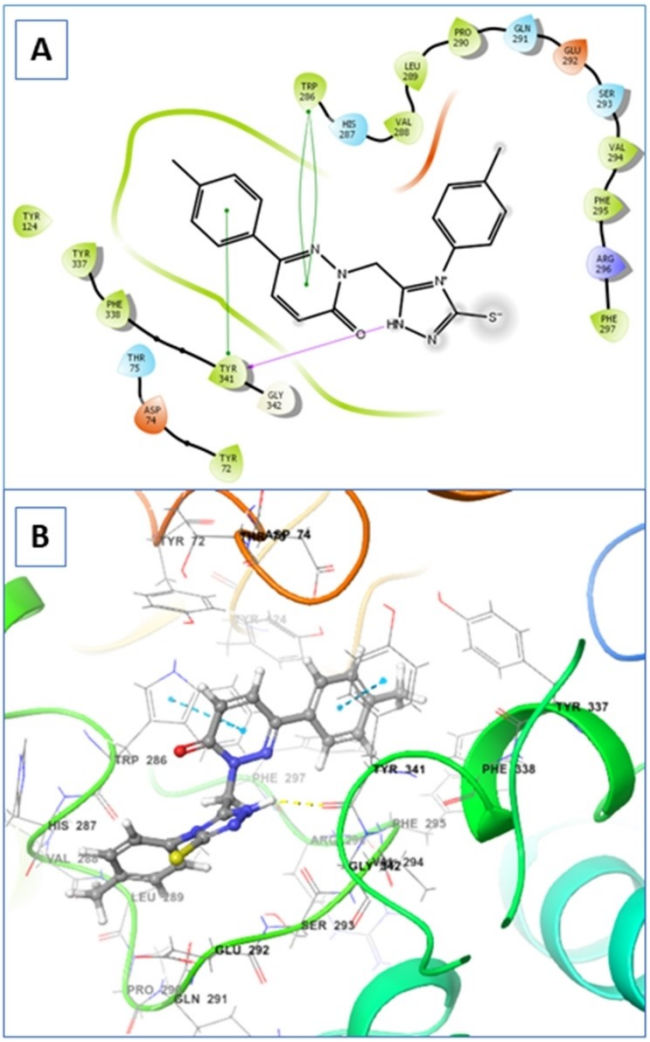

Figure 5. 5 h-AChE (PDB ID:4 M0E) complex (**A**) 2D interaction diagram. (**B**) 3D representation.

Results and Discussion

Chemistry

In accordance with our goal, starting from the aromatic ketone structure, *N*-substituted-(*p*-tolyl)pyridazin-3(2*H*)-one derivatives were synthesized as a result of a five-step reaction (Figure 1). It was started the direct aldol reaction of 4-methylacetophenone and glyoxylic acid to reach the main substrate. The next step, (*p*-tolyl)pyridazin-3(2*H*)-one (**2**) synthesized with hydrazine via nucleophilic substitution reaction. In the other step of the synthesis, the unpaired electrons of the nitrogen atom$^{(2nd)}$ of pyridazinone group attacked the bromine-bonded carbon of ethyl 2-bromoacetate by the nucleophilic substitution S_N2 reaction mechanism to obtain the ethyl 2-(6-oxo-3-(p-tolyl)pyridazin-1(6*H*)-yl)acetate (**3**). The acetatohydrazide compound (**4**) prepared to complete the heterocyclic ring system was synthesized by the reaction of the compound (**3**) and hydrazine hydrate. The final products were synthesized by the

Table 2. Binding parameter values of studied compounds *via* molecular docking.

Compounds	Docking Score (kcal/mol)	XP Gscore (kcal/mol)	Glide Energy (kcal/mol)	Glide Emodel (kcal/mol)	MM-GBSA ΔG_{Bind} (kcal/mol)
5f	−7.821	−10.116	−46.819	−70.221	−60.09
5g	−6.018	−8.282	−43.087	−62.339	−60.53
5h	−6.249	−8.528	−43.993	−60.920	−66.32
Tacrine	−6.566	−6.566	−29.918	−42.832	−70.96

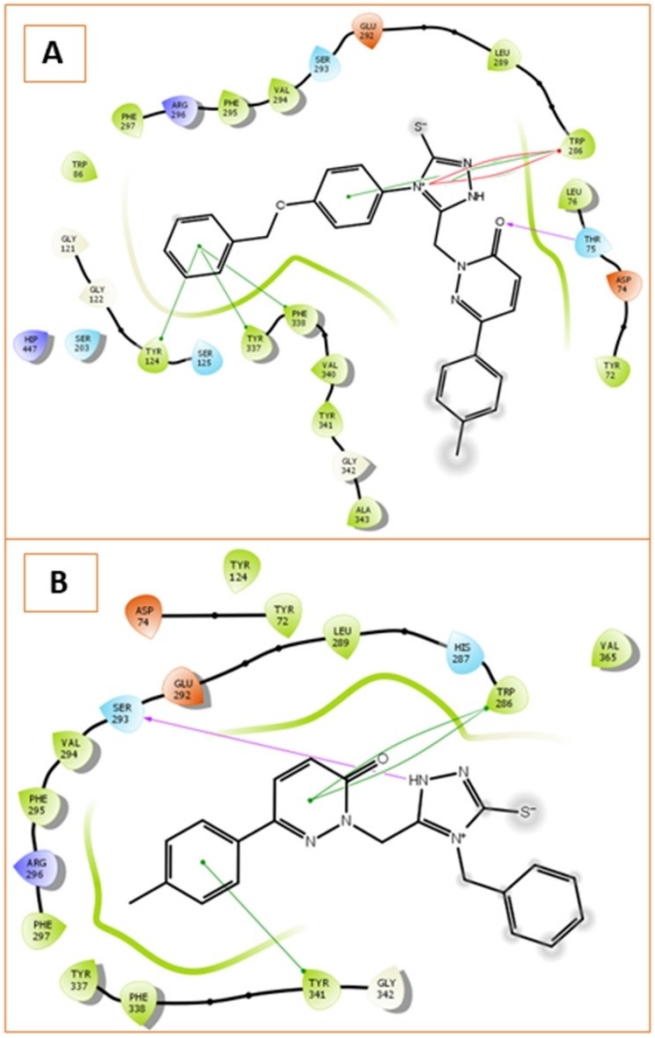

Figure 6. (**A**) 2D interaction diagram of the 5f-AChE complex. (**B**) 2D interaction diagram of the 5 g-AChE complex.

intramolecular condensation reaction of the structure that obtained as a result of the reaction of aromatic isothiocyanates and the compound (**4**).

The melting points of the white colored compounds **5**(**a**–**m**), which were synthesized in yields ranging from 91–63% in general, were 268–102 °C. The range of 14.04-10.57 ppm in the ^{1}H NMR spectrum showed triazol-N*H* peaks. Bridge of triazole and pyridazinone rings –C*H*$_2$– peak was observed near 5.3–5.2 ppm in proton nmr. In addition, the *p*-tolyl- group and *N*-substituted aromatic rings attached to pyridazinone were observed in the arene region in the range of 8.07–6.90 ppm. In the ^{13}C NMR spectrum, the pyridazinone ring carbonyl carbon supported the structure accuracy in the range of 159.0-158.0 ppm. The specific thiocarbonyl group of triazole ring was observed in the range of 168.0-166.0 ppm. In the FT-IR spectra, newly synthesized compounds **5**(**a**–**m**) exhibited characteristic ν(C=O) bands at 1671–1639 cm^{-1} for pyridazinone rings. The ν(N–H) stretching bands of the triazole were centered at 3244–3201 cm^{-1}. In addition, characteristic ν(C=S) bands observed at 1163–1160 cm^{-1}.

Investigation inhibition type of AChE

The inhibitory effect of the *N*-substituted-(*p*-tolyl)pyridazin-3(2*H*)-one derivatives was determined by value of IC_{50} and K_i. For AChE enzyme, the obtained IC_{50} and K_i values were given in Table 1. IC_{50} values were determined to be in the range of 1.77-4.50 μM. K_i values were determined to be in the range of 0.56 ± 0.15-4.12 ± 1.42 μM. When the AChE activities of target compounds were evaluated, the IC_{50} and K_i values of most were found to be much lower than the standard compound Tacrine (Figure 3). Among the synthesized compounds, compound **5h** (R = 4-methylphenyl) demonstrated the best inhibitory effect on AChE with K_i values of 0.56 ± 0.15 (Figure 2).

The compounds we synthesized showed different inhibition mechanisms. We identified that **5a**, **5h**, **5k** and **5l** uncompetitively inhibited cholinesterase activity of this AChE. We think that these compounds can be attached only to the enzyme-substrate complex. **5b**, **5c**, **5d**, **5e**, **5f**, **5g**, **5i** and **5m** noncompetitively inhibited cholinesterase activity of this AChE. These compounds can be attached to a region outside the active site of the enzyme. On the other hand, **5j** competitively inhibited cholinesterase activity of this AChE. Therefore, we think that this compound interacts with the active site of the enzyme.

Its contribution to the activity was evaluated by using phenyl ring and substituted derivatives. In the N-substituted-(p-tolyl)pyridazine-3(2*H*)-one derivatives the inhibitory strength order was **5h** (4-methyl derivative) > **5k** (2-bromo derivative) > **5a** (non-derivative) > **5l** (2-methyl derivative) > **5g** (fenethyl derivative) > **5f** (4-benzoksi derivative). In compounds with methyl R substituent, the inhibitory effect was better than tacrine (K_i = 3.34 ± 0.82 μM), but the activity order decreased as -p (**5h**) >-o (**5l**) >-m (**5m**). Meta-substituted methyl contributed less to the activity. Considering the inhibitory effect of ortho-substituted phenyl derivatives, they showed an effect in the form of Br (**5k**) > CH_3 (**5l**) > F (**5c**), 1.04 ± 0.12 μM, 1.14 ± 0.40 μM, 2.50 ± 0.31 μM. Among the para-substituted phenyl derivatives the inhibitory strength order was 4-methylphenyl (**5h**) > fenethyl (**5g**) > benzyloxyphenyl (**5f**) > 4-florophenyl (**5d**).

In addition, meta-substituted compounds **5m**, **5b** and **5d** ((4-fluorophenyl)methyl) showed some degree of inhibition with K_i in the range of 3.28 to 4.12.

Computational Studies

After analyzing the experimental activities of the synthesized compounds on the AChE enzyme, docking calculations[17] were made with *in silico* approaches to support the results obtained. All molecular docking studies were performed using the Schrödinger 2021–2 software program.[18]

Molecular Docking

Active binding site and binding parameters were determined by interacting AChE (PDB ID: 4 M0E)[19] crystal structure obtained from the protein database as *in silico* with reference Tacrine. Based on these parameters determined later, AChE (PDB ID: 4 M0E)[19] crystal structure and **5f**, **5g** and **5h** compounds were interacted from the same active site.

The binding parameter values of **5f**, **5g**, **5h** and Tacrine compounds interacted with AChE crystal structure (PDB ID:4 M0E)[19] in molecular docking were presented in Table 2. In Table 2, when the reference Tacrine was analyzed first, it was determined that the docking score and XP Gscore values were −6.566 kcal/mol, the Glide energy value was −29.918 kcal/mol, the Glide emodel value was −42.832 kcal/mol, and the free binding energy value was −70.96 kcal/mol. In molecular docking, the value of the free binding energy is as important as the docking score. The binding energy of the tacrine compound is −70.96 kcal/mol, while the free binding energy value of the **5h** compound was −66.32 kcal/mol. Therefore, it can be said that compound **5h** has the best binding among these three compounds.

In Table 2, XP Gscore values of **5f**, **5g** and **5h** compounds calculated according to the algorithm in the Schrödinger 2021–2 software program were −10.116 kcal/mol, −8.282, respectively. kcal/mol and −8.528 kcal/mol. Likewise, the free binding energy values of compounds **5f**, **5g** and **5h** are −60.09 kcal/mol, −60.53 kcal/mol and −66.32 kcal/mol, respectively. Even if the **5f** compound has the best XP Gscore value, it can be said that the **5h** compound binds better to the AChE crystal structure because its free binding energy value is better. In addition, not only the calculated binding parameter values, but also the amino acids in the active binding site should be analyzed.

Figure 4 and Figure 4B respectively shown 2D interaction diagram and 3D interaction of AChE crystal structure (PDB ID:4M0E)[19] and Tacrine reference compound in molecular docking. When the binding region formed by the interaction between the 4M0E crystal structure and Tacrine was examined, the π-π interaction with Trp286, the π-π interaction with Tyr341, and the hydrogen bond interaction with Asp74 were determined in Figure 4A and **4B**. Figure 4. Tacrine-AChE (PDB ID:4M0E) complex (**A**) 2D interaction diagram. (**B**) 3D representation.

When *in silico* studies performed for the synthesized compounds **5f**, **5g** and **5h**, whose activity against AChE is examined, the presence of amino acid residues in the binding region formed in the Tacrine-AChE (PDB ID:M0E) complex is examined. Figure 5 shows the 2D and 3D interactions of amino acids in the binding site formed between **5h**-AChE (PDB ID: 4 M0E), respectively. In Figure 5A, it is presented that there is a π-π interaction with Trp286, a π-π interaction with Tyr341 and a hydrogen bond interaction with Tyr341, which is an important amino acid residue in this inhibitory feature between the **5h** compound and the AChE crystal structure. In Figure 5B, the 3D interaction of the **5h**-AChE (PDB ID:4M0E) complex is presented.

Figure 6presented the 2D interaction diagram generated by the *in silico* approach of the **5f** and **5g** compounds sequentially interacting with the AChE crystal structure. When Figure 6A was analyzed, it has been determined that both π-π interaction and π-cation interaction exist with Trp286 residue, which is an important amino acid in the inhibitory part of the AChE crystal structure (PDB ID:4 M0E).[19] Also, in Figure 6A, Tyr124, Tyr337, Phe338 amino acids have π-π interaction and Thr75 has hydrogen bonding interaction. In Figure 6B, when the 2D interaction diagram between the 5 g and 4M0E crystal structure was examined, it was determined that the amino acids Tyr341 and Trp286 were important according to Tacrine in Figure 4. When Figure 6B is analyzed, it was said that there was π-π interaction with Trp286 and Tyr341 amino acids, and hydrogen bond interaction with Ser293. When the binding parameters of **5f** and **5g** compounds are examined according to Table 2, it can be mentioned that they may have good inhibitory properties on AChE since they have values close to Tacrine.

Conclusion

In conclusion, it was determined that new *N*-substituted-(*p*-tolyl)pyridazin-3(2*H*)-one derivatives **5**(**a**–**m**) had strong inhibition effects on the activity of AChE, as an important serum ester hydrolase enzyme with antiatherosclerotic activity. In general, all compounds showed great inhibition potency against AChE enzyme with K_i values ranging from 0.56 ± 0.15–4.12 ± 1.42 μM μM. Our results showed that the synthesised compound **5h** showed a better inhibition profile than Tacrine (K_i: 3.34 ± 0.82 μM) against AChE with K_i in 0.56 ± 0.15 μM. Therefore, the tested compounds will provide new ideas for the development of new drugs to scientists working on neurodegenerative disorders. Such biological properties highlight **5h** as a very interesting prototype in the search for new prodrugs in the treatment of AD. In experimental data studies, it was supported by molecular docking from *in silico* approaches that **5h** compound show best inhibitory property against AChE. In the molecular docking analysis, both the binding parameters and the amino acids in the binding site were taken as basis. In addition, since all synthesized compounds may have good inhibitory properties, all of them were analyzed in molecular docking. It can be mentioned that the inhibitory property of the compounds **5f**, **5g** and **5h**, which gave the best three results, against AChE may be like Tacrine.

Supporting Information Summary

Supplementary data (synthetic procedures, biological assay methods and ^{1}H NMR & ^{13}C NMR and HRMS spectra of the synthesized compounds) associated with this article can be found, in the online version, at DOI: xxxxxxxxxx

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

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

Keywords: (*p*-tolyl)pyridazin-3(2*H*)-one · AChE inhibitors · Alzheimer's disease · structure-activity relationship · molecular modeling

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