



Synthesis, Characterization, Investigation of AChE Activities and Molecular Docking Studies of New Schiff Bases Based on Substitute Salicyl Aldehyde

Substitüe Salisil Aldehit Temelli Yeni Schiff Bazlarının Sentezi, Karakterizasyonu, AChE Aktivitelerinin İncelenmesi ve Moleküler Yerleştirme Çalışmaları

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ABSTRACT

In this study, two new Schiff base compounds (4 and 5) based on substituted salicyl aldehyde were synthesized. The structures of the synthesized compounds were determined by FT-IR and ^1H (^{13}C) NMR spectroscopies. The AChE inhibition activities of the compounds were investigated. According to the results obtained, the activities of compounds 4 (IC_{50} : $1.396 \pm 0.35 \mu\text{M}$) and 5 (IC_{50} : $0.795 \pm 0.47 \mu\text{M}$) were found to be significantly effective than Tacrin (TAC) (IC_{50} : $166.10 \pm 17.53 \mu\text{M}$) (currently used drug). The interaction types and binding energies of compound 5, which has higher activity, were investigated by molecular docking studies.

Key Words

Schiff base, AChE inhibition activity, molecular docking.

Öz

Bu çalışmada, substitute salisil aldehit temelli yeni Schiff bazları (4 ve 5) sentezlendi. Sentezlenen bileşiklerin yapıları FT-IR, ^1H (^{13}C) NMR spektroskopileri ile aydınlatıldı. Bileşiklerin AChE inhibisyon aktiviteleri araştırıldı. Elde edilen sonuçlara göre 4 (IC_{50} : $1.396 \pm 0.35 \mu\text{M}$) ve 5 (IC_{50} : $0.795 \pm 0.47 \mu\text{M}$) bileşiklerinin aktiviteleri standart olarak kullanılan Tacrinden (TAC) (IC_{50} : $166.10 \pm 17.53 \mu\text{M}$) çok daha yüksek inhibisyon aktivitesi sergilemiştir. Yüksek aktiviteye sahip olan bileşik 5'in moleküler yerleştirme çalışmaları ile etkileşim türleri ve bağlanma enerjileri hesaplanmıştır.

Anahtar Kelimeler

Schiff bazı, AChE inhibisyon aktivitesi, moleküler yerleştirme.

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INTRODUCTION

Schiff bases are also known as imines formed by the reaction condensation of primary amines with aldehydes or ketones [1]. The reaction is shown in Fig 1.

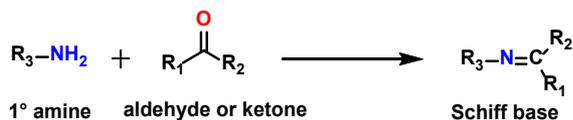


Figure 1. ¹H NMR spectrum of pyrazole 4b.

The widely used Schiff bases constitute an important class of organic compounds. Schiff bases has various applications including paint, catalyst and organic intermediates etc. Moreover, Schiff bases have shown to exhibit various biological activities anti-diabetic, anti-bacterial, Alzheimer's, antimalarial, antifungal, anti-inflammatory, antipyretic, antiviral and antiproliferative [1–7]. Imine groups (-CH=N-) are found in the structure of many natural compounds and the effect of this group is very important for biological activities [8]. Therefore, the number of publications on Schiff bases in the fields of chemistry, pharmacy, biochemistry and industry is quite high and continues to increase [9].

Alzheimer's disease (AD) is a neurodegenerative disease that occurs as the most common cause of dementia in the elderly and causes a decrease in thinking, unders-

tanding and cognitive skills [10,11]. In 2021, there are 2.3 million people over the age of 85 with Alzheimer's disease, making up 36% of all people with Alzheimer's worldwide. It is thought that this number will reach 6.7 million people over the age of 85 by 2060, and this value will correspond to nearly half of all people over the age of 65 with dementia [12]. AD begins with the appearance of plaques and tangles that kill neurons in brain tissue and in some parts of the brain [13]. Neurons cannot renew themselves, so acetylcholine (ACh) levels produced by neurons decrease [11]. ACh is necessary for the proper development of the brain, but the decrease in ACh level in dementia patients causes problems such as thinking, remembering and reasoning [14]. General symptoms seen in 65-year-old patients initially result in short-term memory loss, and in advanced levels, organ failure and death [15,16]. Currently, there is no effective method for the treatment of Alzheimer's, but inhibiting ACh degradation by blocking AChE has proven to be effective in reducing the progression of the disease [17].

It has been reported in the literature that Schiff bases show high activity as AChE inhibitors [18–20]. In this work, we are reporting the synthesis of new Schiff bases based on substituted salicylaldehyde and acetylcholinesterase (AChE) inhibitory activities and molecular docking studies.

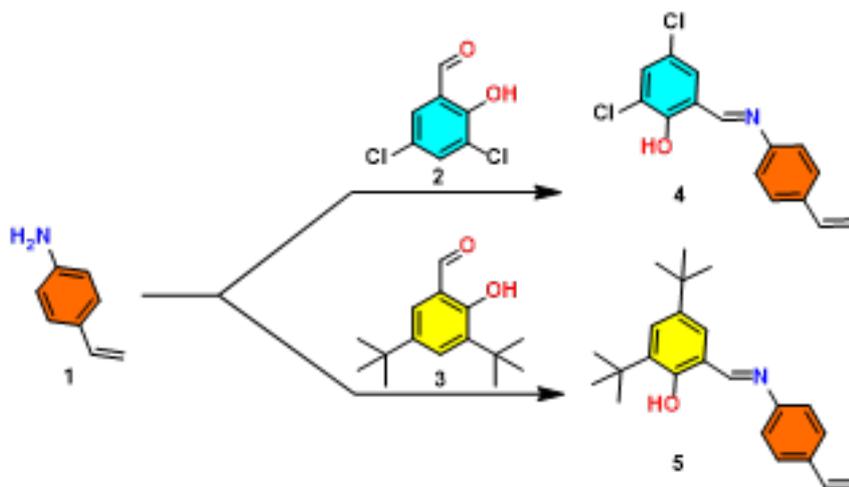


Figure 2. Synthesis scheme of compounds 4 and 5.

MATERIALS and METHODS

Materials and Instrumentation

All reagents and solvents were purchased from commercial companies and were of reagent-grade purity (Aldrich or Merck). A Perkin-Elmer FT-IR spectrometer (Spectrum 400) equipped with an ATR was used to record infrared spectra. ^1H (^{13}C) NMR spectra were obtained on a Bruker 400 MHz equipment and TMS was utilized as an internal standard. In CDCl_3 solvent, the compounds ^1H (^{13}C) NMR spectra were examined. The Hitachi U3900H Spectrophotometer was used to measure the inhibitory activity of AChE.

Synthesis Method of Compounds (4 and 5)

All of the substances were made using a method described in the literature [21]. 1 mmol of aldehyde compound (2 or 3) was dissolved in 20 mL of methanol. 1 mmol of 4-vinylaniline (1) was added and refluxed for 6 hours. The progress of the reaction was checked by TLC. After the reaction was complete, the resulting solids were filtered and dried. Fig. 2 illustrated the general synthesis method.

(E)-2,4-dichloro-6-(((4-vinylphenyl)imino)methyl)phenol (4): m.p.: 125-126 °C. Yield: 95%. Colour: Dark orange solid. FTIR (ν , cm^{-1}): 3393, 3077, 2913, 2852, 1613, 1446, 1237, 1177, 864. ^1H NMR (400 MHz, CDCl_3): δ 14.34 (s, 1H), 8.62 (s, 1H), 7.54–7.48 (m, 2H), 7.35–7.29 (m, 4H), 6.76 (dd, $J = 17.6, 10.9$ Hz, 1H), 5.82 (d, $J_{\text{trans}} = 17.6$ Hz, 1H), 5.34 (d, $J_{\text{cis}} = 10.9$ Hz, 1H). ^{13}C NMR (100 MHz, CDCl_3): δ 159.70, 156.09, 146.07, 137.48, 135.83, 132.71, 129.70, 127.45, 123.38, 122.91, 121.47, 120.25, 114.80.

(E)-2,4-di-tert-butyl-6-(((4-vinylphenyl)imino)methyl)phenol (5): m.p.: 115-116 °C. Yield: 93%. Colour: Light yellow solid. FTIR (ν , cm^{-1}): 3081, 2950, 2905, 2864, 1617, 1439, 1249, 1166, 871. ^1H NMR (400 MHz, CDCl_3): δ 13.74 (s, 1H), 8.68 (s, 1H), 7.51–7.46 (m, 3H), 7.32–7.24 (m, 3H), 6.76 (dd, $J = 17.6, 10.9$ Hz, 1H), 5.79 (d, $J_{\text{trans}} = 17.6$ Hz, 1H), 5.29 (d, $J_{\text{cis}} = 10.9$ Hz, 1H), 1.51 (s, 9H), 1.36 (s, 9H). ^{13}C NMR (100 MHz, CDCl_3): δ 163.32, 158.31, 148.07, 140.62, 137.01, 136.15, 136.08, 128.07, 127.22, 126.82, 121.39, 118.34, 113.83, 35.12, 34.21, 31.49, 29.45.

Pharmalogical-Biological Studies

Acetylcholinesterase (AChE) inhibition studies

The inhibition activities of the test compounds on the cholinergic enzyme AChE were determined using the Ellman method [22]. The enzyme solution was prepared

as 0.22 units/mL. Tacrin and test compounds were studied at concentrations of 1.36-0.085 μM . Standard Tacrin (TAC) and test compounds (4 and 5) were dissolved in a water-DMSO mixture (9:1). 100 μL of phosphate buffer (pH: 6.7) was added to each well of 96 well-plates. Different concentrations of test compounds (20 μL) and AChE (20 μL /well) were added to it and incubated at 25 °C for 10 minutes. The chromatographic reagent was started by adding 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB) (3mM, 50 μL /well) and the substrates acetylthiocholine iodine (ATCI) (3mM, 50 μL /well) to the enzyme-inhibitor mixture. Yellow anion formation was recorded at 412 nm for 10 minutes. An identical solution of the enzyme was prepared without compounds, designated as control. Control and inhibitor readings were corrected with a blank read. The study was repeated three times. The concentrations of the samples that inhibited the degradation of the substrate (acetylcholine) by 50% (IC_{50}) were determined by linear regression analysis between percent inhibition and sample concentration using the excel program [23,24].

RESULTS and DISCUSSION

In the content of this work, new two imine compounds were prepared in high yield and purity. These compounds were characterized by common spectroscopic methods. In the FT-IR spectra of the compounds (4 and 5), characteristic vibrational bands belonging to the aliphatic -CH group were observed between ν 3081 and 2852 cm^{-1} . The characteristic stretchings of the imine groups of the synthesized compounds (4 and 5) were detected at 1613 and 1617 cm^{-1} , respectively. Characteristic vibrational peaks belonging to the vinyl group were observed for compound 4 at ν 1662 and 864 cm^{-1} , and for compound 5 at ν 1650 and 871 cm^{-1} . The existence of characteristic bands in the compounds FT-IR spectra validates their structures.

In the ^1H NMR spectrum of compound 5, the 18 protons belonging to the t-butyl groups resonated as two separate singlets at δ 1.36 and δ 1.51 ppm. The cis-proton in the vinyl group was observed as a doublet at δ 5.29 ppm, and the trans-H at δ 5.79 ppm. The vinyl group proton (-CH=C) resonated at δ 6.76 ppm as a doublet of the doublet. The aromatic C-H protons were observed in the range of δ 7.51-7.46 ppm and δ 7.32-7.24 ppm as overlapped multiplet peaks. The singlet peak observed at δ 8.68 ppm belongs to the imine proton, and the peak observed at δ 13.74 ppm belongs to the hydroxyl group.

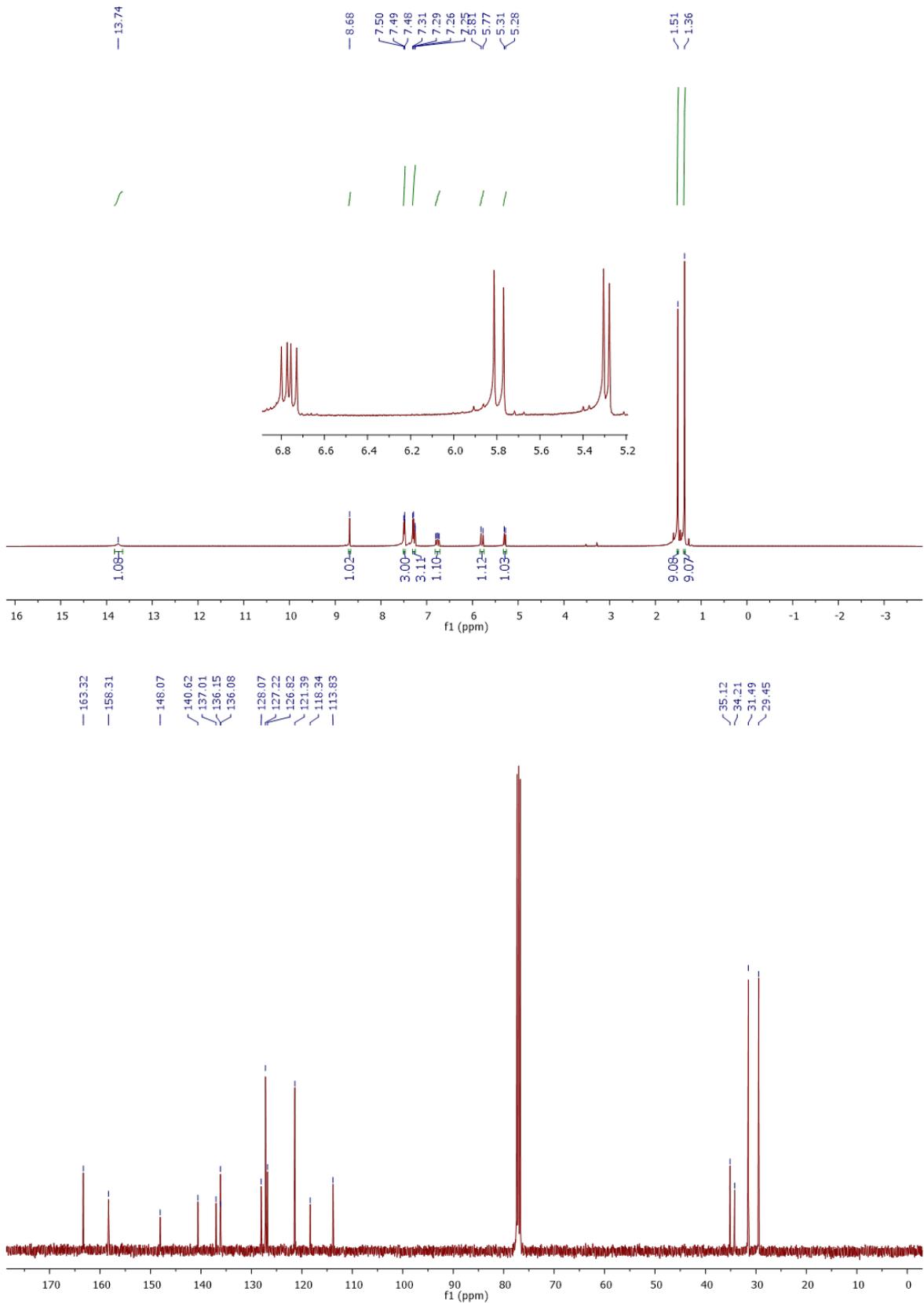


Figure 3. ¹H and ¹³C NMR spectrum of compound 5 (CDCl₃).

In the ^{13}C NMR spectrum of compound 5, aliphatic carbon atoms were observed in the range of δ 29.45-35.12 ppm, aromatic carbon atoms and vinyl carbons were observed in the range of δ 113.83 ppm and δ 158.31 ppm. The signal of the carbon atom in the imine bond resonated at 163.32 ppm. The $^1\text{H}(^{13}\text{C})$ NMR spectrum of compound 5 is given in Fig 3. FT-IR and $^1\text{H}(^{13}\text{C})$ NMR analysis results of the compounds (4 and 5) confirm the proposed structures.

Biological Activity Studies

AChE inhibition activity results

The AChE inhibitions of salicylaldehyde-based compounds evaluated in this study are quite interesting. It has been observed that both compounds effectively inhibit AChE enzyme activity at micromolar level. It was determined that compound 4 (IC_{50} : 1.396 ± 0.35) and compound 5 (IC_{50} : 0.795 ± 0.47) effectively inhibited the AChE enzyme (Table 1). The acetylcholinesterase inhibitory effect of the compounds is given in Fig. 4. It was found that the inhibition activity of compound 5, which has a di-*t*-butyl group, is more active than compound 4, which has two chloride groups in its structure. It is thought that the activity difference is due to the alkyl groups in the structure. In addition, it was determined that tacrine (TAC), used as a standard AChE inhibitor in

the study, had an IC_{50} value of $166.10 \pm 17.53\mu\text{M}$ against AChE. The AChE inhibition values of the synthesized compounds are dramatically higher than the TAC inhibition values, making them compounds that can be used as an alternative to TAC after evaluating the further pharmacologic properties of these compounds.

Molecular Docking Results

Molecular docking studies were performed using the AutoDock 4.2 program to determine the interaction mode, binding affinity and binding site of the most active inhibitor after *in vitro* experimental study of the compounds. First, acetylcholinesterase (AChE) co-crystallized ligand Galantamine (GNT) was removed and redocked to confirm the binding site. The fact that the redocked ligand was in close site proximity to the co-crystallized ligand confirmed the docking process (Fig 5). Confirmation of docking procedure is useful for drug-receptor interactions.

Molecular docking study of compound 5 which is experimentally more active than compound 4 was performed. Compound 5 docking to the active site of AChE the best conformation binding affinity is -10.17 kcal/mol. The 3D docking pose of compound 5 is shown in Fig 6. As a result of docking, the active site of AChE of compo-

Table 1. Determination of compound 4, compound 5 and standard Tacrin (TAC) AChE inhibition values.

Comp.	AChE (μM)	R^2
4	1.396 ± 0.35	0.9738
5	0.795 ± 0.47	0.9906
TAC	166.10 [25]	0.9665

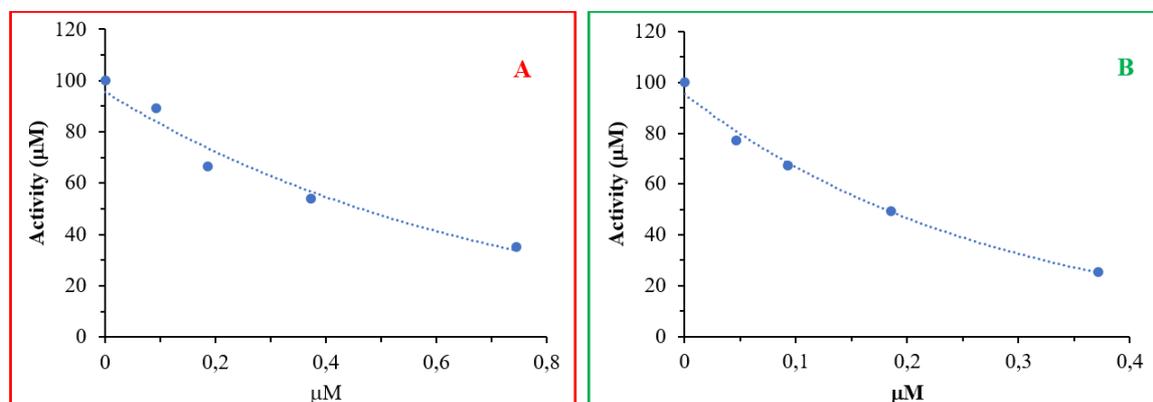


Figure 4. The effect of 4 and 5 compounds acetylcholinesterase (AChE) enzyme. A (for 4) and B (for 5) is IC_{50} values of schiff base compounds.

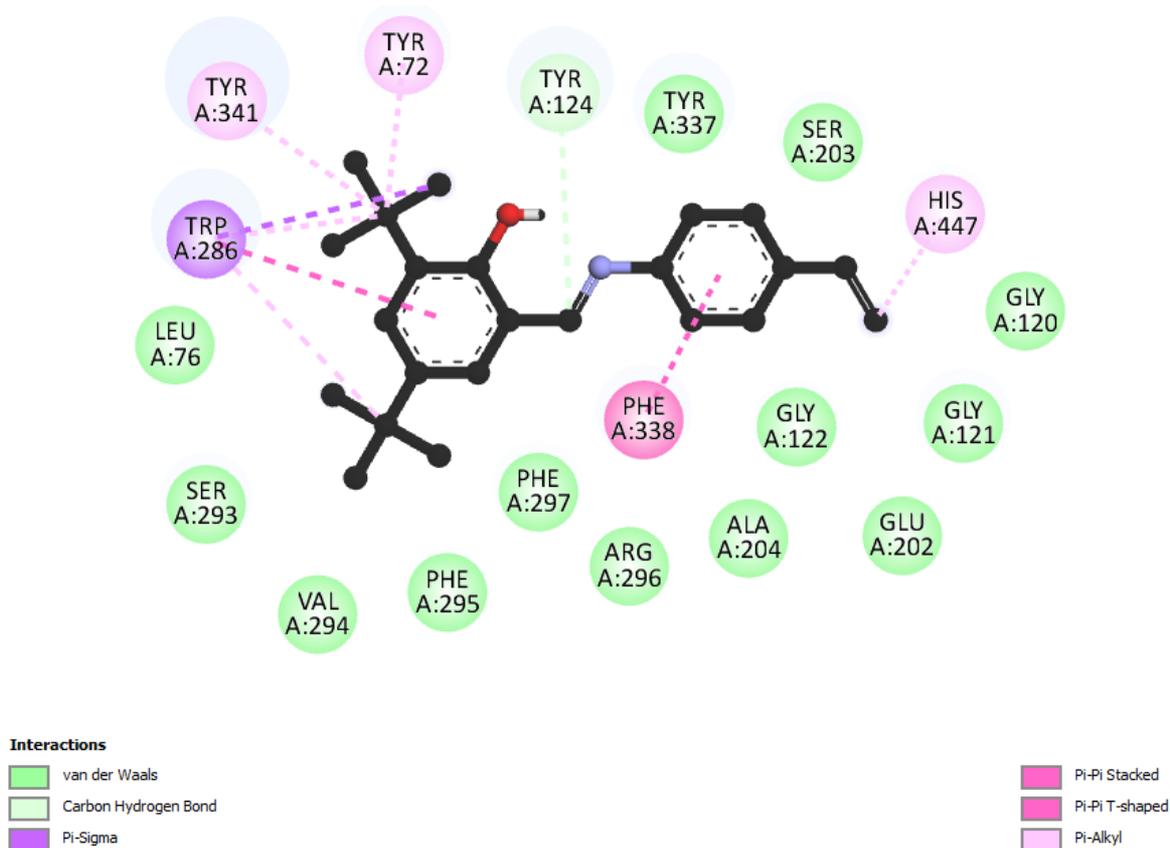


Figure 7. 2D interaction profile of the lowest energy best conformation of compound **5** in the catalytic active site in AChE.

und **5** interacts with some amino acid residues. Compound **5** formed π - π interaction with important amino acid residues in the peripheral anionic subregion (PAS) Trp286 (π - π interaction distances are 5.81 Å, 4.45 Å) and in the acyl binding pocket Phe338 (5.00 Å). Compound **5** made two hydrogen bonds with D \cdots A distances of (1.87 Å and 2.10 Å) with Tyr124 in the entrance region of the PAS area. Also compound **5** formed very hydrophobic interactions with residues in the active site of AChE (Fig 7). There are several subsites that can be distinguished in the active site of AChE: the esteratic subsite, also called the catalytic triad (Ser203, His447, Glu334), the oxanion hole (Gly121, Gly122, Ala204), the anionic subsite (Trp86, Tyr133, Glu202, Gly448), Ile451), acyl binding pocket (Trp236, Phe295, Phe297, Phe338), peripheral anionic subregion (Asp74, Tyr72, Tyr124, Ser125, Trp286, Tyr337, Tyr341) [26–29].

CONCLUSION

In this study, two new Schiff base compounds based on salicylaldehyde were synthesized and their structures were characterized by spectroscopic methods. The compounds were then screened for their AChE inhibition activities. The compounds (**4** and **5**) showed considerably higher AChE inhibition activity compared to the standard drug (Tacrin). The inhibitory activity of compounds **4** and **5** are comparable. Finally, molecular docking studies showed that compound **5** interacts with AChE *via* H-bond and π - π stacking interactions.

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