Journal of Tropical Pharmacy and Chemistry

Journal homepage: https://jtpc.farmasi.unmul.ac.id

Microwave Assisted Synthesis of BenzilideneBenzylamine and Its Acetylcholinesterase and Butyrylcholinesterase Activity

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Abstract

BenzilideneBenzylamine the derivative of Schiff bases contain azomethine group already used widely for industrial purposes and have wide range of biological activities. Benzilidene Benzylamine were synthesized by microwave irradiation reacting different aromatic and aniline purified pure crystal, 85% yield obtained reaction monitor by TLC. The Anticholinesterase activity utilized spectrophotometric Ellman assay for determination of butyrylcholinesterase and acetylcholinesterase. The synthesis compound **1-6** showed a wide range of inhibitory activity the compound 3((E)-N-(4-fluorobenzylidene)aniline) at 1000µg/mL, 71.62±0.74 percent inhibitory acetylcholinesterase potential while compound **6** ((E)-4 ((phenylimino)methyl) benzaldehyde) at 500 and 1000 µg/mL at IC_{50} show 71.68±0.22, 77.84±0.32 percent inhibitory potential comparatively greater than standard Galanthamine at 62.5µg/mL, 74.10±0.90 at IC_{50}. The butyrylcholinesterase activity of compound **6** ((E)-4 ((phenylimino)methyl)benzaldehyde) at 1000 μg/mL, show 75.83±1.07 percent inhibitory potential which is similar to standard compound at 62.5 µg/mL concentration of 75.45±0.90 percent butyrylcholinesterase inhibitory activity.

Keywords: BenzilideneBenzylamine, Microwave irradiation, Butyrylcholinesterase, Acetylcholinesterase, TLC

Submitted: 08 January 2020 **Accepted**: 25 February 2020 **DOI**: https://doi.org/10.25026/jtpc.v5i2.236

■ Introduction

A German scientist Alois Alzheimer in 1907 discovered a disease of central nervous system in which degeneration and dysfunction of neuron cell occur. This disease is named after Alois Alzheimer as Alzheimer disease (AD).Later on it was come to know that this disease effect more than twenty million people all over the world and rank 3rd after cancer and cardiac disease [1].The patient who suffer from (AD) at their early stages can be diagnosis by decrease in cerebral function, short

term memory loss. This make the victim incompetent to speak, read and think properly in later stages of the disease [2]. The current study which are though hypothetical propose a cholinergic pathway give stress on decrease in acetylcholine (ACh) in brain due to hydrolysis by acetylcholinesterase enzyme (AChE) [2,3]. Butyrylcholinesterase have the same function as actylcholinesterase, deactivate acetylcholine in brain. With the decrease of acetylcholine in brain the activity of acetylcholinesterase also decrease. Unlike, AChE Butyrylcholinesterase maintain at optimum level or above. Therefore Butyrylcholinesterase is an important factor of decrease of acetylcholine in brain [4]. Along with this Butyrylcholinesterase inhibition also decrease $_{\beta}$-amyloid peptide [5, 6]. The symptoms of (AD) are matured by Butyrylcholinesterase [7]. The selective inhibition of Butyrylcholinesterase in later stages of disease further decrease mental abilities due to dysfunctioning of neurons. Thus targeted acetylcholinesterase and Butyrylcholinesterase inhibitors open new routes for control measure of Alzheimer disease.

Many researchers workout for discovering drugs to minimize or demolish Alzheimer disease which have no side effects and usual. Tacrine is one of the common drug use for treating Alzheimer disease but due to its side effects their use become limited. Beside Tacrine other drugs used for AD are Rivastigmine, Donepezil, Memantine, Galanthamine and Caproctamine. None of these drugs are without side effects and may cause dizziness, diarrhea, gastro problems, hepato-toxicty, vomiting, nausea etc [7-10]. The free radical produce oxidative stress in cell due to disproportionation of anti-oxidants which are involve in many diseases [11]. This give the idea that control measure of excessive free radical in cell which are captured by AChE, BChE inhibitor and anti-oxidants should be used for treatment of Alzheimer disease [12,13]. Along with this the literature study show that polyamine derivatives present in larg number of standard drugs while Acetylcholinesterase and Butyrylcholinesterase contain aromatic rings residues that have capability to form positive(+ve)-π bond with basic polyamine counterpart [14,15].

Our current study is based o preparation and characterization of other such compounds that have capability of Acetycholinesterase and Butyrylcholinesterase binding reserve sites and compare activities of such compounds with Galanthamine (used as standard drug).

Experimental

General procedure for the synthesis of Benzilidene-benzylamines

Benzilidene-benzylamines were synthesized through the reaction of different aromatic aldehyde (1mmol) and aniline (1mmol) mixed in toluene use as a solvent. The reactants were taken in microwave cuvet and put it in microwave irradiation(250W) at room temperature (25 °C) and 1 atm pressure with constant shaking. The reaction was monitored by TLC after every one minut till the completion of reaction. After completion of reaction, solvent evaporated in rotatory under reduce pressure, and the product will be purified by using column chromatographic technique. When the pure product obtained the reaction mixture were cooled at -25 °C temperature in freezer for 48 hours. Pure crystal of product was obtained. 85% yield were obtained.

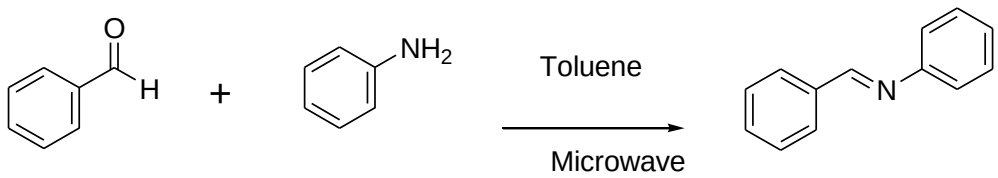


Figure 1. Synthesis of reaction

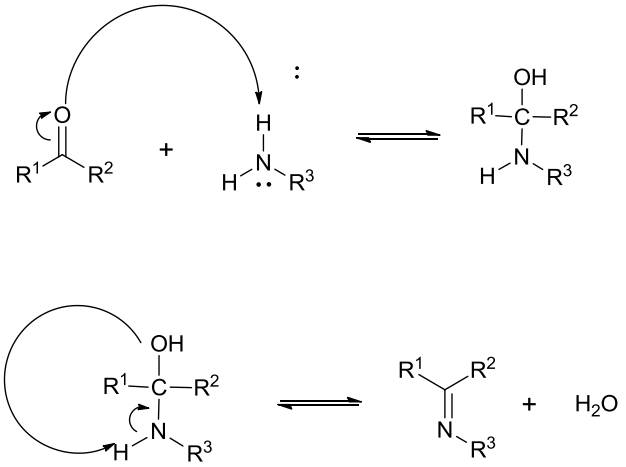


Figure 2. Mechanism of Schiff base formation

Chemistry of reaction

Rate of reaction was effected by substituent(s) nature on benzaldehyde derivatives and also the yield. The electron with drawing groups on aromatic ring increase the electrophilic nature of carbonyl carbon of benzaldehyde derivatives making the attack of nucleophile easier. Opposite effect was observed with electron donating groups on aromatic ring of benzaldehyde. So the rate and yields of Schiff bases with benzaldehyde derivatives having EWG as their substituents were found to be slightly higher as compared to benzaldehyde derivatives with EDG substituents. The ligands on the other hand have no significant differences in their nucleophilicity towards electrophilic carbonyl center as both have EDGs attached as their substituents.

Mechanism

It has been suggested that the establishment of Schiff base takes place in two alterable phases. Initially, a single brace of electrons of Nitrogen bonded with amine behaves as a nucleophile produces a carbinol-amine by attacking the electrophilic carbon bonded carbonyl-group. In the second stage the chemical reaction is that carbinol-amine (alcohol) receeds water molecules by desiccation and hence establishment of C=N dual bond of an imine compound occurs. Abundant of Schiff bases are converted to aldehyde or ketone and amine by hydrolysis of aqueous acids or bases. The slow step that determines the rate of reaction of Schiff base formation is dehydration of carbinalamine. Due to the basic nature of amines, however, the concentration of acid must be appropriate. At high concentration of acid, amine gets protonated and loses its nucleophilicity as a consequence the formation of carbinalamine cannot take place because equilibrium is shifted to the left side. That's why Schiff bases are best synthesized using weak acids. Carbinalamine dehydration can also be achieved using base as a catalyst. Though the reaction is not concerted and takes place in two steps through an anionic intermediate, yet it is rather similar to *E2* elimination of alkyl halides. In general we can say that the formation of Schiff bases occurs are as a consequence of two contrasting reactions i.e. addition reactions trailed by elimination reactions[16].

Anticholinesterase assays

The butyrylcholinesterase activity (BChE) performed on equine serum while the acetylcholinesterase(AChE) activity on electric eel (Electrophorus electricus) are used for synthesized compound 1-6, butyrylcholinesterase and acetylcholinesterase assay determination. The assay phenomenon is based on butyrylthiocholine iodide for BChE and acetylthiocholine iodide by AChE hydrolysis promotions. In the process 5-thio-2-nitrobenzoate anion is formed from hydrolysis which can be detected by spectrophotometer after complexation with

DTNB (5, 5-dithio-bis-nitro-benzoic acid) produced a yellow color.

Solution Preparation

All the synthesized compound separately dissolved in ranging from concentration 62.5 to 1000 µg/mL in a phosphate buffer 0.1 M solution. Prepared a 0.1 M buffer phosphate solution of KH_2PO_4 from 13.6 g/L and K_2HPO_4 from 17.4 g/L having pH value 8.0 ± 0.1 both solutions mixed in a 6 % KH_2PO_4 and 94 % K_2HPO_4 ratio respectively. For adjustment of PHmentainance used a potassium hydroxide (KOH) solution. The BChE from 7–16 U/mg and AChE from solid 518 U/mg diluted to the final concentration 0.01 U/mL and 0.03 U/mL were obtained in pH 8.0 freshly prepared buffer. The BTchI of 0.0005 M and ATchI of also 0.0005 M, DTNB of 0.0002273 M solution in distilled water were prepared and eppendorf tubes kept in refrigerator at 8 °C. For comparison positive control made same concentrations in methanol from standard Galanthamine as the compound.

Spectroscopic Analysis

For each butyrylcholinesterase activity (BChE) and acetylcholinesterase(AChE) activity 5 µl solution of enzyme to the cuvette added then a 205 µL test compound added and finally followed by a 5 µL DTNB reagent. The mixture of solution at 30 °C for 15 minute maintained in controllable electrical water bath followed by 5µl substrate subsequent addition. Analyzed absorbance at 412 nm via spectrophotometer of double beam. For the positive control 10 µg/mL of standard inhibitor cholinesterase a Galanthamine was used while for negative control all the reagent utilized except compound for test. The reaction time condition 30^0C with absorbance is for four minute taken and repeated at least three times. Finally rate of absorption with time were used for calculation of sample tested, inhibition and activity of enzyme.

$$V = \frac{\Delta Abs}{\Delta t}$$

% Enzime Inhibition = 100 - % activity of enzime

$$\%\ \text{Enzime activity} = 100 \times \frac{V}{V_{Max}}$$

Where Vmax is activity of enzyme in absence of drug inhibitor and V is reaction rate in presence of inhibitor drug.

IC_{50} (Median Inhibitory Concentration) Estimation

The butyrylcholinesterase activity (BChE) and acetylcholinesterase (AChE) substrate inhibited hydrolysis concentration by a 50 % (IC_{50}) is different for various compound.

Results and Discussion

Benzilidene-benzylamines were synthesized through the reaction of different aromatic aldehyde (1mmol) and aniline (1mmol) mixed in toluene use as a solvent. The reactants were taken in microwave cuvet and put it in microwave irradiation(250W) at room temperature (25 °C) and 1 atm pressure with constant shaking. The reaction was monitored by TLC after every one minut till the completion of reaction. After completion of reaction, solvent evaporated in rotatory under reduce pressure, and the product will be purified by using column chromatographic technique. When the pure product obtained the reaction mixture were cooled at -25 °C temperature in freezer for 48 hours. Pure crystal of product was obtained. 85% yields were obtained.

The pure synthesized compound obtained were evaluated via spectrophotometric Ellman assay for anticholinesterase inhibition potential (BChE and AChE) as shown in table 2. The compound **1–6** possess acetylcholinesterase inhibition potential in a wide range at tested five different concentrations i.e. 62.5, 125, 250, 500, 1000 µg/mL given in (table 2 and figure 1 and figure 2.

The compound **6** ((E)-4-((phenylimino)methyl)benzaldehyde) show acetylcholinesterase highest inhibition potential at concentration of 1000 μg/mL have 77.84±0.32 percent inhibition activity IC_{50},62.88 which is comparatively greater than a standard anticholinesterase a Galanthamine used at concentration 62.5 μg/mL, have 74.10±0.90, IC_{50},<0.1 shown in **Table 2** and **Figure 1**. While the compound **3** ((E)-N-(4-fluorobenzylidene) aniline) at 1000 μg/mL possess 71.62±0.74 and compound **6** ((E)-4-((phenylimino)methyl) benzaldehyde) at 500 μg/mL have 71.68±0.22 percent acetylcholinesterase inhibition potential which is nearly equal to a standard one

Galanthamine. The compound **1 – 6** also possess butyrylcholinesterase inhibition at different concentration discussed in **Table 2** and **Figure 2**. The entire compounds show a wide range of BChE activity. The most active compound found is **6** ((E)-4 ((phenylimino)methyl)benzaldehyde) at concentration 1000 μg/mL, have 75.83±1.07 percent butyrylcholinesterase potency of inhibition at IC_{50},69.48 which is similar to a standard positive control a Galanthamine used at concentration 62.5 µg/mL possess 75.45±0.90 percent potential of inhibition.

Acetylcholine (ACh), is an organic compound that works as a neurotranspoter and is important for usual purpose of nervous system, which is prepared from acetyl-coenzyme A and choline in the presence of cholineacetyltransferase (ChAT) as a catalyst. Vesicular acetylcholine trannsmitter (VAChT) transmittACh into synaptic vesicles [17]. Acetylcholine, the firstly ever marked neurotranspoter, operates as a neuro regulator in the Central Nervous System (CNS) plus Peripheral Nervous System (PNS). ACh organized with linked neurons in CNS produces a cholinergic system, which is motivated to recruit inhibitory activities. It stimulates muscles in PNS and is one of the most significant neuro transporters in the smooth muscles of heart and glands i.e. Autonomic Nervous System (ANS) [18].

Table 1: Preparation of Benzilidene Benzylamine derivatives in microwave irradiation

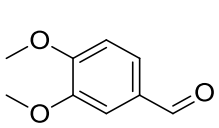
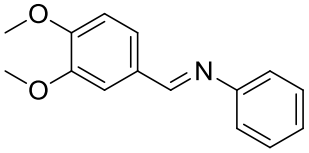
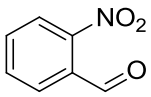
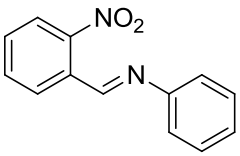
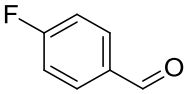
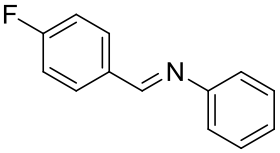
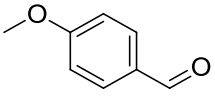
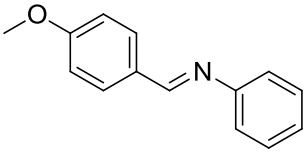
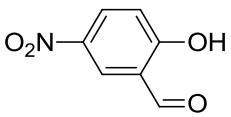
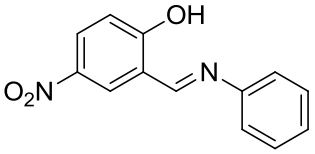
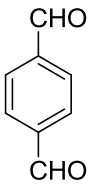
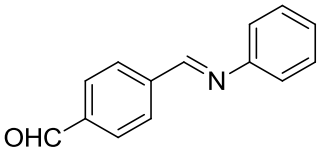
Compounds	Benzaldehyde	Product	Time	% yield
1			7 min	84%
2			6 min	85%
3			7 min	87%
4			5 min	84%
5			8 min	85%
6			7 min	86%

Table 2. Compound acetylcholinesterase inhibition activity result.

Compounds	Concentration (µg/mL)	AChE Percent Inhibition (Mean ± SEM)	IC50 (µg/mL)	BChE Percent Inhibition (Mean ± SEM)	IC50 (µg/mL)
1	1000	67.37±0.26	109.03	64.96±0.32	155.38
	500	61.52±0.95		59.74±1.13	
	250	55.90±0.46		53.33±0.29	
	125	51.02±0.18		47.11±0.06	
	62.5	46.04±0.14		43.68±0.05	
2	1000	64.20±0.23	338.15	61.03±0.35	464.80
	500	51.13±0.20		47.08±0.47	
	250	44.87±1.27		41.91±0.88	
	125	39.76±0.61		37.90±0.96	
	62.5	34.91±1.30		32.98±0.72	
3	1000	71.62±0.74	252.01	66.79±0.63	319.70
	500	63.86±0.60		59.67±0.61	
	250	44.48±0.64		41.69±0.77	
	125	37.54±0.50		35.54±0.50	
	62.5	31.74±0.61		29.00±0.30	
4	1000	64.79±0.62	314.78	62.61 ± 0.77	369.86
	500	56.45 ± 0.49		54.60 ± 0.80	
	250	45.75 ± 0.58		43.83 ± 0.56	
	125	37.51±0.77		35.69±0.77	
	62.5	31.53±0.71		29.67±0.61	
5	1000	65.17±0,72	185.83	63.34±0.98	342.34
	500	57.85±0.97		56.32±1.06	
	250	51.37±1,65		48.05±0.75	
	125	46.73±0.78		44.70±1.25	
	62.5	41.34±1.01		38.74±0.68	
6	1000	77.84±0.32	62.88	75.83±1.07	69.48
	500	71.68±0.22		69.54±0.46	
	250	65.54±0.16		64.36±0.21	
	125	57.83±1.07		55.84±0.32	
	62.5	49.54±0.46		48.68±0.22	
Galanthamine	1000	93.10±0.60	<0.1	91.90±0.96	03
	500	87.58±0.63		87.08±0.47	
	250	83.76±0.71		82.40±0.20	
	125	79.44±0.58		77.61±0.43	
	62.5	74.10±0.90		75.45±0.90	

Date are expressed as means ± SEM. Calculated Values significantly different when compared to standard drug Galanthamine at the same concentration a standard drug ($p > 0.05$) i.e., $p < 0.05$ and $p < 0.01$. Data not significantly different when compared to standard drug ($p > 0.05$).

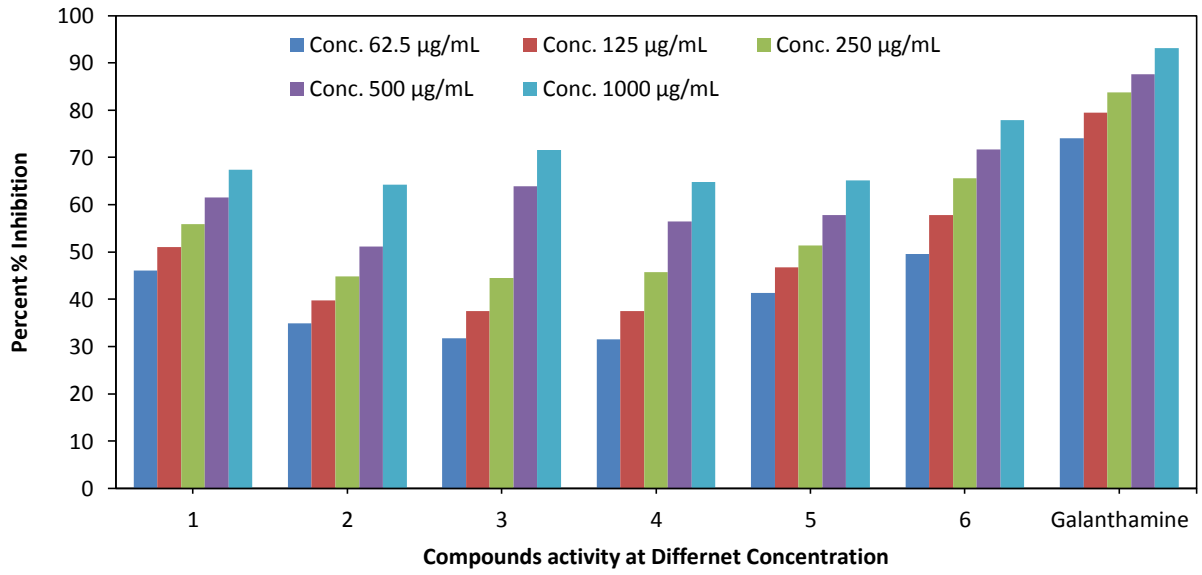


Figure 1: Acetylcholinesterase Activity Result

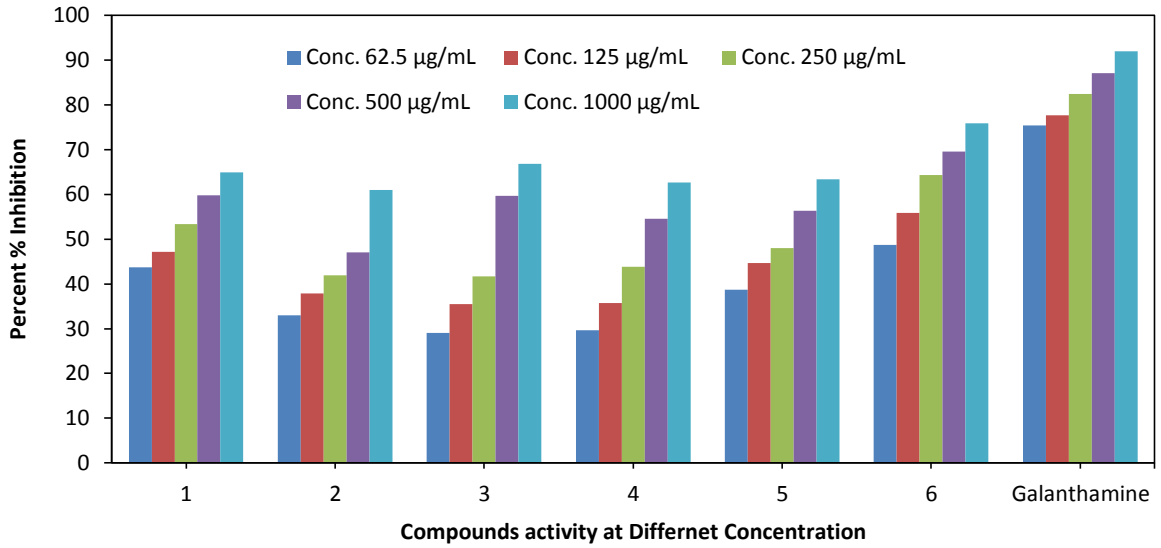


Figure 2: Butyrylcholinesterase Activity Results

In various mammals comprising humansACh is hydrolised by two different cholinesterase enzymes which are acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) [19]. At the junction of neuron and muscle, an arising membrane confined enzyme AChE (also known as true cholinesterase) synchronises the amount of the transmitters by deactivating acetylcholine. AChE becomes active when Ach in reply to an action potential is acquited from the post-synaptic nerve process. After transmission of ACh inside the synaptic cleave, Ach muddles to acetylcholine receptors (AChRs), generally establish on pre-synaptic cells. The ACh receptor-intermediated activity is straightly completed through hydrolysis by acetylcholinesterase (AChE) [20, 21]. AChE is rich in blood cells, nervous system and muscles [22, 23].

Butyrylcholinesterase is a synthetic compound not present in the human body naturally, they are known as common cholinesterase or false cholinesterase which is an extra cholinesterase that hydrolyzes acetylcholine and it specially performances on butyrylcholine. BChE is mostly present in liver, heart, lung, kidney and intestine [23, 24]. Surgeons considered butyrylcholinesterase for its hydrolysis of an antibiotic succinylcholine to succinylmonocholine and then to succinic acid, the antibiotic is being used as a short time inhibitor of the acetylcholine receptor in surgical operations [25]. BChE controls cholinergic conduction in the lack of AChE and hindercarbamate and organophosphate inhibitors afore they spread to AChE. BChE also aids in the initiation of drugs like bambutarol, herion while deactivates others such as cocaneasperin, amitriptylene[26].Similarly AChE and BChE are combinely obstructed by 1,5-bis(4 allyldimethylamminopropyl)pentan-3-on di-bromide and 10-[2-diethylamino-propyl]-phenothiazide (ethopropazine), [27].

Conclusion

The current experimental study evaluated on synthesized Benzilidene Benzylamine the derivative of Schiff bases contain azomethine group and have wide range of biological activities, for a pathological anti – Alzheimer disease. The Anticholinesterase activity is performed on different concentration of synthesized compound. The percent inhibition potential of butyrylcholinesterase and acetylcholinesterase is determined using spectrophotometric Ellman assay. The entire compounds possess a wide range butyrylcholinesterase, acetylcholinesterase inhibitory potential. The compound **3** ((E)-N-(4-fluorobenzylidene)aniline) show acetylcholinesterase (AChE) activity 71.62±0.74

percent which is nearly closed to standard positive control Galanthamine Anticholinesterase 74.10±0.90 percent at IC_{50}. While a compound **6** ((E)-4-((phenylimino)methyl)benzaldehyde) at IC_{50} show 71.68±0.22, 77.84±0.32 percent inhibitory potential comparatively greater than standard Galanthamine 74.10±0.90. The butyrylcholinesterase (BChE) activity of compound **6** ((E)-4 ((phenylimino)methyl)benzaldehyde) 75.83±1.07 percent inhibitory potential which is similar to standard compound 75.45±0.90 percent butyrylcholinesterase inhibitory activity. These synthesized compound show anti – Alzheimer potency concluding from Anticholinesterase assay. There further analysis can provide more sustainable result as compared to a standard one already being available.

Conflict of Interest

The authors declare no conflict of interest regarding the publication of this paper.

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