

## Original Research Article

### Synthesis of some phenylisoindoline-1,3-diones and their acetylcholinesterase and butyryl cholinesterase inhibitory activities

#### ABSTRACT

**Aims:** To synthesize some phthalimides derivatives and evaluate the compounds for their possible biological properties.

**Methods:** The substituted phenylisoindoline-1,3-dione were synthesized from the reactions of N-phenyl phthalimide with different substituted aromatic aldehyde. The synthesized compounds were characterized using nuclear magnetic resonance spectroscopic analysis. The acetylcholinesterase and butyryl cholinesterase inhibitions were determined by Spectrophotochemical analysis of acetylthiocholine and butyryl choline chloride.

**Results:** Compounds 6 ( $IC_{50} = 30 \pm 3 \mu\text{g/mL}$ ) and 4 ( $IC_{50} = 141 \pm 60 \mu\text{g/mL}$ ) were found to be the most active inhibitors against acetylcholinesterase, while compounds 4 ( $IC_{50} = 102 \pm 10 \mu\text{g/mL}$ ), 5 ( $IC_{50} = 105 \pm 20 \mu\text{g/mL}$ ) and 2 ( $IC_{50} = 190 \pm 10 \mu\text{g/mL}$ ), were found to be most active inhibitor against butyryl cholinesterase.

**Conclusion:** The considerable acetylcholinesterase and butyryl cholinesterase inhibitory activities of the synthesized compounds makes them good candidates for the development of selective acetylcholinesterase and butyryl cholinesterase inhibitors.

**Keywords:** Alzheimer's disease, acetylcholine, acetylcholinesterase, butyryl cholinesterase, eserine, chalcones.

#### Introduction

Phthalimide derivatives have been reported to belong to a class of compounds possessing various pharmacological properties such as antimalarial (4), antihypertensive, antimicrobial (5), antiviral

and as herbicides (6). Similarly, compounds derived from Chalcones have been found to possess useful pharmacological properties such as cytotoxicity[1], antioxidant[2], antibacterial [3], antileishmanial [4], anticancer [5], antiangiogenic [6], anti-infective, anti-inflammatory [7], antifungal [8], anti-malarial [9], anti-tumor [10], anti-protozoal [11] and cytotoxic properties [12].

## EXPERIMENTAL

Melting points were determined with open capillary tube on a Gallenkamp (variable heater) melting point apparatus and were uncorrected. Infrared spectra were recorded as KBr pellets on a Bruker 2000 Spectrometer. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR was run on a Bruker 600 MHz spectrometer ( $\delta$  in ppm relative to  $\text{Me}_4\text{Si}$ ) at the Department of Chemistry, Portland state University, Portland U.S.A. The purity of the compounds was routinely checked by TLC on silica gel G plates using n-hexane/ethyl acetate (1:1, v/v) solvent system and the developed plates were visualized by UV light. All reagents used were obtained from Sigma–Aldrich Chemical Ltd, except Glacial acetic acid, ethanol, oxalic acid and vanillin which were obtained from BDH Chemical Limited.

### Synthetic work

#### Synthesis of 2-(4-acetylphenyl) isoindoline-1,3-dione

Phthalic anhydride (5 g), 4-ethoxyaniline (4.0g) in 50 mL glacial acetic acid reacted under reflux for 6 hours. The reaction mixture was poured into crushed ice to obtain 2-(4-acetylphenyl) isoindoline-1,3-dione. **IR Spectra (KBr)** 3073  $\text{cm}^{-1}$  (C-H aromatic), 1736  $\text{cm}^{-1}$  (C=O) imide, 1708  $\text{cm}^{-1}$  (C=O) imide, 1689  $\text{cm}^{-1}$  (C=O) ketone, 1593  $\text{cm}^{-1}$  (C=C), 1384  $\text{cm}^{-1}$  (C-N) imide.  **$^1\text{H}$  NMR (DMSO- $d_6$ ):** 7.92(d, 2H, J=7.5,1.5, ArH), 7.85(d, 2H, J=7.5,1.5, ArH), 7.88(t, J=7.5,1.5,

ArH), 7.28(d, 2H, J=7.5, 1.5, ArH), 2.50(s, 3H, COCH<sub>3</sub>). <sup>13</sup>C-NMR: 197(C=O), 167(CO-amide), 137, 136,132, 129, 124, 123, 26(CH<sub>3</sub>)

### **Synthesis of 2-(4-(3-(4-hydroxyphenyl) acryloyl) phenyl) isoindoline-1,3-dione 1**

2-(4-acetylphenyl) isoindoline-1,3-dione (1.5 g, 0.00565 mol) was allowed to react with 4-hydroxybenzaldehyde (0.689 g, 0.00565 mol) in 20mL ethanol after which 10 % sodium hydroxide solution was added and the reaction mixture was stirred at room temperature for 48hours. The reaction mixture was poured into distilled water and hydrolyzed with 10 % hydrochloric acid solution to obtain a yellow product (2-(4-(3-(4-hydroxyphenyl) acryloyl) phenyl) isoindoline-1, 3-dione. The product was filtered, recrystallized and then oven-dried.

**IR Spectra (KBr)** 3469 cm<sup>-1</sup>(O-H) p-hydroxyl phenol, 3074 cm<sup>-1</sup> (C-H aromatic), 1735 cm<sup>-1</sup>(C=O) imide, 1709 cm<sup>-1</sup> (C=O) imide, 1680 cm<sup>-1</sup>(C=C) chalcones, 1593 cm<sup>-1</sup> (C=C), 1384 cm<sup>-1</sup> (C-N) imide. **<sup>1</sup>H NMR (DMSO-d<sub>6</sub>):** 8.06(d, 1H, J=15.1), 7.88(t, 2H, J=7.5, ArH), 7.87(t, J=7.5, ArH), 7.85(d, 2H, J=7.5, ArH), 7.65(d, 2H, J=7.5), 7.36(d, 2H, J=7.5), 7.59(d, 1H, J=15.1). **<sup>13</sup>C-NMR:** 190(C=O), 167(CO-amide), 145(C=C), 132, 131, 124, 121(C=C), 115.

### **Synthesis of 2-(4-(3-(3-hydroxyphenyl) acryloyl) phenyl) isoindoline-1,3-dione 2**

2-(4-acetylphenyl)isoindoline-1,3-dione(1.5 g, 0.00565 mol) of was allowed to react with 3-hydroxybenzaldehyde ( 0.689 g, 0.00565 mol) in 20mL ethanol after which 10 % sodium hydroxide solution was added and the reaction mixture was stirred at room temperature for 48hours. The reaction mixture was poured into distilled water and hydrolysed with 10 % hydrochloric acid solution to obtain a yellow product (2-(4-(3-(3-hydroxyphenyl) acryloyl)phenyl) isoindoline-1,3-dione. **IR Spectra (KBr)** 3448 cm<sup>-1</sup>(O-H) m-hydroxyl phenol,

3074  $\text{cm}^{-1}$  (C-H aromatic), 1735  $\text{cm}^{-1}$ (C=O) imide, 1709  $\text{cm}^{-1}$  (C=O) imide, 1680  $\text{cm}^{-1}$ (C=C) chalcones, 1593  $\text{cm}^{-1}$  (C=C), 1384  $\text{cm}^{-1}$  (C-N) imide.  $^1\text{H NMR}$  (DMSO- $d_6$ ): 8.06(d, 1H, J=15.1), 7.88(t, 2H, J=7.5, ArH), 7.87(t, J=7.5, ArH), 7.85(d, 2H, J=7.5, ArH), 7.65(d, 2H, J=7.5), 7.36(d, 2H, J=7.5), 7.59(d, 1H, J=15.1).  $^{13}\text{C-NMR}$ : 189.7(C=O), 167(CO-amide), 135(C=C), 132, 131, 124, 121(C=C), 117, 115.

### **Synthesis of 2-(4-(3-(3aH-indol-3yl)acryloyl)phenyl)isoindoline-1,3-dione(compound 3)**

2-(4-acetylphenyl)isoindoline-1,3-dione (1.5 g, 0.00565 mol) was allowed to react with Indolecarbaldehyde (2.19 g, 0.015mol) in 20mL ethanol after which 10 % sodium hydroxide solution was added and the reaction mixture was stirred at room temperature for 48hours. The reaction mixture was poured into distilled water and hydrolysed with 10 % hydrochloric acid solution to obtain a yellow product **2-(4-(3-(3ah-indol-3yl)phenyl)isoindoline-1,3-dione**.

**IR Spectra (KBr)** 3221  $\text{cm}^{-1}$ (N-H) indole, 3074  $\text{cm}^{-1}$  (C-H aromatic), 1735  $\text{cm}^{-1}$ (C=O) imide, 1709  $\text{cm}^{-1}$  (C=O) imide, 1680  $\text{cm}^{-1}$ (C=C) chalcones, 1593  $\text{cm}^{-1}$  (C=C), 1384  $\text{cm}^{-1}$  (C-N) imide.

$^1\text{H NMR}$  (DMSO- $d_6$ ): 8.05(d, 1H, J=15.1), 7.87(t, 2H, J=7.5, ArH), 7.85(t, J=7.5, ArH), 7.84(d, 2H, J=7.5, ArH), 7.66(d, 2H, J=7.5), 7.38(d, 2H, J=7.5), 7.19(t, 1H, J=15.1), 7.14(d, 1H, J=7.5), 6.93(t, 1H, J=7.5).  $^{13}\text{C-NMR}$ : 192(C=O), 169(CO-amide), 145(C=C), 137, 133, 132, 127(C=C), 124, 111.

### **Synthesis of 2-(4-(3-(4-chlorophenyl) acryloyl) phenyl) isoindoline-1,3-dione 4**

2-(4-acetylphenyl) isoindoline-1,3-dione (1.5 g, 0.00565 mol) was allowed to react with 4-chlorobenzaldehyde (1.12 g, 0.00795mol) in 20mL ethanol after which 10 % sodium hydroxide solution was added and the reaction mixture was stirred at room temperature for 48hours. The reaction mixture was poured into distilled water and hydrolyzed with 10 % hydrochloric acid solution to obtain a yellow product 2-(4-(3-(4-chlorophenyl) acryloyl) phenyl)

isoindoline-1,3-dione 5. **IR Spectra (KBr)** 3074  $\text{cm}^{-1}$  (C-H aromatic), 1735  $\text{cm}^{-1}$ (C=O) imide, 1709  $\text{cm}^{-1}$  (C=O) imide, 1680  $\text{cm}^{-1}$ (C=C) chalcones, 1593  $\text{cm}^{-1}$  (C=C), 1384  $\text{cm}^{-1}$  (C-N) imide, 1091  $\text{cm}^{-1}$  (C-Cl)

**$^1\text{H}$  NMR (DMSO- $d_6$ ):** 8.03(d, 1H, J=15.1), 7.88(t, 2H, J=7.5, ArH), 7.87(t, J=7.5, ArH), 7.85(d, 2H, J=7.5, ArH), 7.68(d, 2H, J=7.5), 7.59(d, 2H, J=15), 7.44(d, 1H, J=7.5), 7.36(d, 2H, J=7.5).

**$^{13}\text{C}$ -NMR:** 188(C=O), 165(CO-amide), 145(C=C), 133, 132, 131, 128, 121(C=C), 117, 115.

### **Synthesis of 2-(4-(3-(3-methoxyphenyl) acryloyl) phenyl) isoindoline-1,3-dione 5**

2-(4-acetylphenyl) isoindoline-1,3-dione (1.53g, 0.00577mol) was allowed to react with m-methoxybenzaldehyde (0.785 g, 0.00577mol) in 20mL ethanol after which 10% sodium hydroxide was stirred at room temperature for 48hours. The mixture was poured into distilled water and hydrolyzed with 10% hydrochloric acid solution to obtain a red product, **2-(4-(3-(3-methoxyphenyl) acryloyl) phenyl) isoindoline-1,3-dione**

**IR Spectra (KBr)** 3074  $\text{cm}^{-1}$  (C-H aromatic), 1735  $\text{cm}^{-1}$ (C=O) imide, 1709  $\text{cm}^{-1}$  (C=O) imide, 1680  $\text{cm}^{-1}$ (C=C) chalcones, 1593  $\text{cm}^{-1}$  (C=C), 1384  $\text{cm}^{-1}$  (C-N) imide, 1234  $\text{cm}^{-1}$  C-O-C) methoxy, 1126  $\text{cm}^{-1}$  (C-O-C) methoxy.

**$^1\text{H}$  NMR (DMSO- $d_6$ ):** 8.05(d, 1H, J=15.1), 7.88(t, 2H, J=7.5, ArH), 7.87(t, J=7.5, ArH), 7.85(d, 2H, J=7.5, ArH), 7.68(d, 2H, J=7.5), 7.59(d, 2H, J=15), 7.44(d, 1H, J=7.5), 7.36(d, 2H, J=7.5), 7.16(s, 1H), 3.83(s, 3H(CO- $\text{CH}_3$ ))  **$^{13}\text{C}$ -NMR:** 188(C=O), 160(CO-amide), 145(C=C), 133, 132, 131, 128, 121(C=C), 117, 115, 55(CO- $\text{CH}_3$ )

### **Synthesis of 2-(4-(3-(2-hydroxy-4-methoxyphenyl) acryloyl) phenyl) isoindoline-1,3-dione 6**

2-(4-acetylphenyl) isoindoline-1,3-dione (1.53g, 0.00577mol) was reacted with o-vanillin (0.975g, 0.00641mol) (0.785g, 0.00577mol) in 20mL ethanol after which 10% sodium hydroxide was stirred at room temperature for 48hours. The mixture was poured into distilled water and

hydrolyzed with 10% hydrochloric acid solution to obtain a red product **2-(4-(3-(2-hydroxy-4-methoxyphenyl)acryloyl)phenyl)isoindoline-1,3-dione 7**

**IR Spectra (KBr)** 3460  $\text{cm}^{-1}$  (O-H) phenolic, 3074  $\text{cm}^{-1}$  (C-H aromatic), 1735  $\text{cm}^{-1}$  (C=O) imide, 1709  $\text{cm}^{-1}$  (C=O) imide, 1680  $\text{cm}^{-1}$  (C=C) chalcones, 1593  $\text{cm}^{-1}$  (C=C), 1384  $\text{cm}^{-1}$  (C-N) imide, 1233  $\text{cm}^{-1}$  C-O-C) methoxy, 1124  $\text{cm}^{-1}$  (C-O-C) methoxy.  **$^1\text{H}$  NMR (DMSO- $d_6$ ):** 8.33(d, 1H,  $J=15.1$ ), 7.99(d, 1H), 7.88(t, 2H,  $J=7.5$ , ArH), 7.87(t,  $J=7.5$ , ArH), 7.85(d, 2H,  $J=7.5$ , ArH), 7.68(d, 2H,  $J=7.5$ ), 7.42(d, 2H,  $J=15$ ), 7.44(d, 1H,  $J=7.5$ ), 7.36(d, 2H,  $J=7.5$ ), 3.43(s, 3H(CO- $\text{CH}_3$ )).  **$^{13}\text{C}$ -NMR:** 189(C=O), 168(CO-amide), 146(C=C), 133, 132, 131, 128, 121(C=C), 117, 115, 55(CO- $\text{CH}_3$ )

## **Biological Assays**

### **In vitro acetylcholinesterase and butyryl cholinesterase Inhibitory Assays**

The anti-cholinesterase (acetylcholinesterase and butyryl cholinesterase) inhibiting activities of the synthesized compounds were determined by using modified method of Ellman et al. (1961) as described by Obuotor (2004). The synthesized compounds were prepared in a stock solution of DMSO in buffer and was used for the cholinesterase inhibition assay, while Eserine prepared in buffer was used as the reference compound (positive control).

**Procedure:** To triplicate test tube was added 240 $\mu\text{l}$  of buffer (50 mM Tris-HCl, pH 8.0) and 20  $\mu\text{l}$  of varying concentration of the test compounds (10, 5, 2.5 and 1.25 mg/ mL), 20 $\mu\text{l}$  of the enzyme preparation, the reaction mixture was then incubated for 30mins at 37 $^{\circ}\text{C}$ , after which 20  $\mu\text{l}$  of 10 mM 5,5'-dithiobis (2-nitrobenzoic acid), was added.

The reaction was then initiated by the addition of 20 $\mu\text{l}$  of 25mM ATChI (1.042 mM final concentration). The rate of hydrolysis of ATChI was then determined spectrophotometrically by measuring the change in the absorbance per minute ( $\Delta A/\text{min}$ ) due to the formation of the yellow

5-thio-2-nitrobenzoate anion at 412 nm over a period of 4min at 30s interval. A solution of buffer was used as negative control. The percentage inhibition (% I) of the synthesized compounds were obtained using the formula:

$$I (\%) = [(V_0 - V_i) / V_0] * 100$$

Where: I (%) = Percentage inhibition

$V_i$  = enzyme activity in the presence of synthesized compounds

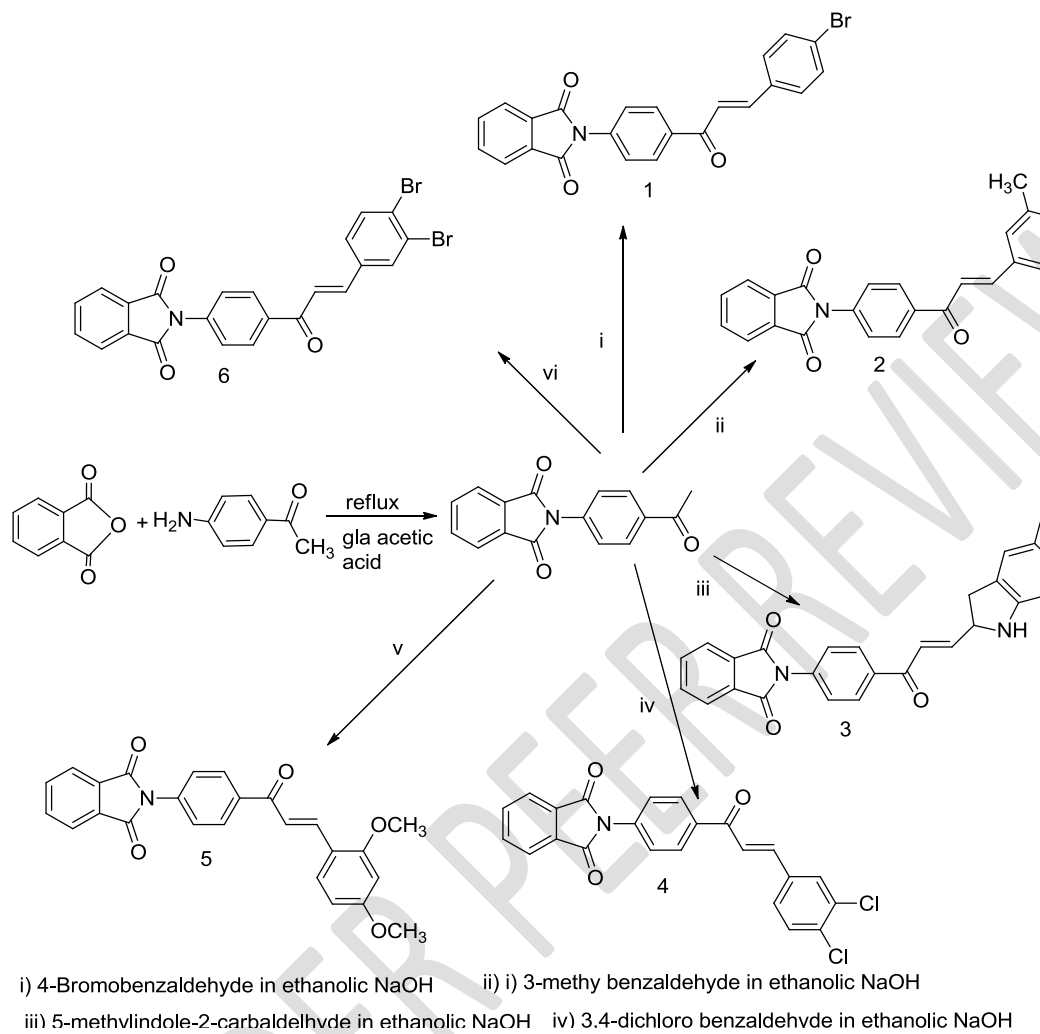
$V_0$  = enzyme activity in the absence of synthesized compounds

## Results and Discussion

### Chemistry

The respective chalcones were synthesized by reacting the phthalimide derivative 1, obtained from the reaction of 4-aminoacetophenone with phthalic anhydride in glacial acetic acid with different substituted aromatic aldehydes in the presence of ethanolic NaOH using the method described by Singh *et. al*,2010 (Scheme 1). All the desired chalcones (**1-6**) were obtained in high yields with the percentage yield between 66.30% - 72.12%. The melting point of compounds (**1-6**) ranged between 155 and 238<sup>0</sup>C (Table 1). The structures of the compounds were partially characterized using infrared, <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic methods. The diagnostic bands for presence of chalcones HC=CH bonds in the infrared spectral data was observed at 1680 cm<sup>-1</sup>. The results of the <sup>1</sup>H spectroscopic data showed the presence of the olefinic protons of the chalcones appearing downfield between 7.36 and 7.68 ppm for the compounds synthesized. The results of the <sup>13</sup>C NMR spectroscopic data showed the presence of the olefinic carbons of the chalcones appearing at 145 ppm.

# Scheme 1





## **Biology**

### **Acetylcholinesterase activity**

The activity of the compounds ranged from  $1.03 \pm 0.3$  to  $4.83 \pm 1.0$  (Figure 1). It was observed that compound **6** has the lowest  $IC_{50}$  value. This study indicated that compound **6** having  $IC_{50}$  value of  $1.03 \pm 0.3$  has the highest activity compared to other compounds and eserine which is a standard drug used as a positive control. Moreover, all the six compounds showed activity that is higher than the standard drug Eserine that was used. The activity of compound **6** could be as a result of the presence of an electron donating groups at the ortho (hydroxyl) and para (methoxy) of the chalcone phenyl ring. The lower activity exhibited by compound **3** may be due to the presence of a bulky indole moiety of the chalcone which may exhibit some steric hindrance.

### **Butyrylcholinesterase activity**

The activities observed ranged from  $1.02 \pm 0.1$  to  $4.99 \pm 0.3$  (Figure 2). It was observed that compound **5** has the lowest  $IC_{50}$  value of  $1.02 \pm 0.1$  which indicated that compound **5** has the highest Butyrylcholinesterase inhibitory activity compared to rest of the compounds. Also, all the six compounds showed higher inhibitory properties than the standard drug Eserine used in this test. The higher inhibitory activity exhibited by compound **5** could be as a result of the presence of an electron withdrawing chloro- group that has 7 electrons in his valence orbitals of the chalcone ring. Looking at the lower activity obtained for compound **3** ( $4.99 \pm 0.3$ ) this could be as a result of steric hindrance caused by a bulky indole ring attached to the chalcone.

## CONCLUSION

It can be concluded that the synthesis of compounds **1-6** was successful and the workup stage was environmentally friendly. The bioactivities of all synthesized chalcones analogues **1-6** were tested in vitro by using the standard activity protocol. It was observed that this study identifies a new class of potential AChE, BuChE, nitric oxide and FRAP actives that could be developed into drugs.

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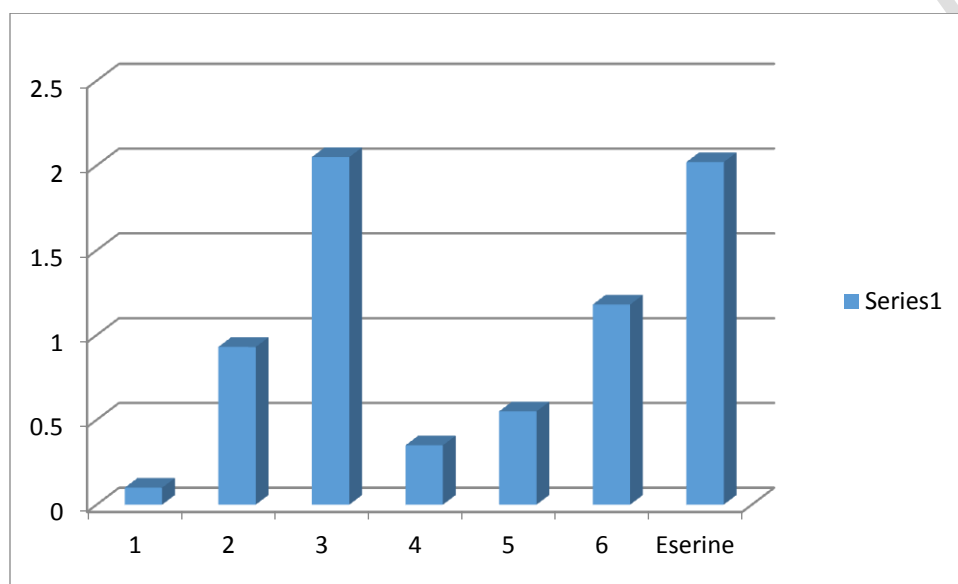
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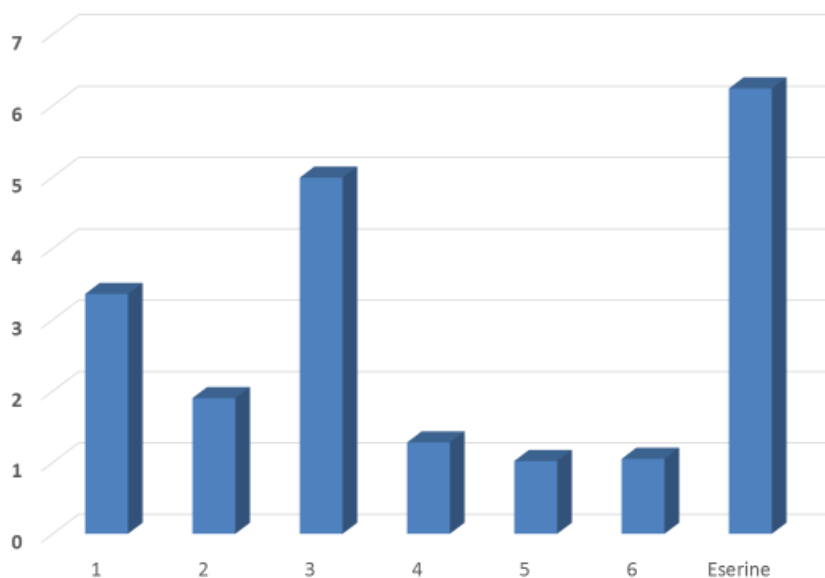
**Table 1: Some physical properties of the synthesized compounds.**

<b>COMPOUND NO</b>	<b>MOLECULAR FORMULA</b>	<b>MOLECULAR WEIGHT (gmol<sup>-1</sup>)</b>	<b>YIELD (%)</b>	<b>MELTING POINT(°C)</b>
1	C <sub>23</sub> H <sub>15</sub> NO <sub>4</sub>	369.0	72.12	155-158
2	C <sub>23</sub> H <sub>15</sub> NO <sub>4</sub>	369.0	71.80	174-176
3	C <sub>25</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub>	392.0	67.57	235-238
4	C <sub>22</sub> H <sub>14</sub> NO <sub>3</sub> Cl	375.5	70.57	165-167
5	C <sub>23</sub> H <sub>17</sub> NO <sub>4</sub>	371.0	71.50	229-231
6	C <sub>24</sub> H <sub>18</sub> NO <sub>3</sub>	400.0	66.30	235-238

**FIGURE 1: The IC<sub>50</sub> of Acetylcholinesterase plotting compounds against the concentration**



**FIGURE 2: The IC<sub>50</sub> of Butrylcholinesterase plotting compounds against the concentration**



**Table 2: The selectivity value of AChE and BUCHE**

Sample	AChE inhibition IC <sub>50</sub> Mg/ml	BUCHE inhibition IC <sub>50</sub> Mg/ml	Selectivity for AChE	Selectivity for BUCHE
<b>1</b>	4.09±0.06	3.36±0.4	0.82	1.22
<b>2</b>	2.76±0.6	1.90±0.01	0.69	1.45
<b>3</b>	4.83±1.0	4.99±0.3	1.03	0.97

<b>4</b>	1.41±0.6	1.28±0.01	0.91	1.1
<b>5</b>	2.12±0.5	1.02±0.1	0.48	2.08
<b>6</b>	1.03±0.3	1.05±0.2	1.01	0.98
<b>Eserine</b>	9.97±1.3	6.24±0.3	0.63	1.6

## APPENDIX

### Result of Acetylcholinesterase inhibitory activity of the Chalcones

COMPOUNDS	% INHIBITION
1	82.88±3.0
2	98.24±3.0
3	58.24±1.0

4	81.36±4.0
5	86.24±0.01
6	87.84±0.9
Eserine	77.04±0.02

**Results of IC50 values for acetylcholinesterase activity of the Chalcones**

COMPOUNDS	IC50(mg/ml)
1	4.09±0.06
2	2.76±0.6
3	4.83±1.0



4	1.41±0.6
5	2.12±0.5
6	1.03±0.3
Eserine	9.97±1.3

**Results of butyrylcholinesterase inhibitory activity of the Chalcones**

<b>COMPOUNDS</b>	<b>% INHIBITION</b>
<b>1</b>	<b>72.11±4.0</b>
<b>2</b>	<b>91.83±3.0</b>
<b>3</b>	<b>77.00±2.0</b>

<b>4</b>	<b>85.28±5.0</b>
<b>5</b>	<b>98.72±3.0</b>
<b>6</b>	<b>79.22±2.</b>
<b>Eserine</b>	<b>77.00±2.0</b>

Result of IC50 values for butyrylcholinesterase activity of the Chalcones

COMPOUNDS	IC50(mg/ml)
1	3.36±0.4
2	1.90±0.01

3	4.99±0.3
4	1.28±0.01
5	1.02±0.1
6	1.05±0.2
Eserine	6.24±0.3

UNDER PEER REVIEW

UNDER PEER REVIEW