

# Acetylcholinesterase inhibitory potencies of new pyrazoline derivatives

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**ABSTRACT:** Alzheimer's disease (AD) has no current cure and its mechanism is not fully known, but treatments for symptoms are available. Acetylcholinesterase (AChE) has been reported to be an applicable therapeutic target in patient with AD. Acetylcholinesterase inhibitors (AChEIs) are commonly used for it. For this purpose, novel series of pyrazoline based compounds [2-(3-(4-methoxyphenyl)-5-aryl-4,5-dihydro-1H-pyrazol-1-yl)benzo[d]thiazole, **1-9**] were synthesized and AChE inhibitory potencies were reported here. The results indicated that compound **1** ( $K_i = 0.13 \pm 0.004 \mu\text{M}$ ) possessed the highest AChE inhibitory effect in series, which is two times more potent than the reference compound Tacrin ( $K_i = 0.26 \pm 0.045 \mu\text{M}$ ). So, pyrazoline derivative **1** can be considered as a lead inhibitor in designing new AChE inhibitors.

**KEYWORDS:** Acetylcholinesterase; alzheimer disease; benzothiazol; pyrazoline.

## 1. INTRODUCTION

Alzheimer's disease (AD) is a destructive brain disorder among elderly people. The first of the pathophysiological factors associated with AD is the collection of  $\beta$ -amyloid protein exterior to the neurons in the encephalon, another is the collection of twisted tau protein (tangles) strands interior of the neurons. In addition, AD patients undergo significant loss of neurons especially in the hippocampus. Many neurotransmitter levels are significantly reduced in this disease. These are serotonin, dopamine, glutamate, noradrenaline and acetylcholine (ACh). Decreased neurotransmitter levels also cause memory, cognition, motor and functional capacity loss. This disease often affects people who are exposed to stress. The number of people suffering from this disease will certainly increase in future due to aging population, insufficient personal care, deficient health support for patients and their families [1-4].

ACh is a critical neurotransmitter in the brain, which is especially important for attention, memory and motivation. Studies have shown that this neurotransmitter decreases dramatically in the brain cortex and hippocampus of Alzheimer's patients. The cholinergic supposal proposes that molecules that goal and prevent the acetylcholinesterase enzyme (AChE) will be useful for the treatment of AD for instance rivastigmine, galantamine, and donepezil (Figure 1). They cause increasing of ACh concentration by inhibiting AChE enzyme and prevent acetylcholine (ACh) hydrolysis. Although neurotransmitter therapy causes improvements in cognitive skills, such improvements are usually temporary. The drugs used in therapy aim to reduce AD symptoms rather than disease progression. Therefore, the design and development of new compounds that may be acetylcholinesterase inhibitors are important and good choice in the treatment of AD and development of new drug candidates for AD [3-5].

2-Pyrazolines are most popular heterocyclic structure in drug design. They have various important biological activities such as antimicrobial [6], antifungal [7], antimalarial [8], antiinflammatory [9-10], analgesic [10], anti-Alzheimer's disease [11], anti-Parkinson's disease [12], antidepressant [13-14], anticancer [15-16] and monoamine oxidase (MAO) inhibitory activities [17-18]. In recent years, pyrazoline derivatives are shown to have inhibiting or activating effects on several enzymes such as cholinesterases [19], MAOs, and carbonic anhydrases [20-21].

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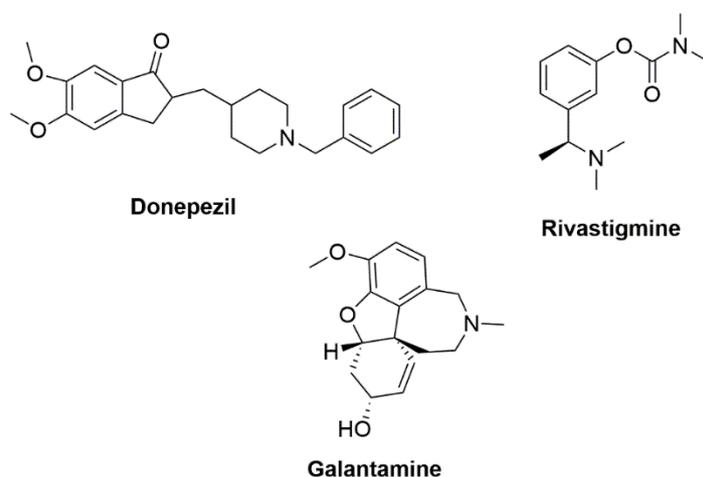


Figure 1. Drugs commonly prescribed for Alzheimer's disease.

In addition, benzothiazole, the subject of this research, was considered as an important pharmacophoric group because of its wide range of bioactivities such as antifungal activity [22], carbonic anhydrase inhibition [23], antihypoxic [24] and monoamine oxidase type A (MAO-A) inhibition [25].

It was aimed to synthesize new 2-(3-(4-methoxyphenyl)-5-aryl-4,5-dihydro-1H-pyrazol-1-yl)benzo[d]thiazoles (**1-9**) to investigate their AChE inhibitory effects to ascertain possible lead compounds in this study.

## 2. RESULTS AND DISCUSSION

### 2.1. Chemistry

2-(3-(4-Methoxyphenyl)-5-aryl-4,5-dihydropyrazol-1-yl)benzo[d]thiazoles **1-9** were synthesized successfully as presented in Figure 2. In the study, phenyl (**1**), 4-methylphenyl (**2**), 4-chlorophenyl (**3**), 4-fluorophenyl (**4**), 4-bromophenyl (**5**), 3-bromophenyl (**6**), 2-bromophenyl (**7**), 2-chlorophenyl (**8**), 2,6-dichlorophenyl (**9**) were used as aryl part. Compounds synthesized are original, except **1** and **3**.

When the  $^1\text{H}$  NMR spectra were examined, the proton of the C-5 carbon of the pyrazoline ring resonated as a doublet of doublet between the range of 6.40–5.61 ppm. The other protons on C-4 resonated as a doublet of the doublet. One of these protons resonated in the range of 4.01–3.95 ppm, while the other proton resonated in the range of 3.15–3.46 ppm. Both protons were found to have two  $J$  values.

One of the protons of the pyrazoline ring of the many compounds was coincided with the methoxy peak existed in the main structure. It was observed that the protons belonging to the methoxy group had a resonance in the range of 3.87–3.78 ppm.

It has been observed that C-4, C-5 and methoxy groups of the pyrazoline ring resonated in the range of 63.6–43.2 ppm in the  $^{13}\text{C}$  NMR. Chemical shift values of compounds (**2**, **4-9**) were presented for the first time in detail in the experimental section. These values showed that the synthesized compounds were coherent with their chemical structure.

### 2.2. Acetylcholinesterase inhibitory activity

Recombinant human AChE enzyme was used to evaluate AChE inhibitory activity of the compounds [20]. Tacrine (TAC) was utilized as a reference drug. Table 1 shows that the compounds had  $\text{IC}_{50}$ = 1.30–2.78  $\mu\text{M}$  and  $\text{K}_i$ = 0.13 $\pm$ 0.004–0.85 $\pm$ 0.070  $\mu\text{M}$  values. In addition, reference compound TAC had value of  $\text{IC}_{50}$ = 0.84  $\mu\text{M}$  and  $\text{K}_i$ = 0.26 $\pm$ 0.045  $\mu\text{M}$ .

When the effect of the groups on the phenyl ring was evaluated in terms of  $\text{IC}_{50}$ , both the electron donating group (compound **2** which had 4- $\text{CH}_3$  substituent,  $\text{IC}_{50}$ = 2.49  $\mu\text{M}$ ) and the electron withdrawing groups (compound **3** which had 4-Cl substituent,  $\text{IC}_{50}$ = 2.43  $\mu\text{M}$ , and compound **5** which had 4-Br substituent,  $\text{IC}_{50}$ = 2.45  $\mu\text{M}$ ) in para position of phenyl ring reduced the inhibitory potency compared to the unsubstituted phenyl analog **1** ( $\text{IC}_{50}$ = 1.30  $\mu\text{M}$ ). When para position halogenated compounds **3**, **4**, **5** ( $\text{IC}_{50}$ = 2.43, 1.48, 2.45  $\mu\text{M}$ , respectively) were compared, compound **4** had the strongest inhibitory effect among others according to its  $\text{IC}_{50}$  value. On the other hand, compound **5** has shown the lowest inhibitory property. The reason that compound **5** with bromine at 4-th position has the lowest inhibitory property among the

halogenated ones may be due to the bigger atomic diameter of bromine than chlorine and fluorine atoms. On the other hand, when the effect of position of bromine substitution was compared, 4-bromo derivative compound **5**, 3-bromo derivative compound **6**, and 2-bromo derivative compound **7**; compound **6** showed great AChE inhibitory potency than other brominated ones with IC<sub>50</sub> value of 1.42 μM.

Compound **1** displayed the best AChE inhibition potency with IC<sub>50</sub> value of 1.30 μM among others. When chlorine substituent (compound **3**, **8** and **9**) at different positions of the phenyl ring was evaluated in terms of IC<sub>50</sub>, compound **8**, which has chlorine at orto position, had the strongest inhibitory effect among others. The reason for lower inhibition observed in other derivatives (**3** and **9**) may be that compound **8** might have interacted with different active sites of the enzyme.

Majority of the compounds evinced notable inhibition properties toward AChE enzyme in micromolar concentrations. The compound **1** was ascertained preeminent AChE inhibitor with the lowest Ki value of 0.13±0.004 μM. In addition, compound **1** exhibited better inhibition profile toward AChE than Tacrine. Furthermore, the halogen size has been found to be considerable in reducing the inhibition potential on AChE. Compound **4** (with fluorine) showed more potent inhibitory activity than other halogenated ones with Ki value of 0.19±0.056 μM.

**Table 1.** Inhibitory effects of pyrazoline derivatives **1-9** on AChE.

Compounds	IC <sub>50</sub> (μM)		Ki (μM)
	AChE	r <sup>2</sup>	AChE
<b>1</b>	1.3	0.9236	0.13±0.004
<b>2</b>	2.49	0.9889	0.52±0.560
<b>3</b>	2.43	0.9846	0.29±0.006
<b>4</b>	1.48	0.944	0.19±0.056
<b>5</b>	2.45	0.9863	0.68±0.137
<b>6</b>	1.42	0.9646	0.53±0.058
<b>7</b>	1.88	0.9518	0.85±0.070
<b>8</b>	2.00	0.972	0.50±0.055
<b>9</b>	2.78	0.9861	0.51±0.054
TAC <sup>a</sup>	0.84	0.9844	0.26±0.045

<sup>a</sup>Tacrine (TAC) was used as a standard inhibitor for AChE. IC<sub>50</sub> value is the half maximal inhibitory concentration of inhibitors used for enzyme inhibition. Ki is the inhibition constant and the dissociation equilibrium constant of the enzyme-inhibitor complex. r-squared (r<sup>2</sup>) value presents a variety of goodness-of-fit statistics.

When the Ki results of the compounds were evaluated, nonsubstituted compound **1** has drawn attraction in series. When substitution effects of the groups on the phenyl ring was taken into account, methyl substitution at compound **2**, which is a electron releasing group, inhibition of AChE by **2** decreased 4.17 times comparing to **1**, which is a nonsubstituted compound. However, halogenated compound **3** (with chlorine), **4** (with fluorine), **5** (with bromine) were considered in terms of their Ki value; fluorinated compound **4** was found more effective on AChE than the other two halogenated compounds **3** and **5**. This situation most probably results from the small size of fluorine atom than chlorine and bromine atoms. In this study, halogenated compounds (compounds **5**, **6**, **7**), which have halogen atom of different position of phenyl ring, were synthesized. The effect of bromine position at the compound **5**, **6**, and **7** was also evaluated. By considering Ki values of them, in case of compound **6**, which has bromine at the 3rd position of phenyl, was seen as the most potent inhibitor among compounds **5**, **6**, and **7**. In addition, the same situation was evaluated for the compounds **3**, **8** and **9**. In this case, compound **3**, which has chlorine at the 4th position of phenyl ring, was found as the most effective compound while compound **8**, which is 2-chloro substituted and compound **9**, which is 2,6-dichloro substituted, have shown similar inhibition property in series. According to Ki values, compound **1** has been found as the most effective compound of series.

### 3. CONCLUSION

This study reported synthesis and AChE inhibitory potency of the new pyrazoline derivatives (**1-9**). The results indicated that compound **1** had the greatest inhibitory potency with both IC<sub>50</sub> (1.30 μM) and Ki (0.13±0.004 μM) values. Based on the results of enzyme inhibition, the pyrazoline derivative compound **1** can be taken into consideration as a candidate compound to improve new AChE inhibitors.

### 4. MATERIALS AND METHODS

#### 4.1. Chemistry

During the synthesis studies, to monitor of the reaction and check the purity of the synthesized compounds, Thin Layer Chromatography (TLC) plates (60 HF254, Merck KGaA) were used. Chloroform:methanol (4.8:0.2) was used as mobile phase system. Nuclear Magnetic Resonance (NMR) spectra of the compounds were taken with the Varian Mercury Plus spectrometer (Varian inc., Palo Alto, California, U.S.). Shimadzu's LCMS-TOF-ESI (Shimadzu, Kyoto, Japan) device was used for HRMS. Electrothermal 9100/IA9100 instrument (Bibby Scientific Limited, Staffordshire, UK) was used to determine melting points.

##### 4.1.1. Synthesis of 2-hydrazinylbenzo[d]thiazole (Compound A, Figure 2)

The starting compound 2-mercaptobenzothiazole (3.0 g) and hydrazine hydrate (10 ml / 80%) were refluxed for 24 hours by conventional method in ethanol (20 ml). At the end of the specified period, the contents of the flask were kept at room temperature and the separated product was filtered and dried. Light brown compound A was used for the next reaction without purification [26].

##### 4.1.2. General procedure for the preparation of chalcones, 1a-9a, Figure 2

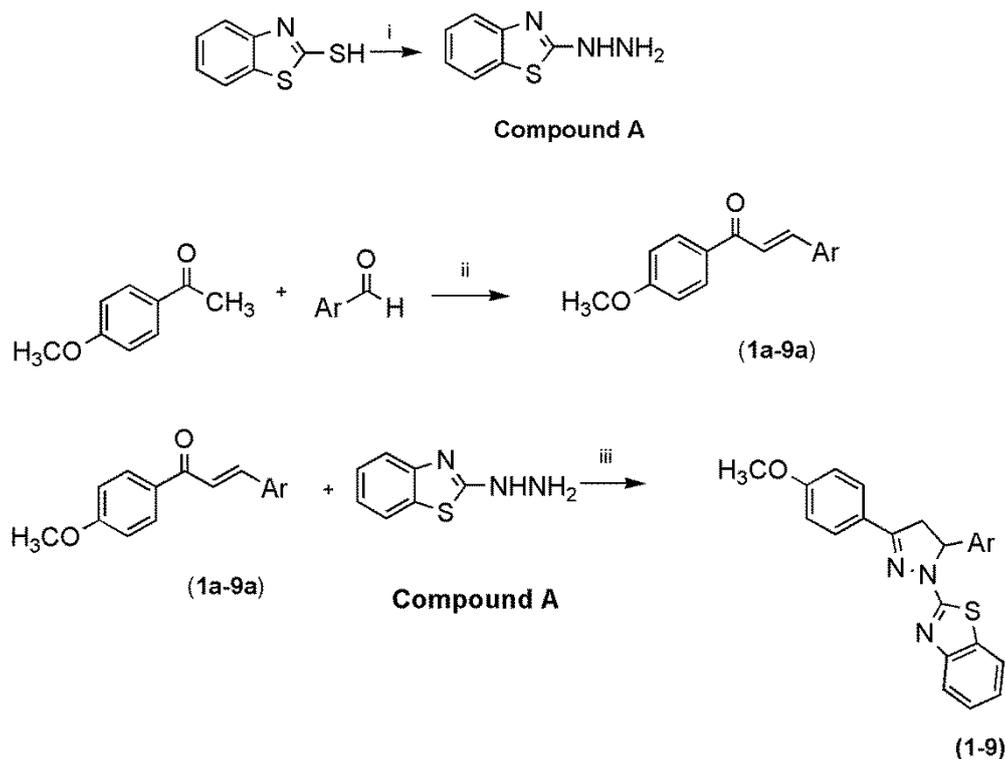
Starting compounds of the series, which are chalcones, were synthesized by Claisen-Schmidt condensation [27-31]. 4-Methoxy acetophenone and the appropriate aldehyde derivative [benzaldehyde (**1a**), 4-methylbenzaldehyde (**2a**), 4-chlorobenzaldehyde (**3a**), 4-fluorobenzaldehyde (**4a**), 4-bromobenzaldehyde (**5a**), 3-bromobenzaldehyde (**6a**), 2-bromobenzaldehyde (**7a**), 2-chlorobenzaldehyde (**8a**), 2,6-dichlorobenzaldehyde (**9a**)] were mixed within ethyl alcohol (6 ml) in 1: 1 mole ratio. The mixture was cooled on ice bath afterward aq. NaOH solution (6 ml, 10%) was added drop by drop to the flask. The mixing was sustained at room temperature throughout the night. After 24 hours, the content was taken into the cold water (50 ml). The contents of the flask were acidified with concentrated HCl acid (pH = 6-7). The collapsed solid was filtered and washed with water and ethanol. After the intermediate compound was dried, it was used as a starting material in the third step.

##### 4.1.3. Synthesis of the pyrazoline type compounds 1-9, Figure 2

Synthesis of pyrazoline derivative compounds (**1-9**) was carried out in acidic medium using a conventional method with a protic solvent. Briefly, favorable chalcone derivative (1 mmol) (**1a-9a**) and 2-hydrazinylbenzo[d]thiazole (1.1 mmol) in ethanol (25 mL) with acetic acid (0.05 mL) were heated for 19-36h (for **1-9**) [20-21]. Then, ethanol was evaporated until half volume and the flask was left at room temperature. After the collapsed solid was filtered, it was purified by crystallization from the suitable solvent or solvent mixture (methanol or methanol-ether). <sup>1</sup>H NMR, <sup>13</sup>C NMR, and HRMS were used to confirm their chemical structures.

##### 2-(3-(4-Methoxyphenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)benzo[d]thiazole (1)

A bright green solid, yield 9.3%. Mp: 188-190 °C; Lit m.p: 149-150 °C [32]. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, δ ppm): 7.71 (d, 2H, Ar-H, J = 8.8 Hz), 7.63 (d, 1H, Ar-H, J = 7.7 Hz), 7.50 (d, 1H, Ar-H, J = 8.1 Hz), 7.46-7.22 (m, 6H, Ar-H), 7.07 (t, 1H, Ar-H, J = 7.5 Hz), 6.94 (d, 2H, Ar-H, J = 8.8 Hz), 5.78 (dd, 1H, pyrazoline ring, J = 12.1, 5.1 Hz), 3.84 (s, 3H, OCH<sub>3</sub>), 3.27 (dd, 1H, pyrazoline ring, J = 17.2, 5.1 Hz), (one of the proton peak of the pyrazoline ring was under methoxy peak). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, δ ppm): 152.9, 152.7, 141.6, 131.9, 129.1, 128.7, 128.4, 127.9, 126.2, 125.8, 124.1, 121.8, 121.4, 120.9, 120.2, 114.4, 63.7, 55.6, 44.1, HRMS (ESI-MS) calc. for C<sub>23</sub>H<sub>19</sub>N<sub>3</sub>OS [M+H]<sup>+</sup> 386.1322; found 386.1336.



**Reagents:** i: Hydrazine hydrate (%80), ethanol, reflux, ii: NaOH (%10), ethanol, rt, iii: Ethanol, glacial acetic acid, reflux.  
**Ar:** Phenyl (1), 4-methylphenyl (2), 4-chlorophenyl (3), 4-fluorophenyl (4), 4-bromophenyl (5), 3-bromophenyl (6), 2-bromophenyl (7), 2-chlorophenyl (8), 2,6-dichlorophenyl (9).

**Figure 2.** Synthesis of compounds 1-9.

*2-(3-(4-Methoxyphenyl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)benzo[d]thiazole (2)*

A bright yellow solid, yield 11.3%. Mp: 200-201 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, δ ppm): 7.71 (d, 2H, Ar-H, J = 8.8 Hz), 7.62 (d, 1H, Ar-H, J = 8.1 Hz), 7.50 (d, 1H, Ar-H, J = 8.1 Hz), 7.25-7.21 (m, 3H, Ar-H), 7.12 (d, 2H, Ar-H, J = 8.1 Hz), 7.06 (t, 1H, Ar-H, J = 7.5 Hz), 6.94 (d, 2H, Ar-H, J = 8.8 Hz), 5.74 (dd, 1H, pyrazoline ring, J = 11.7, 5.0 Hz), 3.85 (s, 3H, OCH<sub>3</sub>), 3.26 (dd, 1H, pyrazoline ring, J = 17.2, 5.0 Hz), 2.30 (s, 3H, CH<sub>3</sub>), (one of the proton peak of the pyrazoline ring was under methoxy peak). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, δ ppm): 163.6, 161.4, 152.9, 152.6, 138.7, 137.6, 131.9, 129.7, 128.3, 126.2, 125.7, 124.2, 121.8, 120.9, 120.2, 114.4, 63.5, 55.6, 44.1, 21.3, HRMS (ESI-MS) calc. for C<sub>24</sub>H<sub>21</sub>N<sub>3</sub>OS [M+H]<sup>+</sup> 400.1478; found 400.1487.

*2-(5-(4-Chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzo[d]thiazole (3)*

A cream colour solid, yield 8.4%. Mp: 176-177 °C; Lit m.p:128-129 [32]. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, δ ppm): 7.70 (d, 2H, Ar-H, J = 8.8 Hz), 7.64 (d, 1H, Ar-H, J = 8.1 Hz), 7.50 (d, 1H, Ar-H, J = 8.1 Hz), 7.28-7.23 (m, 5H, Ar-H), 7.09 (t, 1H, Ar-H, J = 8.1 Hz), 6.94 (d, 2H, Ar-H, J = 8.8 Hz), 5.71 (dd, 1H, pyrazoline ring, J = 12.1, 5.5 Hz), 3.85 (s, 3H, OCH<sub>3</sub>), 3.22 (dd, 1H, pyrazoline ring, J = 17.6, 5.5 Hz), (one of the proton peak of the pyrazoline ring was under methoxy peak). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, δ ppm): 163.5, 161.5, 152.8, 152.6, 140.2, 133.7, 131.9, 129.3, 128.4, 127.8, 125.9, 123.9, 122.0, 121.0, 120.2, 114.4, 63.2, 55.6, 43.9, HRMS (ESI-MS) calc. for C<sub>23</sub>H<sub>18</sub>N<sub>3</sub>OCl [M+H]<sup>+</sup> 420.0932; found 420.0936.

*2-(5-(4-Fluorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzo[d]thiazole (4)*

A dark yellow solid, yield 6.4%. Mp: 163-165 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, δ ppm): 7.71 (d, 2H, Ar-H, J = 8.8 Hz), 7.63 (d, 1H, Ar-H, J = 7.7 Hz), 7.50 (d, 1H, Ar-H, J = 8.1 Hz), 7.34-7.22 (m, 3H, Ar-H), 7.08 (t, 1H, Ar-H, J = 7.7 Hz), 7.02-6.93 (m, 4H, Ar-H), 5.74 (dd, 1H, pyrazoline ring, J = 11.7, 5.1 Hz), 3.85 (s, 3H, OCH<sub>3</sub>), 3.25 (dd, 1H, pyrazoline ring, J = 17.2, 5.1 Hz), (one of the proton peak of the pyrazoline ring was under methoxy peak). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, δ ppm): 163.5, 162.5 (<sup>1</sup>J<sub>CF</sub>=245 Hz), 161.5, 152.8 (<sup>2</sup>J<sub>CF</sub>=22.7 Hz), 131.9, 128.4, 128.1, 127.9, 125.9, 123.9, 121.9, 120.9, 120.2, 116.1, 115.9, 114.4, 63.1, 55.6, 44.0, HRMS (ESI-MS) calc. for C<sub>23</sub>H<sub>18</sub>N<sub>3</sub>OFS [M+H]<sup>+</sup> 404.1227; found 404.1235.

2-(5-(4-Bromophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzo[d]thiazole (5)

A cream colour solid, yield 7.3%. Mp: 184-185 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, δ ppm): 7.69 (d, 2H, Ar-H, J = 8.8 Hz), 7.64 (d, 1H, Ar-H, J = 7.7 Hz), 7.50 (d, 1H, Ar-H, J = 8.1 Hz), 7.44 (d, 2H, Ar-H, J = 8.1 Hz), 7.27-7.21 (m, 3H, Ar-H), 7.08 (t, 1H, Ar-H, J = 7.7 Hz), 6.94 (d, 2H, Ar-H, J = 8.8 Hz), 5.70 (dd, 1H, pyrazoline ring, J = 12.1, 5.5 Hz), 3.85 (s, 3H, OCH<sub>3</sub>), 3.22 (dd, 1H, pyrazoline ring, J = 17.6, 5.5 Hz), (one of the proton peak of the pyrazoline ring was under methoxy peak). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, δ ppm): 163.5, 161.5, 152.8, 152.6, 140.7, 132.2, 131.9, 128.4, 128.1, 125.9, 123.9, 122.0, 121.8, 121.0, 120.2, 114.4, 63.2, 55.6, 43.9, HRMS (ESI-MS) calc. for C<sub>23</sub>H<sub>18</sub>N<sub>3</sub>OSBr [M+H]<sup>+</sup> 464.0427; found 464.0421.

2-(5-(3-Bromophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzo[d]thiazole (6)

A cream colour solid, yield 13.7%. Mp: 200-201 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, δ ppm): 7.70 (d, 2H, Ar-H, J = 8.8 Hz), 7.64 (d, 1H, Ar-H, J = 7.7 Hz), 7.53-7.51 (m, 2H, Ar-H), 7.40-7.37 (m, 1H, Ar-H), 7.27-7.16 (m, 2H, Ar-H), 7.09 (t, 3H, Ar-H, J = 8.0 Hz), 6.94 (d, 1H, Ar-H, J = 8.8 Hz), 5.71 (dd, 1H, pyrazoline ring, J = 12.1, 5.5 Hz), 3.85 (s, 3H, OCH<sub>3</sub>), 3.24 (dd, 1H, pyrazoline ring, J = 17.6, 5.5 Hz), (one of the proton peak of the pyrazoline ring was under methoxy peak). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, δ ppm): 163.5, 161.5, 152.8, 152.6, 143.9, 132.0, 131.1, 130.7, 129.4, 128.4, 125.9, 124.9, 123.9, 123.1, 122.0, 121.0, 120.3, 114.4, 63.2, 55.6, 43.9, HRMS (ESI-MS) calc. for C<sub>23</sub>H<sub>18</sub>N<sub>3</sub>OSBr [M+H]<sup>+</sup> 464.0427; found 464.0412.

2-(5-(2-Bromophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzo[d]thiazole (7)

A cream colour solid, yield 18.5%. Mp: 219-221 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, δ ppm): 7.71-7.62 (m, 4H, Ar-H), 7.51 (d, 1H, Ar-H, J = 8.1 Hz), 7.27-7.08 (m, 5H, Ar-H), 6.93 (d, 2H, Ar-H, J = 8.4 Hz), 6.03 (dd, 1H, pyrazoline ring, J = 11.7, 5.3 Hz), 4.01 (dd, 1H, pyrazoline ring, J = 17.2, 11.7 Hz), 3.83 (s, 3H, OCH<sub>3</sub>), 3.15 (dd, 1H, pyrazoline ring, J = 17.6, 5.3 Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, δ ppm): 163.3, 161.5, 152.95, 152.91, 140.2, 133.5, 132.1, 129.3, 128.4, 128.1, 127.0, 125.9, 123.9, 122.04, 122.0, 121.0, 120.4, 114.4, 63.6, 55.6, 43.2, HRMS (ESI-MS) calc. for C<sub>23</sub>H<sub>18</sub>N<sub>3</sub>OSBr [M+H]<sup>+</sup> 464.0427; found 464.0430.

2-(5-(2-Chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzo[d]thiazole (8)

A bright yellow solid, yield 18.2%. Mp: 184-185 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, δ ppm): 7.70 (d, 2H, Ar-H, J = 8.8 Hz), 7.64 (d, 1H, Ar-H, J = 7.3 Hz), 7.51 (d, 1H, Ar-H, J = 8.1 Hz), 7.35-7.22 (m, 5H, Ar-H), 7.09 (t, 1H, J = 8.3 Hz), 6.94 (d, 2H, Ar-H, J = 8.8 Hz), 5.72 (dd, 1H, pyrazoline ring, J = 11.7, 5.1 Hz), 3.85 (s, 3H, OCH<sub>3</sub>), 3.24 (dd, 1H, pyrazoline ring, J = 17.2, 5.5 Hz), (one of the proton peak of the pyrazoline ring was under methoxy peak). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, δ ppm): 163.5, 161.5, 152.8, 152.6, 143.7, 134.9, 132.0, 130.4, 128.4, 128.2, 126.5, 125.9, 124.4, 123.9, 122.0, 121.0, 120.2, 114.4, 63.2, 55.6, 43.9, HRMS (ESI-MS) calc. for C<sub>23</sub>H<sub>18</sub>N<sub>3</sub>OSCl [M+H]<sup>+</sup> 420.0932; found 420.0935.

2-(5-(2,6-Dichlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzo[d]thiazole (9)

A white solid, yield 27 %. Mp: 286-287 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, δ ppm): 7.74 (d, 2H, Ar-H, J = 8.8 Hz), 7.60 (d, 1H, Ar-H, J = 7.7 Hz), 7.42 (d, 1H, Ar-H, J = 7.7 Hz), 7.26-7.14 (m, 4H, Ar-H), 7.04 (t, 1H, Ar-H, J = 7.5 Hz), 6.96 (d, 2H, Ar-H, J = 8.8 Hz), 6.40 (dd, 1H, pyrazoline ring, J = 20.5, 8.8 Hz), 3.86 (s, 3H, OCH<sub>3</sub>), 3.46 (dd, 1H, pyrazoline ring, J = 17.2, 8.8 Hz), (one of the proton peak of the pyrazoline ring was under methoxy peak). HRMS (ESI-MS) calc. for C<sub>23</sub>H<sub>17</sub>N<sub>3</sub>OSCl<sub>2</sub> [M+H]<sup>+</sup> 454.0542; found 454.0547.

#### 4.2. Acetylcholinesterase inhibitory assay

The effects of compounds on AChE enzyme were investigated. Two substrates which are 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) and acetylcholiniodate (AChI) were used in the inhibition tests. In addition, Tris / HCl (100 ml, 1M, pH=8) buffer was used in this method for AChE enzyme. The final volume was completed to 100 μL and changes in the absorption were followed at 412 nm. The IC<sub>50</sub> values of the compounds were calculated *via* activity (%) versus compounds concentration plots [33].

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