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Synthesis, characterization, crystal structure of schiff base compound as potent acetylcholinesterase inhibitors and their molecular modeling study.

Basma M. Abd Razik

College of Pharmacy, Al-Mustansiriyah University, Baghdad, Iraq

Abstract

In this study new Schiff base compounds **3(a-d),** have been synthesized by reaction between aldehyde **1 (a-d)** and 5-methoxy tryptamine (**2**) in the neutral ionic liquid [BMIM]Br. These compounds were characterized by using Fourier Transform Infrared (FTIR), ^{1}H, ^{13}C Nuclear Magnetic Resonance NMR, CHN elemental analysis and single crystal X-ray diffraction for **3d** compound which crystallizes into monoclinic crystal system with P21/C space group. The unit cell dimensions, a= 6.6144(7) Å, b= 30.014(3) Å, c= 9.9371(10) Å, α= 90°, ß= 97.615°, γ= 90°, V= 1955.4 (3) Å^3 and Z= 4. The crystal packing show that the compound are consolidate by a combination of van der waals forces and weak hydrogen bond. There is several bonding type appear such as hydrogen bonding(N1—H1N1··N2) (Table 4) and non covalent bonding C—H, C--π, C—H--π and π--π interaction.

All compounds were tested for their ability to inhibit acetylcholinesterase (AChE) by Ellman's method and variation in this ability were described. Among them, compounds **3c** and **3d** displayed higher activity than Galanthamine as a refrense drug, with IC_{50} values of (1.88, 2.05) µM respectively, while compounds **3a** and **3b** shows high to moderate inhibition with IC_{50} values of (2.45 and 5.40) µM respactivly. Molecular docking simulations were proceed to understand the molecular basis for this difference. The results were obtained by docked into the active site of AChE which completely coincided with in vitro results.

Keywords: Schiff base, Elemental analysis, Crystal packing, ionic liquid, acetylcholinesterase (AchE),docking studay.

1.Introduction

A wide variety of heterocyclic with structural diversity is one of the major interests for designing pharmaceutical molecule[1-2]. Heterocyclic compounds also serve as synthons in the construction of complex organic molecules. A large number of these compounds also find important applications as intermediates in the synthesis of more complex heterocycles and important in biological part such as anticancer, antimicrobial, antibacteria, antitubercular, anti-inflammatory, antifungal and many more [3-4]. On the other hand, the imine derivative or schiff base have attracted the interest over the year as their synthetic availability along with antibacterial, anti-inflammatory, anticancer [5,6] and antimycobacterial properties[7,8,9].

From reports, Alzheimer's disease (AD) affectes more than 37 million humans around the world wild [10]. The cholinesterases enzyme, acetylcholinesterase (AChE) is responsible for collapsing of acetylcholine in humans bodies [11] and the decrease in ACh level associates with the cognitive impairments observed in AD patient [12]. There are three acetylcholinesterase inhibitors accepted for treatment of AD, galanthamine, rivastigmine and donepezil drugs [13,14,15,16].

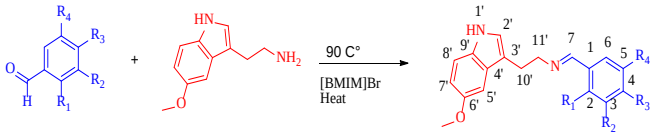
In this paper, we describe the crystallocraphy structure of novel Schiff base compounds synthesized via catalyst-free techniques in ionic liquid by condensation reaction of aromatic aldehydes in the neutral ionic liquid [BMIM]Br and primary amines [17,18,19]. Currently, the usage of ionic liquid have received more attention as it is reusable, ecofriendly catalyst and also well known as green solvent [20,21]

Molecular docking analysis or in silico calculations is a efficient implement in drug discover to estimate the binding energy and checking the conformation of a ligand which is docked into the active site of the target enzyme which wiedly used in drug design to assessment medicinal potency of new products [22]. A novel schiff base products. Thus, we report the direct synthesis of the compound by using normal reflux in [BMIM]Br, spectral properties and also crystallographic analysis for **3d** compound.

2.Materials and General Methods

2.1. Chemistry

Chemical and solvents were obtained from commercial sources and were used without further purification. Melting point was determined using open capillary tubes by Stuart Scientific SMP1. Elemental analysis (CHN) was measured from Perkin Elmer 2400 Series II Analyzer. IR spectrum were recorded with Perkin Elmer 2000 FT-IR Spectrometer within the range of 400-4000 cm^{-1}. The 1D NMR Spectra were obtained from Bruker-Avance by 500 MHz instrument using TMS as internal standard.



1 (a-d) **2** **3(a-d)**

a: R_1 =OH , R_2= H , R_3=H , R_4= CH_3
b: R_1 =OH , R_2= tert-butyl, R_3=H , R_4= H
c: R_1 =OH , R_2= H , R_3=H , R_4= tert-butyl
d: R_1 =H , R_2= H , R_3=n-butyl , R_4= H

Figure 1: Reaction between amine(1) and aldehyde(2 (a-d)) to afford schiff base(3)

To a mixture of different benzaldehydes **(1)** and 5-methoxy tryptamine **(2)** in a semi-micro boiling tube, containing 6.5 mmol of [BMIM]Br was additional and the mixture was uniformly grounded for 15 min in an oil bath. After accomplishment the reaction as checked by TLC, the reaction mixture was cooled down and extracted using diethyl ether dried over $MgSO_4$ and then evaporated the solvent *in vacuo* to give the final compounds. The finaly compound, **3** were afterward recrystallized by using boiling toluene with very good yields percintage . Single crystal afforded as brownish needles from $CHCl_3$/Mt-OH (3:2) after one weak.

3a (E)-5-(((2-(5-methoxy-1H-indol-3-yl)ethyl)imino)methyl)-4-methylphenol

Brown solids; Yield: 92%; mp 77-79 ºC; IR (KBr) υmax: 3412, 1583, 1218, 1021 cm^{-1}; Anal. for $C_{19}H_{20}N_2O_2$; Calculated(found): C: 74.00(74.20), H: 6.54(6.50), N: .9.08(9.30). ^{1}H NMR (500 MHz, DMSO): δH 2,18(3H, s, CH_3), 3.04 (2H, t, *J*=6.9 Hz, CH_2-10´), 3.85 (3H, s, OCH_3), 3.83 (2H, t, *J*=6.8 Hz, CH_2-11´), 6.72 (1H, d, *J*= 8.7, 2Hz, H-3), 7.01 (1H, d, *J*=8.6 Hz, H-4), 7.25 (1H, d, *J*=2.3 Hz, H-6), 7.12 (1H, s, H-2´), 7.25 (1H, d, J=8.7 Hz, H-8´), 7.33-7.30 (2H, m, H-7´, H-5´), 8.30 (1H, s, H-7), 10.66 (1H, s, NH). ^{13}C NMR (125 MHz, DMSO): δc 19.78, 26.50, 55.18, 59.05, 100.6,

111.61, 112.01, 116.23, 118.16, 123.55, 127.44,132.38, 152.96, 158.61, 165.46.

3b (E)-3-(tert-butyl)-6-(((2-(5-methoxy-1H-indol-3-yl)ethyl)imino)methyl)phenol

Brown solids; Yield: 90%; mp 72-74 ºC; IR (KBr) υmax: 3415, 1587, 1216, 1027 cm^{-1}; Anal. for $C_{22}H_{26}N_2O_2$; Calculated(found): C: 75.40(75.31), H: 7.48(7.44), N: 7.99(8.10). ^{1}H NMR (500 MHz, DMSO): δH 1.39(9H, s, tert-butyl), 3.07 (2H, t, J=7.1 Hz, CH_2-10´), 3.73 (3H, s, OCH_3), 3.80 (2H, t, J=6.8 Hz, CH_2-11´), 6.56 (1H, t, J=7.7 Hz, H-5), 6.73 (1H, dd, J=8.7, 2.4 Hz, H-7´), 6.76 (1H, dd, J=7.9, 1.5 Hz, H-4), 6.81 (1H, dd, J=7.9, 1.5 Hz, H-6), 7.07 (1H, d, J=2.4 Hz, H-5´), 7.13 (1H, s, H-2´), 7.24 (1H, d, J=8.7 Hz, H-8´), 8.39 (1H, s, H-7), 10.69 (1H, s, NH). ^{13}C NMR (125 MHz, DMSO): δc 26.4, 55.2, 57.0, 100.1, 111.1, 111.2, 112.0, 116.5, 116.9, 117.2, 121.7, 123.6, 127.3, 131.3, 146.3, 153.0, 153.6, 165.8.

3c (E)-5-(tert-butyl)-2-(((2-(5-methoxy-1H-indol-3-yl)ethyl)imino)methyl)phenol

Brown solids; Yield: 87%; mp 75-78 ºC; IR (KBr) υmax: 3415, 1587, 1216, 1027 cm^{-1}; Anal. for $C_{22}H_{26}N_2O_2$; Calculated(found): C: 75.40(75.33), H: 7.48(7.34), N: 7.99(8.01). ^{1}H NMR (500 MHz, DMSO): δH 1,25(9H, s, tert-butyl),3.05 (2H, t, J=6.9 Hz, CH2-10´), 3.75 (3H, s, OCH_3), 3.87 (2H, t, J=6.8 Hz, CH2-11´), 6.73 (1H, dd, J= 8.7, 2.4 Hz, H-3), 6.81 (1H, d, J=8.6 Hz, H-4), 7.07 (1H, d, J=2.3 Hz, H-6), 7.12 (1H, s, H-2´), 7.25 (1H, d, J=8.7 Hz, H-8´), 7.36-7.32 (2H, m, H-7´, H-5´), 8.48 (1H, s, H-7), 10.70 (1H, s, NH). ^{13}C NMR (125 MHz, DMSO): δc 26.6,31.15, 33.6,55.2, 59.1, 100.1, 111.1, 111.5,111.9, 115.9, 123.5, 127.4, 127.7,129.2,131.3, 140.4, 152.9, 158.5, 165.9.

3d (E)-N-(4-butylbenzylidene)-2-(5methoxy-1H-indol-3yl)ethanamine.

Brown single crystal; 85% yield; mp 210-215°C; IR(KBr) υmax: 3119, 2918, 1645, 1484, 1213 cm^{-1}; Anal. for $C_{22}H_{26}N_2O$; Calculated(found): C: 79.00(79.04), H: 7.84(7.78), N: 8.38(8.36). ^{1}H NMR (*δ*,ppm,500MHz,DMSO-d_6): 10.65(1H,s,NH), 8.23(1H,s,HC=N), 7.64 (2H,d,*J*=8.10Hz,H-2,H-6), 7.26-7.22(3H,m,H-3,H-5,H-8´), 7.12 (1H,d,*J*=2.50Hz,H-5´), 7.06(1H,d,*J*=2.50Hz,H-2´), 6.73 (1H, dd, *J*= 8.70, 2.40 Hz, H-7´), 3.85 (2H,t,*J*=6.90Hz,CH2-11´), 3.73 (3H, s, OCH_3), 3.02 (2H, t, *J*=7.22Hz,CH2-10´), 2.58(2H,t,J=5.30Hz,CH_2-8), 1.56-1.51(2H, m, CH_2-9), 1.31-1.22 (2H, m, CH_2-10), 0.89 (3H, t, *J*= 7.35Hz, CH_3-11); ^{13}C NMR (*δ*,ppm,75MHz,DMSO-d_6): 160.46(C=N), 152.93(C-6´), 145.02(C-4), 133.89(C-1), 131.34(C-9´), 128.45(C-2,C-6,C-4´),127.79(C-3,C-5),123.45(C-2´), 112.21(C-3´), 111.90(C-7´), 111.03(C-8´), 100.28(C-5´), 61.47(CH_2N), 55.23(OCH_3), 34.72(CH_2-8), 32.91(CH_2-9), 26.77(CH $_2$-10´), 21.71(CH_2-10), 13.71(CH_3-10).

X-ray Crystallography Determinations

The single crystal was grown through slow evaporation of methanol solution and was mounted on the glass wall afforded brown crystal. Crystal suitable for x-ray diffraction experiments were performed on a Bruker APEX-II CCD diffractometer. With data collection: Bruker APEX ; cell ingstructure refinement: from Bruker SAINT; data reduction: from Bruker SAINT; program used for computing structure solution: SHELXS-97; program used for computing structure refinement: SHELXS-2013; molecular graphics: by Bruker SHELXTL and software for publication material: by Bruker SHELXTL. A brown crystal was formed on glass tube and collected for data collection. Data were collected at a temperature of 294K. Crystal data and refinement detail of the crystal are given in Table 1.

Table 1. Crystal data and refinement detail of the crystal compound 3d.

Emperical formula	$C_{22}H_{26}N_2O$
Formula Weight	334.45
Crystal System	Monoclinic
Space Group	P21/c
Crystal Size (mm)	0.08 x 0.30 x 0.42
Temperature	294 (2) K
Unit cell dimensions	
a (Å)	6.6144 (7)
b (Å)	30.014 (3)
c (Å)	9.9371 (10)
α (°)	90
ß (°)	97.615(2)
γ (°)	90
Volume	1955.4 (3) $Å^3$
Z	4
Density (calculated)	1.136 [g/cm^3]
Absorption coefficient	0.070 mm^{-1}
F(000)	720
θ range for data collection (°)	1.4 to 29.0
Index ranges	$-9 \le h \le 9, -40 \le k \le 40, -13 \le l \le 13$
Reflection with I > 2σ*(I)*	
Number of parameters	3142
Largest diff. peak and hole (e $Å^{-3}$)	5096 (R_{int}= 0.037) -0.16 and 0.19

2.2. Cholinesterase inhibitory test.

Ellman's method was cried out to evaluate the cholinesterases inhibitory activityb with galanthamine as a reference standard [23]. The solutions of test samples and galanthamine were prepared in DMSO at concentration of 1 mg/mL with a concentration of DMSO in final reaction mixture at 1%, in this concentration no inhibitory effect on AChE were observed. Next, for inhibitory assay, 140 µL of 0.1 M sodium phosphate buffer of pH 8 was added first to a 96-wells microplate followed by addition of 20 µL of each test samples and 20 µL of 0.09 units /mL acetylcholinesterase enzyme. Furthermore, after 15 min of incubation at 25 °C, 10 µL of 10 mM 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) was added into each well followed by 10 µL of 14 mM acetylthiocholine iodide. The observation of absorbance of the colored end-product was measured using BioTek PowerWave X 340 Microplate Spectrophotometer at 412 nm for 30 min after the initiation of enzymatic reaction. Each test was conducted in triplicate. Absorbance of the test samples was refined by subtracting the absorbance of their respective blank. Percentage inhibition was calculated using the following formula:

$$\text{percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.3. Molecular docking

The compounds were docked into the active site of *Tc*AChE resulting from the crystal structures form of the enzyme in-complex with donepezil drug (PDB ID: 1EVE) using Glide™, (version 5.7, Schrödinger, LLC, New York, NY, 2011). Hetero groups and water molecules were deleted from the enzyme beyond the radius of 5 Å of the reference ligand (donepezil). Protein structure was minimized by Protein Preparation Wizard™ application using the OPLS-2005 force field and the Receptor Grid Generation program was used to prepare *Tc*AChE grid. Next, all ligands were optimized by Ligand Preparation™ application using the OPLS-2005 force field to resultant the lowest energy state of each respective ligand. Docking simulations were carried out to generate 5 poses per ligand, and the best pose (with the highest score) displayed for each compound.

3. Results and Discussion

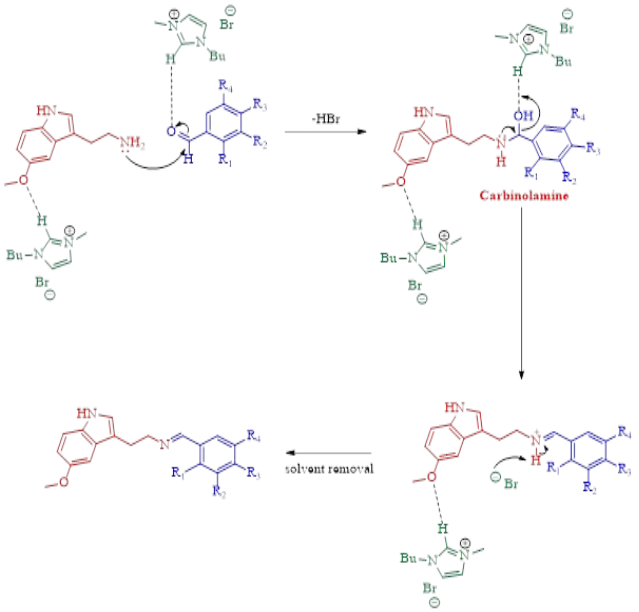
Schiff base derivatives were prepared by the reaction of diffrenet benzaldehydes **1(a-d)** with 5-methoxy tryptamine (2) in ecofrendly solvent 1-butyl-3-methylimidazolium bromide ([BMIM]Br) as conventional organic solvents. The ecofrendly solvent is the factor to stabiliz of intermediate compound by forming strong hydrogen bond interactions between acidic hydrogen of ecofrendly solvent and electronegative of the oxygen atom for starting materials. According to this concept, in any reaction with ecofrendly solvent employed as reaction medium, product yields will clearly increased. In addition, reaction times with 15 to 20 mints., noticeably exhibited the unique catalytic skill of [BMIM]Br to perform this reaction in suitable conditions Table 2.

Table 2: Yield and reaction time data for azomethine 3(a-d) in ionic solvent.

Compounds	Yield (%)	Reaction time (min.)
3a	92	15
3b	90	15
3c	78	20
3d	85	15

The suggested mechanism for this reaction can be demonstrated as the carbinolamine intermediate compound for the advancement of the reaction, this produced by nucleophilic addition of (5-methoxy tryptamine 2) (amine group) to carbonyl group of the different benzaldehydes (1), and then dehydration step as shown in Scheme 1.

All benzaldehyde, indole starting materials and reaction intermediate products are strongly solvated by ecofriendly solvent molecules. Solvent ion had an important part to complete the dehydration process giving final products. The [BMIM]Br solvent is acting as ecofriendly solvent, because of its unique catalytic result to stabilize the negative charges of oxygen atoms, shows a benefit over other original organic solvents such as methanol and ethanol which are mostly used in chemical synthesis reactions to increase the yields and decrease times of the reaction as summarized in Table 2.



Scheme 1: The mechanism for formation of azomethine 3(a-d)

As presented in above scheme 1, the carbonyl group and the oxygen in methoxy group for the starting materials; are added to acidic hydrogen of the solvent [bmim]Br with hydrogen bonds. This interactions were decreased the activation energy and the stabilizaation of starting chemicals ' initial energy states of the reaction and activated carbonyl moiety of benzaldehyde. For that, generation of reaction intermediate "carbinolamine", *via* attacking active nitrogen is strongly facilitated**.**

The structure of the final product **3c** was matches with CHN, 1D NMR and IR spectra. The ^{1}H NMR spectrum of **3c** was displayed singlet signal (1.25 ppm) due to tert-butyl. In addition, showed a triplet signals at 3.05 and 3.87 ppm assignable to H-10´and H-11´ respectively and 3.75 ppm due to OCH_3. Aromatic protons of H-2´and H-8´ displayed up to a doublet in 7.12 and 7.25 ppm respectively, wile H-5´ as well as H-7´, showed up two multiplate as seen in ^{1}H NMR spectra at 7.36-7.32 ppm. H-7 appeared as singlet at 8.48 ppm as well as NH show singlet at10.70 ppm. Structure elucidation was further substantiated and confirmed by the ^{13}C-NMR. From the ^{13}C , it was recorded two exceptionally downfield signals at δ 158.54 ppm were characteristic of carbon in (C=N) . The most upfield signal appear at δ31.16 ppm, that was assigned to tert-butyl group in addition 55.20 ppm for OCH_3 . Furthermore, there are many aromatic carbon that gave rise to different peaks within the range of δ 11198-158.54 ppm. The signal corresponded to C=N appered in 165.99 ppm.

The stereochemistry structure of azomethine compounds **3(a-d)** were similarly checked by single crystal X-ray crystallographic analysis of product **3d** (Crystal structure data of compound **3d (Figure 1**).

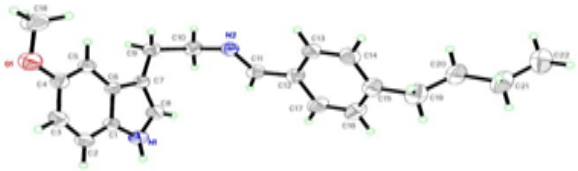


Figure 2. ORTEP view of atom numbering for compound (3d)

The planarity of the benzene ring allows enough space for the aliphatic chain and methoxy group to accomodate. The nitrogen atom which numbered as N2 was twisted with interplanar angle around 90° thus all the compound arranged without any major deformation. As the compound have long aliphatic chain as substituen with two benzene ring and a cyclic ring, it was expected to be strained but it show only a little strain along the molecule. Table 3 and Table 4 show bong length and bond angle for the compound. Bond angle around aliphatic chain which is C19,C20,C21 and C22 was in the range of 113° to 115°. Through X-ray diffraction, there is several bonding type appear such as hydrogen bonding(N1—H1N1··N2) (Table 5) and non covalent bonding C—H, C--π, C—H--π and π--π interaction (**Figure 3).**

Table 3: Selected bond length [Å] for compound *(3)*

O1 – C4	1.373(2)	O1- C18	1.404(3)
N1 – C1	1.404(3)	N1 – C8	1.360(2)
N2 – C10	1.463(2)	N2 – C11	1.260(3)
NI - HINI	0.87(3)	C6 – C7	1.428(2)
C7 – C8	1.364(2)	C7– C9	1.496 (2)
C11 – C12	1.464(2)	C15 – C19	1.511(3)

Table 4: Selected bond angle [°] for compound *(3)*

C4-O1-C18	117.64(15)	C7-C9-C10	114.74(14)
C1-N1-C8	108.71(13)	N2-C10-C9	110.92(13)
C10-N2-C11	118.76(13)	N2-C11-C12	123.99(14)
C1-N1-H1N1	127.60(15)	C14-C15-C19	120.83(18)
C8-N1-H1N1	123.70(15)		

Table 5: Hydrogen bonds and O—H··π packing interaction

D—H··A	d(D—H)	d(H··A)	d(D··A)	<(DHA)
N1—H1N1··N2	0.87(3)	2.08(2)	2.881(19)	154(18)

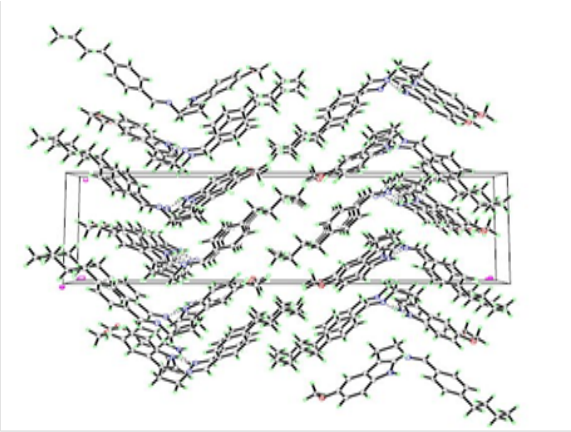


Figure 3: Two dimensional supramolecular styructure formed by intermolecular hydrogen bonding

The AChE inhibitions potency at 10 µg/mL for series 3(a-d) compounds were shown IC_{50} values and were summarized in Table 4.15. In compromise, compounds 3(a-d) were relatively exhibited good inhibitory potency toward AChE, therein compound 3c with R_1 = OH and R_4= tert-butyl showed the highest inhibition with IC_{50} values of 1.88 µM. Product **3d** with R_1= R_3=H and R_3= n-butyl and 3a with R_1= OH, R_2= tert-butyl exhibited significant AChE inhibitions because of IC_{50} values of less than 5 µM. while product **3b** exhibited lower inhibition with IC_{50} values of 5.40 µM

the presence of electron donating group OH at R_1 and tert-butyl substituents at R_3 position as well as R_2 had excellent effect on the AChE inhibitory potency observed in this compounds but, compound having n-butyl substituent at R_3 position, showed significant AChE inhibition activity in these compounds. In addition, the synthesized compounds 3c and 3d in this products showed greater AChE inhibitory than galanthamine drug.

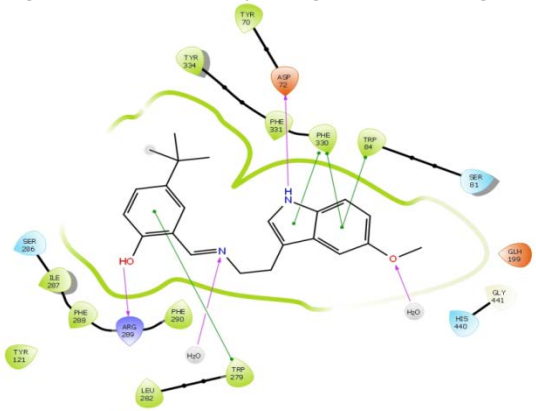


Figure 4: Binding orientation and interaction of compound 3c at the active site of *Tc*AChE

Compound **3c**, exhibited strong inhibitory activity was successfully docked inside the cavity of AChE enzyme. This compound was bounded strongly to the peripheral anionic site of the AChE enzyme via H-bonding interactions to Asp 72 (2.02 Å) , Arg 289 (2.24 Å) as well as two H-bonding through H_2O. In addition, hydrophobic connections with Trp 279, Tyr 334 pi-pi stacking bonding between **Trp 84, Phe 330 and Trp 279** aromatic ring as well as indol ring at peripheral anionic site of the enzyme **Figure 4**. This product can be selected as a candidate for PAS inhibition because of its wide interactions to the PAS. Amino acid for example **Tyr 121**. Hydrophobic interactions with moderate polar interaction with **His 440** at CT (catalytic triad) of the enzyme and additional great interaction noted for this product (**Figure 5)** .

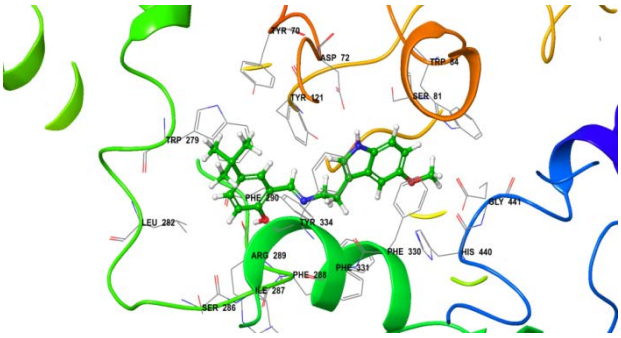


Figure 5: Ribbon structure of *Tc*AChE with ompound 3c

4. Conclusion

Ionic liquid mediated as an eco-friendly procedure for the preparing of Schiff bases products with high yield has been reported. In comparison with usual methods, the present method is cleaner, suitable, safer, and involves mild reaction environments resulting in maximum efficacy. All compounds of Schiff base products were tested for their AChE inhibitory efficacy. The result of compound **3c** exhibited most effective inhibitory activity with IC_{50} values of 1.88 µM against AChE. And molecular docking of products 3**a, 3b, 3c and 3d** in the active site of *Tc*ACh enzyme were totally agreed with the activities which observed in experimental part.

Supplementary Data

These paper contain supplementary ^{1}H-NMR,^{13}C-NMR for synthesis compounds **3a** and **3c** and crystallographic data for **3d** ((E)-N-(4-butylbenzylidene)-2-(5methoxy-1H-indol-3yl)ethanamine).

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