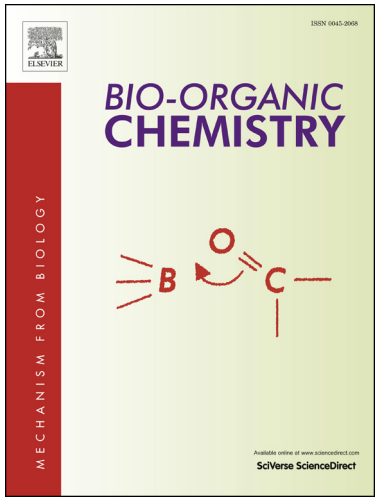

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Synthesis, Molecular Docking, Acetylcholinesterase and Butyrylcholinesterase Inhibitory Potential of Thiazole Analogs as New Inhibitors for Alzheimer Disease

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ABSTRACT

A series of thirty (**30**) thiazole analogs were prepared, characterized by ^{1}HNMR, ^{13}C NMR and EI-MS and evaluated for Acetylcholinesterase and butyrylcholinesterase inhibitory potential. All analogs exhibited varied butyrylcholinesterase inhibitory activity with IC_{50} value ranging between 1.59 ± 0.01-389.25 ± 1.75 μM when compared with the standard eserine (IC_{50}, 0.85±0.0001μM). Analogs **15, 7, 12, 9, 14, 1, 30** with IC_{50} values 1.59 ± 0.01, 1.77 ± 0.01, 6.21 ± 0.01, 7.56 ± 0.01, 8.46 ± 0.01, 14.81 ± 0.32 and 16.54 ± 0.21 μM respectively showed excellent inhibitory potential. Seven analogs **15, 20, 19, 24, 28, 30** and **25** exhibited good acetylcholinesterase inhibitory potential with IC50 values 21.3 ± 0.50, 35.3 ± 0.64, 36.6 ± 0.70, 44.81 ± 0.81, 46.36 ± 0.84, 48.2 ± 0.06 and 48.72 ± 0.91 μM respectively. All other analogs also exhibited well to moderate enzyme inhibition. The binding mode of these compounds was confirmed through molecular docking.

Keywords: Alzheimer's disease, Acetylcholinesterase inhibition, Butyrylcholinesterase Inhibition, Thiazole, Synthesis, Molecular docking

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1. Introduction

Alzheimer's disease is an irretrievable, complex neurodegenerative disorder characterized by progressive cognitive impairment, various neuropsychiatric and behavioral disturbances, and restrictions in activities of daily life. It is a pathologically complex disease implicating interactions between environmental and genetic risk factors [1]. Alzheimer's is an age related disease and is the most common reason of dementia in old people, being diagnosed after the age of 56, and affecting up to 10 % of the population over the age of 65. The disease affects 30% or more of the population over the age of 80. In the developed world, AD is the fourth major cause of death after cardiovascular disease, cancer, and cerebral accidents. Worldwide there are approximately 35 million peoples with AD, and that number is expected to grow to 107 million by 2050 [2]. Alzheimer disease was reported to be associated with cardiovascular risk factors such as hypertension and increased serum cholesterol [3]. It involves the degeneration of cholinergic neurons and loss of cholinergic transmission. Since AD is a multi-pathogenic illness, a current drug-discovery strategy is to develop novel anti-Alzheimer agents with multiple potencies such as inhibition of both acetylcholinesterase and butyrylcholinesterase [4]. Acetylcholine plays an important role in cognitive functions particularly in memory. It reduced cholinergic neurotransmission in the brain which have an important role in cognitive impairment associated with Alzheimer's disease [5, 6]. *BChE* (EC 3.1.1.8) is a sister enzyme of AChE [7]. Interest in *BChE* has been growing because of its possible role in Alzheimer disease and the introduction of anticholinesterase treatments for this disorder [8]. *BChE* enzymatic activity was higher in patients who had hypertension, hyperlipidemia, and high body weight and lower in patients who had suffered acute myocardial infarction or undergone treatment with beta blockers [9–11]. *BChE* inhibit the activity of the enzyme which affects the transmission of the neurotransmitter and makes patient discomfort including dizziness, blurred vision, vomiting, fever and even death [12, 13]. *BChE* specific inhibition is unlikely to be associated with adverse events and may show efficacy without remarkable side effects [14]. Therefore *BChE* may be considered as an important target for novel drug development to treat Alzheimer disease. In the future, the development of specific *BChE* inhibitors and the continued use of cholinesterase inhibitors may lead to improved clinical outcomes [15].

The heterocyclic compounds are reported to posses various biological activity [16]. Thiazole moiety is the key pharmacophore and intermediate for synthesizing pharmaceuticals and in the

field of agro-chemicals [17–19]. Thiazole are important class of heterocyclic compounds found in many potent biologically active molecules such as thiobendazol (anthelmintic drug) [20], riluzolr (anticonvulsant drug) [21] and talipexzol (antiparkinsonian drug) [22]. Its application also found in drug development for the treatment of inflammation [23]. Recently thiazole analogs have been also reported as antiglycating agent, anti-diabetic and as a potent inhibitor for cholinesterase [24-26].

Our research group is continuously doing an effort in search of lead molecules [27]. Herein we are going to report thiazole derivatives as new class of Acetylcholinesterase and butyrylcholinesterase inhibitors.

2. Results and discussion

2.1 Chemistry

Different acetophenone/benzaldehyde (1mmol) were reacted and refluxed with thiosemicarbazide (1mmol) in methanol in the presence of catalytic glacial acetic acid for 3-5 hours. After completion of reaction the mixture were filtered and washed with hexane to yield pure product. The obtained products (1mmol) were then reacted and refluxed with (1mmol) of methoxy or chloro substituted phenacyl bromide for 3-5 hours. After reaction completion the mixture was filter and wash with hexane to yield pure products (**1-30**) [23, 28, 29]. Different spectroscopic techniques, such as EI-MS, ^{1}H NMR and ^{13}C NMR were used to determine the structure of all analogs.

Insert Scheme-1 here

Insert Table-1 here

2.2. Acetyl cholinesterase and Butyrylcholinesterase inhibition

Analogs **1-30** showed acetylcholinesterase inhibition with IC_{50} values ranging between 21.3±0.08 to 452.1±0.27 μM as compared with standard eserine with IC_{50}, 0.04 ± 0.0001 μM. Seven analogs **15, 20, 19, 24, 28, 30** and **25** exhibited good acetylcholinesterase inhibitory potential with IC_{50} values 21.3 ± 0.50, 35.3 ± 0.64, 36.6 ± 0.70, 44.81 ± 0.81, 46.36 ± 0.84, 48.2 ± 0.06 and 48.72 ± 0.91 μM respectively. Analog **15** was found the most potent among the series. This compound has two hydroxyl groups on one phenyl ring and one chloro group on other

phenyl ring. The presence of these two hydroxyl group seems to be play an important role in this inhibition. The hydroxyl might be involved in hydrogen bonding. Analog **20** was found to be second active among the series. This compound illustrate activity have one methoxy group on one phenyl ring and also methoxy group on other phenyl part. Compound **19** have two chloro groups on one phenyl ring and one methoxy group on other phenyl ring. Analog **30** have two hydroxyl groups on one phenyl ring. Similarly other active analogs have either EWG or EDG on phenyl ring, whose position, nature and arrangement on phenyl ring greatly affect the inhibition.

Analogs **1-30** also showed a variable degree of butyrylcholinesterase inhibition with IC_{50}values ranging between 1.59 ± 0.01-389.25 ± 1.75 μM as compared with standard eserine (IC_{50},0.85 ± 0.0001 μM). Seven analogs **15, 7, 12, 9, 14, 1** and **30** exhibited potent butyrylcholinesterase inhibitory potential with IC_{50} values 1.59 ± 0.01, 1.77 ± 0.01, 6.21 ± 0.01, 7.56 ± 0.01, 8.46 ± 0.01, 14.81 ± 0.32 and 16.54 ± 0.21 μM respectively. Compound **15** was also found to be the most potent among the series. This compound has two hydroxyl groups on one phenyl ring and one chloro group on other phenyl ring. The presence of these two hydroxyl group seems to be play an important role in this inhibition. The hydroxyl group is might be involved in hydrogen bonding. Analog **7** was found to be second most active among the series. The compound have anthracene moiety and chlorinated phenyl ring. The activity is may be due to arene-interaction. Analog **12** have one hydroxyl group on one phenyl ring and one chloro group on other phenyl ring. Its potential is less than compound **15**; this might be due less number of hydroxyl groups. Analog **9** having secondary amine group on one phenyl part and chloro group on the other phenyl part. Analog **14** illustrate activity have one methoxy and one hydroxyl group on one phenyl ring and also chloro group on other phenyl part. Analogs **1** and **30** also showed good potential, but surprisingly in analog **1** on both phenyl parts electron withdrawing groups are present and in analog **30** on both phenyl parts electron donating groups are present. It means that those analogs which have electron donating or withdrawing groups on both the phenyl part show less potency as compared to those analogs which have electron donating group on one phenyl part and electron withdrawing group on the other phenyl part. All other analogs also showed well to moderate activities. All analogs except **3, 5** and **29** showed both acetylcholinesterase and butyrylcholinesterase inhibitory potential, which might be due to position of substituent on phenyl ring that not suitably interact with the active site of enzyme. The binding interaction of these analogs for butyrylcholinesterase inhibition was confirmed through molecular docking.

2.3. Molecular Docking

From the docking simulation and *in vitro* studies, it was observed that compound **15** (IC_{50} value 1.59 μM) is the most active analog among all the **30** compounds which included in this study. Compound **07** (IC_{50} value 1.77 µM) also the second ranked most active compound. These compounds were bound extremely into the binding cavity of butyrylcholinesterase enzyme making interactions with the active site residues. In active site, two most important residues of BChE (TYR128 and TYR332) are frequently involved in hydrogen bonding and play an important inhibitory role [30].

The docking results showed that all the compounds fit well in active site of butyrylcholinesterase. The top-ranked docking conformation of the most active compound (compound **15**) showed that the compound established five hydrogen bonds and two arene-arene interations with the active site residues (Figure-1).

The hydroxyl groups attached to the phenyl ring of the compound formed hydrogen bonds with Trp82, Gly115 Thr120 and Tyr128. One hydrogen bond between the amine moiety of the compound and His 438 was also observed. Furthermore, two arene-arene interactions between the compound and the active site residues (Trp82 and Tyr332) were also observed (Figure-1). The highest activity of compound **15** might be due to the presence of two hydroxyl groups attached to the same benzene ring. Both the hydroxyl groups are involved in bonding (backbone acceptor and side chain acceptor). The hydroxyl groups are well-built activating groups which polarize the molecule and facilitate it to make several interactions with other residues.

Insert Figure-1 here

The second most active compound (Compound **07**) in the series was observed to establish four interactions with important active site residues (**Figure-2**). As shown in Figure-2, **Thr284** interact with the NH group of the compound, which is directly attached to the Azomethine group, **Ser287** make interaction with the nitrogen atom of thiazole group and with the NH attach next to azomethine group and **Tyr332** establish π-interaction with the chloro-substituted benzene ring.

Insert Figure-2 here

The third most active compound (Compound **12)** in the series was observed in establishing two hydrogen bonds with the important active site residues, as shown in (**Figure-3**). Glu197 establish interaction with the NH next attach to the azomethine group and Leu286with *para*-hydroxyl moiety of the compound **12**.

The fourth, fifth and sixth most active compound (Compound **09**, Compound **14** and Compound **01**) in the series of the synthesized compounds was observed in making interactions with the active site residues of the BChE enzyme, Trp82 and His438 was found in phi-interaction with benzene ring of the compound (compound **09**) through arene-arene bond and His438 interact with thiazole ring through arene-cation bond. Trp82& Leu286 making interactions with thiazole moiety of the compound and Leu286 interact with *meta*-hydroxyl moiety attaches to the benzene ring of the compound (compound **14**).

Glu197 was found in interaction with compound **01** through hydrogen bonding as shown in (**Figure-3**).

Insert Figure-3 here

Compound **02**, **08**, **10** and compound **11** also exhibited weak potency against BChE enzyme.

Compound **3**, **4**, **5, 6**, **13** and **16-30** reflects no interaction between compounds and active site residues of BChE, which might be due to the inverted orientation, and these compounds also show least inhibitory activity (IC_{50} value) against the human BChE enzyme which contain much differences, if compared with the standard substrate (Eserine with IC_{50} value 0.85 ± 0.0001).Therefore, these compounds were not further evaluated for further study.

The structural difference between the most active compound **15** and the least active compounds are the *Meta* and *Para*-hydroxyl moiety of the compound **15**. This OH moiety in compound **15** may provide enzymatic potency against butyrylcholinesterase enzyme.

Docking results of compound **15** and **07** with BChE provided valuable information about the nature of the binding interactions that were delightfully associated with the experimental studies. This information could be utilized to design new leads against the BChE.

2.4. Computational Methods

The study was designed to dock thiazole derivatives against butyrylcholinesterase enzyme with the following communications; Intel$^{(R)}$ xenon$^{(R)}$ CPU E5620@2.40GHz system having 3.8GB RAM with the open 11.4 (X 86_64) operating platform. Protein-Ligand docking was carried out using the Molecular Operating Environment (MOE 2010.11) software package. Among thirty-one X-ray crystal structures of human BChE in the protein data bank [31, 32] (Pdb code **1P0P** with 2.30Å resolution) was selected as the target protein based on suitable resolution and co-crystallized ligand, BChE. The entire system (target protein) was energy minimized by MMFF94x force field [33], after adding the missing hydrogen atoms, 3D structures of all 30 synthesized compounds were drawn by molecule builder which is incorporated in MOE modeling package and the structures (Ligands) were subjected to MMFF94x for energy minimization.

Subsequently for the evaluation of potential energy, partial charges were calculated by MMFF94x force field [34]. Both prepared systems (protein and Ligand) were introduced for molecular docking simulation. Docking simulations were performed by using Triangle matcher placement method. A total of 30 conformations were generated for each Ligand protein complex with docking score. Each complex was analyzed for interactions and their 3D images were taken.

2.5. Butyrylcholinesterase Assay

Ellman et al spectrophotometric method was to measure the ChEs inhibitory assay [33]. For (AChE) and (BChE) inhibition the substrate (AChE) and (BChE) substrate were used respectively. 140μl sodium phosphate buffer (pH 8.0), 20 μl of either AChE/BChE solution and test sample of 20μl were incubated for 15 min at room temperature. The reaction was initiated with AChE/BChE 10μl with addition of DTNB respectively. For 15 min,AChE or BChE was hydrolyzing the reaction of DTNB with thiocoline, unconfined by the enzymatic hydrolysis of AChE and BChE. The 10% analytical grade ethanol were dissolve test samples and positive control of serine, tecrine and berberine. The percentage (%) inhibition was calculated from E-S/E ×100, where E&S are enzyme activities with and without test sample. Each sample was expressed

ChEs inhibitory activity in term of IC_{50} value (μg/ml) or μm essential to inhibit the hydrolysis of substrate.

2.6. Acetylcholinesterase Assay

The AChE inhibition activity was performed according to the method [33] with slight modifications. Total volume of the reaction mixture was 100 µL. It contained 60 µL $Na_2H\ PO_4$ buffer with concentration of 50 mM and pH 7.7. Ten µL test compound (0.5 mM $well^{-1}$) was added, followed by the addition of 10 µL (0.005 unit $well^{-1}$) enzyme. The contents were mixed and pre-read at 405 nm. Then contents were pre-incubated for 10 min at 37ºC. The reaction was initiated by the addition of 10 µL of 0.5 mM $well^{-1}$ substrate (acetylthiocholine iodide), followed by the addition of 10 µL DTNB (0.5 mM $well^{-1}$). After 15 min of incubation at 37ºC absorbance was measured at 405 nm using 96-well plate reader Synergy HT, Biotek, USA. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM $well^{-1}$) was used as a positive control. The percent inhibition was calculated by the help of following equation

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

IC_{50} values were calculated using EZ–Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA).

3. Conclusion

Thirty analogs were prepared and evaluate for acetylcholinesterase and butyrylcholinesterase inhibition. Seven analogs **15, 7, 12, 9, 14, 1, 30** with IC_{50} values 1.59±0.003 μM, 1.77 ±0.002μM, 6.21 ±0.005μM, 7.56 ±0.003μM, 8.46 ±0.003μM, 14.81 ±0.02μMand 16.54 ±0.11μM respectively showed excellent butyrylcholinesterase inhibitory potential comparable with the standard eserine (IC_{50}, 0.85±0.0001μM). Seven analogs **15, 20, 19, 24, 28, 30** and **25** also exhibited good acetylcholinesterase inhibitory potential with IC_{50} values 21.3±0.08, 35.3±0.04, 36.6±0.06, 44.81±0.05, 46.36±0.02, 48.2±0.06 and 48.72±0.09 μM respectively. All other analogs also exhibited well to moderate enzyme inhibition. The binding affinity was confirmed through molecular docking studies. All compounds were characterized through ^{1}HNMR, ^{13}C NMR and EI-MS.

4. Experimental Section

4.1. General methods

^{1}H NMR spectra were run on an Avance AV-300-400 MHz in d6-DMSO. Chemical shifts (δ) are reported as values in ppm in DMSO. TMS was used as an internal standard. Electron ionization (EI) mass spectra (MS) were recorded on Jeol JMS-600H. Reactions were checked by thin TLC on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany); spots were visualized by UV at 254 and 365nm.

4.2. General procedure for the synthesis of compounds (1–30)

Different acetophenone/benzaldehyde (1mmol) were reacted and refluxed with thiosemicarbazide (1mmol) in methanol in the presence of catalytic glacial acetic acid for 3-5 hours. After completion of reaction the mixture were filtered and washed with hexane to yield pure product. The obtained products (1mmol) was then reacted and refluxed with (1mmol) of methoxy or chloro substituted phenacyl bromide for 3-5 hours. After reaction completion the mixture was filter and wash with hexane to yield pure products (**1-30**) [26, 27]. Different spectroscopic techniques, such as EI-MS, ^{1}H NMR and ^{13}C NMR were used to determine the structure of all analogs.

4.3. Characterization of compounds

4.3.1. (E)-4-(4-chlorophenyl)-2-(2-(-(3-nitrophenyl)ethylidene)hydraiznyl)thiazole. ***(1)***

Yield: 33%; ^{1}HNMR: (DMSO-d_6, 300 MHz): δ 11.5 (s, 1H, NH), 8.5 (Hr.s, 1H, H-2), 8.2 (t,$J_{4/5,6}$ $_{6/4,5}$ = 8.4 Hz , 2H, H-4/6), 7.9 (d, $J_{3'',2''/5'',6''}$ = 8.4 Hz , 2H, H-3″/5″), 7.7 (t, $J_{5/4,6}$ = 7.6 Hz, 1H, H-5),7.4 (m, 3H, H-5′/2″/6″); IR (ATR): 3012, 1657, 1522, , 720, 695 cm^{-1}; ^{13}C NMR (75 MHz, DMSO-*d6*): 171.4, 154.4, 150.1, 147.6, 138.2, 135.0, 134.2, 131.0,129.5, 129.2, 129.2, 128.4, 128.4, 123.0, 120.6, 105.1, 31.1,; EI-MS: *m/z* (rel. int. %): 372 (M^+, 100), 223 (37), 209 (67), 174 (95), 168 (73).

*4.3.2. (E)-4-(4-chlorophenyl)-2-(2-(2-(4-nitrophenyl)ethylidene)hydrazinyl)thiazole.****(2)***

Yield: 50%; ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.6 (s, 1H, NH), 8.2 (d, $J_{3,2/5,6}$ = 8.7Hz, 2H, H-3/5), 8.1(s, 1H, HC=N), 8.4 (m, 4H,H-2,6/2″,6″), 8.1 (m, 3H, H-5'/2″/6″); IR (ATR): 3018, 1660, 1528, , 740, 691 cm^{-1}; ^{13}C NMR (75 MHz, DMSO-*d6*): 171.5, 154.4, 150.1, 144.6, 143.4, 134.1,

131.0, 129.1, 129.1, 128.8, 128.8, 128.7, 128.5, 123.6, 123.6, 105.0, 32.1; EI-MS: *m/z* (rel. int. %): 210 (M^+, 100), 168 (50), 358 (45), 372 (3), 236 (5).

4.3.3. (E)-2-(2-(1-(2-bromo-4-nitrophenyl)ethylidene)hydrazinyl)-4-(4-chlorophenyl) thiazole. ***(3)***

yield: 50%; Solid m.p. 262 °C; ^{1}H-NMR: (DMSO-*d6*, 300 MHz): δ 12.8 (s, 1H, NH), 8.2 (m, 2H, H-3/5), 8.1 (d, *J*3″,2″/5″,6″ = 6.3Hz , 2H, H-3″/5″), 8.0 (m, 1H, H-6), 8.1 (d, *J*2″,3″/6″,5″ = 8.7Hz , 2H, H-2″/6″), 7.5 (s, 1H, H-5′), 3.3 (s, 3H, Me); IR (ATR): 3030, 1654, 1540, , 680, 555 cm^{-1}; ^{13}C NMR (75 MHz, DMSO-*d6*): 171.4, 168.4, 152.2, 150.0, 141.5, 134.1, 132.2, 131.0, 129.1, 129.1, 128.6, 128.6, 126.5, 123.1, 122.4, 105.1, 16.2; EI-MS: *m/z* (rel. int. %): 449 (M+, 18), 221 (17), 149 (15), 137 (31), 83 (100); MS: as calculated 449.9553 and found 449.9545.

4.3.4. (E)-4-(4-chlorophenyl)-2-(2-(2,4-dichlorohenzylidene)hydrazinyl)thiazole. ***(4)***

Yield: 50%; ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 12.4 (s, 1H, NH), 8.3 (Hs, 1H, HC=N), 7.9 (m, 3H, H-3/3″/5″), 7.6 (s, 1H, H-5'), 7.5 (m, 3H, H-6/2"/6"); IR (ATR): 3030, 1654, 690, 560 cm^{-1}; ^{13}C NMR (75 MHz, DMSO-*d6*): 171.6, 150.0, 143.3, 134.2, 132.6, 131.0, 131.0, 129.1, 129.1129.0, 129.0, 128.7, 128.6, 128.2, 127.0, 105.1; EI-MS: *m/z* (rel. int. %): 381 (M^+, 15), 210 (100), 168 (36).

*4.3.5. (E)-4-(4-chlorophenyl)-2-(2-(2-nitrobenzylidene)hydrazinyl)thiazole.****(5)***

Yield: 50%; Solid m.p. 260 °C; ^{1}H-NMR: (DMSO-*d6*, 300 MHz): δ 12.5 (s, 1H, NH), 8.4 (s, 1H, HC=N), 8.0 (d, *J*3″, 2″/5″,6″ = 8.1 Hz , 2H, H-3″/5″), 8.0 (d, *J*3,4/6,5 = 8.4 Hz , 2H, H-3/6), 7.7 (t, *J4*/3,5 = 7.5 Hz, 1H, H-4), 7.6 (t, *J*5/4,6 = 7.2 Hz, 1H, H-5), 7.4 (m, 3H, H-5′/2″/6″); IR (ATR): 3020, 1674, 1560, , 695, 540 cm^{-1}; ^{13}C NMR (75 MHz, DMSO-*d6*): 171.4, 150.1, 147.6, 143.1, 134.6, 134.1, 131.7, 131.0, 130.2, 129.1, 129.1, 128.6, 128.6, 128.2, 124.1, 105.2; EI-MS: *m/z* (rel. int. %): 358 (M+, 54), 210 (100), 168 (91), 83 (68), 44 (56); MS: as calculated 358.0291 and found 358.0295.

4.3.6. (E)-4-(4-chlorophenyl)-2-(2-(1-(4-methoxyphenyl)ethylidene)hydrazinyl)thiazole .***(6)***

Yield: 81%; ^{1}H-NMR: (DMSO-d_6, 400 MHz): δ7.9 (m, 4H,H-2",3"/5″,6″), 7.5 (d,$J_{2,3/6,5}$ = 8.8 Hz , 2H, H-2/6), 7.4(s, 1H, H-5')7.0 (d,$J_{3,2/5,6}$ = 8.8 Hz , 2H, H-3/5); IR (ATR): 3035, 1658, 1240,

680, 555 cm^{-1}; ^{13}C NMR (75 MHz, DMSO-*d6*): 171.4, 168.6, 162.4, 150.1, 134.1, 131.0, 129.6, 129.1, 129.1, 128.8, 128.8, 128.6, 128.6, 114.2, 114.3, 105.1, 55.6, 17.0; EI-MS: *m/z* (rel. int. %): 357 (M^+,33), 342 (3), 171 (1), 148 (100), 134 (50).

*4.3.7. (E)-2-(2-(anthracen-9-ylmethylene)hydrazinyl)-4-(4-clorophenyl)thiazole.**(7)***

Yield: 81%; ^{1}H-NMR: (DMSO-d_6, 400 MHz): δ11.2 (s, 1H, NH), 9.4 (s, 1H, HC=N) 8.8 (d,$J_{3",2"/5",6"}$ = 8.8 Hz , 2H, H-3"/5"), 8.6 (s, 1H, H-5') 8.1 (d,$J_{2″,3″/6″,5″}$ = 8.4 Hz , 2H, H-2″/6″), 7.9 (d,$J_{2,3/10,9}$ = 8.8 Hz , 2H, H-2/10), 7.6 (m, 4H, H-3,4/9,8), 7.4 (d,$J_{5,4/7,8}$ = 8.4 Hz , 2H, H-5/7), 7.2 (s, 1H, H-6); IR (ATR): 3105, 1680, 690, 564 cm^{-1}; ^{13}C NMR (75 MHz, DMSO-*d6*): 171.6, 105.0, 128.7, 129.1, 134.2, 129.1, 150.1, 128.7, 131.0, 143.2, 128.0, 128.7, 128.0, 125.4, 125.4, 128.7, 131.6, 128.0, 123.7, 125.5, 128.7, 131.6, 126.0, 125.5; EI-MS: *m/z* (rel. int. %): 411 (M^+, 33), 237 (8), 210 (20), 202 (100), 176 (36).

*4.3.8. (E)-4-(4-chlorophenyl)-2-(2-(1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazinyl) thiazole.**(8)***

Yield: 43%; Solid m.p. 280 °C; ^{1}H-NMR: (DMSO-*d6*, 300 MHz): δ 7.9 (m, 4H, H-3″/5″/2/6), 7.4 (m, 4H, H-2″/6″/3/5), 7.2 (s, 1H, H-5′), 3.3 (s, 3H, Me), 3.0 (s, 10H, H-2‴/3‴/4‴/5‴/6‴); IR (ATR): 3095, 1680, 1630, 695, 560 cm^{-1}; ^{13}C NMR (75 MHz, DMSO-*d6*): 171.6, 168.7, 151.8, 150.1, 134.2, 131.0, 130.0, 130.0, 129.1, 129.1, 128.7, 128.7, 127.0, 111.8, 111.8, 105.1, 54.7, 54.7, 25.4, 25.4, 24.3, 17.1; EI-MS: *m/z* (rel. int. %): 410 (M+, 54), 375 (20), 299 (29), 210 (100), 168 (91), 83 (68), 44 (56); MS: as calculated 410.1332 and found 410.1327.

*4.3.9. (E)-4-((2-(4-(4-chlorophenyl)thiazol-2-yl)hydrazono)methyl)-N,N-dimethyl aniline.**(9)***

Yield: 38%; Solid m.p. 270 °C; ^{1}H-NMR: (DMSO-*d6*, 300 MHz): δ 8.0 (s, 1H, HC=N), 7.8 (d, *J*3″,2″/5″,6″ = 6.3 Hz , 2H, H-3″/5″), 7.6 (m, 2H, H-2/6), 7.4 (d, *J*2″,3″/6″,5″ = 6.6 Hz, 2H, H-2″/6″), 7.3 (d, *J*3,2 = 6.3 Hz , 1H, H-3), 7.2 (d, *J*5,6 = 6.3 Hz , 1H, H-5), 7.2 (s, 1H, H-5′), 3.0 (s, 6H, 2Me); IR (ATR): 3045, 2960, 1680, 1060, 685, 548 cm^{-1}; ^{13}C NMR (75 MHz, DMSO-*d6*): 171.5, 153.2, 150.1, 143.2, 134.2, 131.0, 129.1, 129.1, 128.8, 128.8, 128.1, 128.1, 123.1, 111.8, 111.8, 105.0, 41.2, 41.2; EI-MS: *m/z* (rel. int. %): 356 (M+, 3), 210 (100), 168 (39), 147 (46), 43 (87); MS: as calculated 356.0862 and found 356.0854.

*4.3.10. (E)-4(4-chlorophenyl)-2-(2-(naphthalen-2-yl-methylene)hydrazinyl)thiazole.**(10)***

Yield: 32%; Solid m.p. 276 °C; ^{1}H-NMR: (DMSO-*d6*, 300 MHz): δ 8.3 (s, 1H, HC=N, 8.0 (d, *J*3″, 2″/5″,6″ = 8.4 Hz , 2H, H-3″/5″), 7.9 (m, 5H, H-2/3/4/5/8), 7.5 (m, 2H, H-7/6), 7.4 (d, *J*2″,3″/6″,5″ = 8.4 Hz , 2H, H-2″/6″), 7.2 (s, 1H, H-5′); IR (ATR): 3115, 1680, 1640, 700, 568 cm^{-1}; ^{13}C NMR (75 MHz, DMSO*d6*): 171.6, 150.1, 143.2, 134.2, 136.0, 133.8, 131.0, 129.1, 128.6, 128.6, 128.4, 128.0, 127.9, 127.9, 127.1, 126.8, 126.1, 126.1, 105.1; EI-MS: *m/z* (rel. int. %): 363 (M+, 18), 210 (87), 168 (35), 153 (100), 127 (33); MS: as calculated 363.0597 and found 363.0590.

*4.3.11. (E)-2-(2-(4-(benzyloxy)benzylidene)hydrazinyl)-4-(4-chlorophenyl)thiazole.**(11)***

Yield: 81%; Solid m.p. 278 °C; ^{1}H-NMR: (DMSO-*d6*, 300 MHz): δ 8.0 (s, 1H, HC=N), 7.8 (d, *J*3″,2″/5″,6″ = 6.3 Hz , 2H, H-3″/5″), 7.6 (d, *J*2″,3″/6″,5″ = 6.3 Hz , 2H, H-2″/6″), 7.4 (d, *J*2,3/6,5 = 5.4 Hz , 2H, H-2/6), 7.3 (m, 5H, H-2‴/3‴/4‴′/5‴′/6‴′), 7.2 (s, 1H, H-5′), 7.0 (d, *J*3,2/5,6 = 6.6 Hz , 2H, H-3/5), 5.1 (s, 2H, OCH2); IR (ATR): 3025, 1665, 690, 570 cm^{-1}; ^{13}C NMR (75 MHz, DMSO-*d6*): 171.6, 161.2, 150.1, 143.2, 136.5, 134.2, 131.0, 130.1, 130.1, 129.1, 129.1, 128.7, 128.7, 128.7, 128.7, 127.5, 127.0, 127.0, 126.1, 114.2, 114.2, 105.1, 70.7; EI-MS: *m/z* (rel. int. %): 429 (M+, 17), 214 (53), 91 (100), 65 (22); MS: as calculated 419.0859 and found 419.0848.

*4.3.12. (E)-2,6-di-tert-butyl-4-((2-(4-(4-chlorophenyl)thiazol-2-yl)hydrazono)methyl) phenol.**(12)***

Yield: 100%; Solid m.p. 287 °C; 1H-NMR: (DMSO-*d6*, 300 MHz): δ 7.9 (s, 1H, HC=N), 7.7 (d, *J*3″,2″/5″,6″ = 6.3 Hz , 2H, H-3″/5″), 7.4 (s, 2H, H-2/6), 7.0 (s, 1H, H-5′), 6.9 (d, *J*2″,3″/6″,5″ = 6.6 Hz , 2H, H-2″/6″), 3.7 (s, 3H, OMe), 1.3 (s, 18H, 6Me); IR (ATR): 3025, 2972 1655, 1060, 680, 560 cm^{-1}; 13C NMR (75 MHz, DMSO-*d6*): 171.6, 150.1, 156.8, 143.2, 136.1, 136.1, 134.2, 131.0, 129.1, 129.1, 128.7, 128.7, 125.4, 123.6, 123.6, 105.1, 36.4, 36.1, 36.1, 36.1, 34.2, 34.2, 31.4, 31.4; EIMS: *m/z* (rel. int. %): 441 (M+, 18), 216 (35), 210 (100), 174 (37), 168 (59); MS: as calculated 441.1642 and found 441.1650.

*4.3.13. 1-((E(2-(4-(4-chlorophenyl)thiazol-2-yl)hydrazono)methyl)-3-((Z)-2-(4-(4-chloro phenyl)thiazol-2-yl)hydrazono)methyl)benzene**.(13)***

Yield: 85%; Solid m.p. 278 °C; ^{1}H-NMR: (DMSO-*d6*, 300 MHz): δ 12.2 (s, 2H, 2xNH), 8.0 (s, 2H, 2xHC=N), 7.8 (d, *J*3″,2″/5″,6″ = 8.4 Hz , 4H, 2xH-3″/5″), 7.6 (s, 2H, 2xH -5′), 7.4 (m, 8H,

H-2/3/5/6, 2xH-2″/6″); IR (ATR): 3030, 1650, 695, 580 cm^{-1}; ^{13}C NMR (75 MHz, DMSO-*d6*): 171.6, 171.6, 150.1, 150.1, 143.1, 143.1, 136.1, 136.1, 134.2, 134.2, 133.7, 133.7, 131.4, 131.3, 129.2, 129.2, 129.2, 129.2, 128.7, 128.7, 128.7, 128.7, 128.6, 128.2, 105.1, 105.1; EI-MS: *m/z* (rel. int. %): 549 (M+, 18), 338 (22), 210 (100), 168 (59), 82 (13); MS: as calculated 549.4973 and found 549.4964.

4.3.14. (E)-5-((2-(4-(4-chlorophenyl)thiazol-2-yl)hydrazono)methyl)-2-methoxyphenol. ***(14)***

Yield: 52%; Solid m.p. 281 °C; 1H-NMR: (DMSO-*d6*, 400 MHz): δ 8.1 (s, 1H, HC=N), 7.8 (d, *J*3″,2″/5″,6″ = 6.3 Hz , 2H, H-3″/5″), 7.4 (d, *J*2″,3″/6″,5″ = 6.3 Hz , 2H, H-2″/6″), 7.3 (m, 2H, H-2/5), 7.1 (dd, *J* 6,2 = 1.8, *J*6,5 = 6.3 Hz , 1H, H-6), 7.0 (s, 1H, H-5′); IR (ATR): 3040, 1670, 1290, 690, 575 cm^{-1}; 13C NMR (75 MHz, DMSO-*d6*): 171.6, 152.1, 150.1, 147.1, 143.1, 134.2, 131.0, 130.1, 129.1, 129.1, 128.7, 128.7, 122.7, 115.8, 112.2, 105.1, 56.0; EI-MS: *m/z* (rel. int. %): 359 (M+, 18), 210 (60), 101 (32), 59 (81), 43 (100); MS: as calculated 359.0495 and found 359.0486.

*4.3.15. (E)-4-((2-(4-(4-chlorophenyl)thiazol-2-yl)hydrazono)methyl)benzene-1,2-diol.****(15)***

Yield: 32%; ^{1}H-NMR: (DMSO-d_6, 400 MHz): δ 11.8 (s, 1H, NH), 7.8 (m, 3H, H-3"/5"/HC=N), 7.4 (d,$J_{2″,3″/6″,5″}$ = 8.4 Hz , 2H, H-2″/6″), 7.3 (s, 1H, H-5'), 7.1 (s 1HH-2) 6.8 (m, 1H, H-6), 6.7 (d, J_2 = 8 Hz , 1H, H-5); IR (ATR): 3460, 3030, 1650, 1270, 695, 570 cm^{-1}; 13C NMR (75 MHz, DMSO-*d6*): 171.6, 150.1, 149.5, 146.0, 143.2, 134.2, 131.2, 131.0, 129.1, 129.1, 128.7, 128.7, 123.1, 116.1, 117.2, 105.0; EI-MS: *m/z* (rel. int. %): 355 (M^+, 30), 206 (100), 191 (27), 164 (54), 149 (43).

4.3.16. (E)-4-(4-Methoxyphenyl)-2-(2-(1-(3-nitro phenyl) ethylidene) hydrazinyl)thaizole ***(16)***

Yield: 74%; Solid m.p. 290 °C; ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 8.5 (s, 1H, H-2), 8.2 (t, $J_{4/5,6}$ $_{6/5,4}$ = 7.8 Hz, 2H, H-4/6), 7.8 (d, $J_{2″,3″/6″,5″}$ = 8.7 Hz , 2H, H-2″/6″), 7.7 (t, $J_{5/6,4}$ = 8.1 Hz, 1H, H-5), 7.1 (s, 1H, H-5′), 6.9 (d, $J_{3″,2″/5″,6″}$ = 8.7 Hz , 2H, H-3″/5″), 3.7 (s, 3H, OMe), 2.4 (s, 3H, Me); IR (ATR): 3020, 1660, 1360, 1270, 690, 575 cm^{-1}; ^{13}C NMR (75 MHz, DMSO-d_6): 171.6, 168.6, 160.4, 150.1, 134.8, 134.2, 131.7, 131.7, 128.4, 128.4, 126.0, 126.4, 125.2, 114.7, 114.7,

105.1, 55.7, 17.0; EI-MS: *m/z* (rel. int. %): 368 (M_+, 100), 205 (77), 164 (93), 149 (26), 82 (46); MS: as calculated 368.4096 and found 368.4096.

4.3.17. (E)-4-(4-methoxyphenyl)-2-(2-(4-nitrobenzylidene)hydrazinyl)thiazole ***(17)***
Yield: 51%; ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 11.3 (s, 1H, NH), 8.3 (d, $J_{3,2\,/5,6}$ = 8.7 Hz, 2H, H-3/5), 8.2 (s, 1H, CH=N), 7.9 (d, $J_{2,3/6,5}$ = 8.7 Hz, 2H, H-2/6), 7.8 (d, $J_{2'',3''/6'',5''}$ = 9 Hz, 2H, H-2″/6″), 7.0 (s, 1H, H-5′), 6.9 (d, $J_{3'',2''/5'',6''}$ = 9 Hz , 2H, H-3″/5″), 3.8 (s, 3H, OMe); IR (ATR): 3025, 1670, 1340, 1280, 695, 575 cm^{-1}; 13C NMR (75 MHz, DMSO-*d6*): 171.6, 160.5, 150.1, 150.1, 143.2, 139.7, 125.1, 128.4, 128.4, 124.1, 124.1, 124.0, 124.0, 114.6, 114.6, 105.0, 55.6; EI-MS: *m/z* (rel. int. %): 354 (M^+, 100), 206 (93), 191 (24), 164 (75), 148 (34).

4.3.18. (E)-4-(4-methoxyphenyl)-2-(2-(3-nitrobenzylidene)hydrazinyl)thiazole ***(18)***
Yield: 28 %; ^{1}H-NMR: (DMSO-d_6, 300MHz): δ 11 (s, 1H, NH), 8.5 (d, $J_{2,6}$ = 1.8 Hz, 1H, H-2), 8.2 (s, 1H, CH=N), 8.2 (m, 1H, H-4), 8.1 (d, $J_{6\,,5}$ = 7.8 Hz , 1H, H-6), 7.8 (d, $J_{2'',3''/6'',5''}$ = 6.9 Hz, 2H, H-2″/6″), 7.7 (t, $J_{5/4,6}$ = 8.1 Hz, 1H, H-5), 7.0 (s, 1H, H-5′), 6.9 (d, $J_{3'',2''/5'',6''}$ = 9 Hz, 2H, H-3″/5″), 3.8 (s, 3H, OMe); IR (ATR): 3035, 1655, 1370, 1290, 690, 570 cm^{-1}; 13C NMR (75 MHz, DMSO-*d6*): 171.6, 160.5, 150.1, 148.0, 143.1, 134.3, 132.4, 129.6, 128.3, 128.3, 126.1, 121.5, 125.1, 114.7, 114.7, 105.0, 55.6; EI-MS: *m/z* (rel. int. %): 354 (M^+, 71), 206 (93), 191 (22), 164 (100), 149 (55).

4.3.19. (E)-2-(2-(2,4-dichlorobenzylidene)hydrazinyl)-4-(4-methoxyphenyl)thiazole ***(19)***
Yield: 34 %; ^{1}H-NMR: (DMSO-d_6, 300MHz): δ 8.3 (s, 1H, H-3), 7.9 (d, $J_{5,6}$ = 8.4Hz, 1H, H-5), 7.7 (d, $J_{2'',3''/6'',5''}$ = 8.7Hz, 2H, H-2″/6″), 7.6 (bs, 1H, CH=N), 7.5 (d, $J_{6,5}$ = 8.7Hz, 1H, H-6), 7.1 (s, 1H, H-5′), 6.9 (d, $J_{3'',2''/5'',6''}$ = 8.7Hz, 2H, H-3″/5″), 3.7 (s, 3H, OMe); IR (ATR): 3030, 1650, 1280, 710, 590 cm^{-1}; 13C NMR (75 MHz, DMSO-*d6*): 171.6, 160.5, 150.1, 143.2, 132.6, 131.0, 129.0, 129.0, 128.1, 128.3, 128.3, 127.0, 125.1, 114.6, 114.6, 105.0, 55.6; EI-MS: *m/z* (rel.int. %): 376 (M^+ 45), 341 (11), 205 (100), 163 (48), 148 (23).

4.3.20. (E)-4-(4-methoxyphenyl)-2-(2-(1-(4-methoxyphenyl)ethylidene)hydrazinyl)thiazole. ***(20)***
Yield: 65 %; ^{1}H-NMR: (DMSO-d_6, 300MHz): δ 7.8 (m, 4H, H-2/6/2″/6″), 7.1 (s, 1H, H-5′), 7.0 (m, 4H, H-3/5/3″/5″), 3.8 (s, 6H, OMe), 2.4 (s, 3H, Me); IR (ATR): 3040, 1680, 1265, 690, 580 cm^{-1}; 13C NMR (75 MHz, DMSO-*d6*): 171.6, 168.6, 162.7, 160.4, 150.1, 129.7, 128.6, 128.6,

128.3, 128.3, 125.1, 114.6, 114.6, 114.2, 114.2, 105.0, 55.6, 55.6, 17.0; EI-MS: *m/z* (rel.int. %): 211 (M^+, 29), 196 (42), 153 (73), 137 (100), 122 (67).

4.3.21. (E)-2-(2-(Anthracen-9-yl-methylene)hydrazinyl)-4-(4-methoxyphenyl) thiazole ***(21)***

Yield: 73%; Solid m.p. 277 °C; ^{1}H-NMR: (DMSO-*d6*, 300 MHz): δ 12.3 (s, 1H, NH), 9.2 (s, 1H, HC=N), 8.6 (m, 3H, H-2/6/10), 8.1 (d, *J*5,4/7,8 = 8.1 Hz , 2H, H-5/7), 7.8 (d, *J*2″,3″/6″,5″ = 8.4 Hz , 2H, H-2″/6″), 7.6 (m, 4H, H-3/4/8/9), 7.1 (s, 1H, H-5′), 6.9 (d, *J*3″,2″/5″,6″ = 8.7 Hz , 2H, H-3″/5″), 3.7 (s, 3H, OMe); IR (ATR): 3120, 1670, 1630, 1280, 695, 575 cm^{-1}; ^{13}C NMR (75 MHz, DMSO-*d6*): 171.6, 160.5, 150.1, 143.2, 131.7, 131.7, 128.7, 128.7, 128.7, 128.4, 128.4, 128.0, 128.0, 128.0, 128.0, 125.4, 125.4, 125.4, 125.4, 125.1, 123.8, 114.7, 114.7, 105.1, 55.7; EI-MS: *m/z* (rel. int. %): 409 (M+, 45), 203 (100), 191(21), 176 (26), 101 (15), 88 (25); MS: as calculated 409.5029 and found 409.5020.

4.3.22. (E)-4-((2-(4-(4-Methoxyphenyl)thiazol-2-yl)hydrazono)methyl)-N,N-dimethylaniline ***(22)***

Yield: 38%; Solid m.p. 264 °C; ^{1}H-NMR: (DMSO-*d6*, 300 MHz): *δ* 7.9 (s, 1H, HC=N), 7.7 (d, *J*2,3/6,5 = 8.7 Hz , 2H, H-2/6), 7.4 (d, *J*2″,3″/6″,5″ = 8.7 Hz , 2H, H-2″/6″), 7.0 (s, 1H, H-5′), 6.9 (d, *J*3,2/5,6 = 8.7 Hz , 2H, H-3/5), 6.7 (d, *J*3″,2″/5″,6″ = 9.6 Hz , 2H, H-3″/5″), 3.7 (s, 3H, OMe), 2.9 (s, 6H, 2Me); IR (ATR): 3020, 1680, 1320, 705, 585 cm^{-1}; ^{13}C NMR (75 MHz, DMSO-*d6*): 171.6, 160.5, 153.2, 150.1, 143.2, 128.4, 128.4, 128.1, 128.1, 125.2, 123.1, 114.7, 114.7, 111.7, 111.7, 105.1, 55.7, 41.0, 41.0; EI-MS: *m/z* (rel. int. %): 352 (M+, 100), 206(100), 191 (91), 164 (65), 147 (67); MS: as calculated 352.1358 and found 352.1350.

4.3.23. (E)-4-(4-Methoxyphenyl)-2-(2-(naphthalen-2-yl-methylene)hydrazinyl)thiazole ***(23)***

Yield: 32%; Solid m.p. 291 °C; ^{1}H-NMR: (DMSO-*d6*, 300 MHz): *δ* 12.2 (s, 1H, NH), 8.1 (s, 1H, HC=N), 8.0 (s, 1H, H-8), 7.9 (m, 4H, H-/3/4/5/8), 7.8 (d, *J*2″,3″/6″,5″ = 6.6 Hz , 2H, H-2″/6″), 7.5 (m, 2H, H-6/7), 7.1 (s, 1H, H-5′), 6.9 (d, *J*3″,2″/5″,6″ = 6.6 Hz , 2H, H-3″/5″), 3.7 (s, 3H, OMe); IR (ATR): 3105, 1670, 1270, 695, 570 cm^{-1}; EI-MS: *m/z* (rel. int. %): 359 (M+, 48), 206 (100), 191 (22), 164 (33), 153 (79); MS: as calculated 359.1092 and found 359.1099.

4.3.24. (E)-2(2-(4-(Benzyloxy)benzylidene)hydrainyl)-4-(4-methoxyphenyl)thiazole ***(24)***

Yield: 81%; Solid m.p. 310 °C; ^{1}H-NMR: (DMSO-*d6*, 300 MHz): δ 7.9 (s, 1H, HC=N), 8.0 (d, *J*2,3/6,5 = 6.6 Hz , 2H, H-2/6), 7.5 (d, *J*2‴,3‴/6‴,5‴ = 6.3 Hz , 2H, H-2‴/6‴), 7.4 (d, *J*2″,3″/6″,5″ = 5.4 Hz , 2H, H-2″/6″), 7.3 (m, 2H, H-3‴/5‴), 7.2 (t, *J*4‴/3‴,5‴ = 6 Hz, 1H, H-4‴), 7.1 (s, 1H, H-5′), 7.0 (d, *J*3,2/5,6 = 6.6 Hz , 2H, H-3/5), 6.9 (d, *J*3″,2″/5″,6″ = 6.3 Hz , 2H, H-3″/5″), 5.1 (s, 2H, OCH2), 3.7 (s, 3H, OMe); IR (ATR): 3040, 1660, 1270, 570, 520 cm^{-1}; ^{13}C NMR (75 MHz, DMSO-*d6*): 128.7, 127.5, 128.7, 127.0, 127.0, 136.5, 114.3, 161.2, 144.2, 70.5, 130.1, 126.0, 130.3, 143.1, 171.6, 128.4, 114.7, 105.1, 128.4, 114.7, 125.2, 150.1, 160.4, 55.6; EI-MS: *m/z* (rel. int. %): 415 (M+, 10), 206 (88), 191 (17), 149 (18), 91 (100); MS: as calculated 415.1354 and found 415.1349.

4.3.25.(E)-2,6-di-tert-butyl-4-((2-(4-(4-Methoxyphenyl)thiazol-2-yl)hdrazono)-methyl)phenol ***(25)***

Yield: 99%; Solid m.p. 282 °C; 1H-NMR: (DMSO-*d6*, 300 MHz): δ 7.9 (s, 1H, HC=N), 7.7 (d, *J*2″,3″/6″,5″ = 6.3 Hz , 2H, H-2″/6″), 7.4 (s, 2H, H-2/6), 7.0 (s, 1H, H-5′), 6.9 (d, *J*3″,2″/5″,6″ = 6.6 Hz , 2H, H-3″/5″), 3.7 (s, 3H, OMe), 1.3 (s, 18H, 6Me); IR (ATR): 3020, 2965, 1670, 1260, 670, 520 cm^{-1}; 13C NMR (75 MHz, DMSO-*d6*): 171.6, 160.5, 156.8, 150.1, 143.2, 136.1, 136.1, 128.4, 128.4, 125.2, 125.4, 123.6, 123.6, 114.7, 114.7, 105.1, 55.8, 34.3, 34.3, 31.4, 31.4, 31.4, 31.4, 31.4, 31.4; EI-MS: *m/z* (rel. int. %): 437 (M+, 22), 261 (32), 206 (100), 191 (30), 164 (61); MS: as calculated 437.2137 and found 437.2130.

4.3.26.1-((E)-(2-(4-(4-Methoxyphenyl)thiazol-2-yl)hydrazno)methyl)-3-((Z)-(2-(4-(4-methoxy phenyl)thiazol-2-yl)hydrazono)methyl)benze ***(26)***

Yield: 86%; Solid m.p. 279 °C; 1H-NMR: (DMSO-*d6*, 300 MHz): δ 11.5 (s, 2H, NH), 8.2 (s, 1H, H-2), 8.0 (s, 2H, 2xHC=N), 7.9 (s, 2H, 2xH-5′), 7.7 (d, *J*2″,3″/6″,5″ = 8.7 Hz, 4H, 2xH-2″/6″), 7.6 (d, *J*4,5/6,5 = 7.5 Hz , 2H, H-4/6), 7.5 (m, 1H, H-5), 6.9 (d, *J*3″,2″/5″,6″ = 8.7 Hz , 4H, 2xH-3″/5″), 3.7 (s, 6H, 2-OMe); IR (ATR): 3010, 2965, 1680, 1290, 675, 550 cm^{-1}; 13C NMR (75 MHz, DMSO-*d6*): 171.6, 171.6, 160.4, 160.4, 150.1, 150.1, 143.2, 143.2, 133.7, 133.7, 131.4, 131.1, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 125.2, 125.2, 124.8, 124.6, 114.6, 114.6, 105.1, 105.1, 55.7, 55.7; ESI/MS; calculated for and found EI-MS: *m/z* (rel. int. %): 540 (M+, 59), 309 (66), 206 (100), 191 (42), 149 (46), 128 (37); MS: as calculated 540.1402 and found 540.1410.

4.3.27. 5-(4-methoxyphenyl)-2-((E)-2-(4-((E)-(2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazono)methyl)benzylidene)hydrazinyl)thiazole ***(27)***

Yield: 85%; Solid m.p. 284 °C; ^{1}H-NMR: (DMSO-*d6*, 300 MHz): δ 11.5 (s, 1H, NH), 8.0 (s, 2H, 2xHC=N), 7.9 (s, 2H, 2xH-5′), 7.7 (d, *J*2″,3″/6″,5″ = 8.7 Hz , 4H, 2xH-2″/6″), 7.6 (d, *J* 2,3/6,5 = 7.5 Hz , 2H, H-2/6), 7.6 (d, *J*3,2/5,6 = 7.5 Hz , 2H, H-3/5), 6.9 (d, *J*3″,2″/5″,6″ = 8.7 Hz , 4H, 2xH-3″/5″), 3.7 (s, 6H, 2OMe); IR (ATR): 3010, 2970, 1654, 1260, 690, 570 cm^{-1}; ^{13}C NMR (75 MHz, DMSO-*d6*): 171.6, 160.4, 160.4, 150.1, 150.1, 143.2, 143.2, 133.7, 133.7, 131.2, 131.2, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 125.2, 125.2, 125.1, 125.1, 114.6, 114.6, 105.1, 105.1, 55.7, 55.7; EI-MS: *m/z* (rel. int. %): 540 (M+, 29), 390 (54), 206 (100), 191 (37), 149 (35); MS: as calculated 540.1402 and found 540.1415.

4.3.28. (E)-2-(2-(3,4-dimethoxybenzylidene)hydraziny)-4-(4-methoxyphenyl)thiazole ***(28)***

Yield: 76 %; ^{1}H-NMR: (DMSO-d_6, 400MHz): δ 12.0 (s, 1H, NH), 7.9 (s, 1H, CH=N), 7.7 (d, $J_{2'',3''/6'',5''}$ = 8.8 Hz, 2H, H-2″/6″), 7.2 (d, $J_{2,6}$ = 1.2Hz, 1H, H-2), 7.1 (d, $J_{6,2}$ = 8.4Hz, 1H, H-6), 7.1 (s, 1H, H-5′), 6.9 (m, 3H, H-3″/5″/3), 3.7 (s, 9H, OMe); IR (ATR): 3030, 2980, 1690, 1280, 695, 570 cm^{-1}; ^{13}C NMR (75 MHz, DMSO-*d6*): 171.5, 160.4, 152.0, 150.0, 149.6, 143.2, 130.4, 128.4, 128.4, 125.2, 122.6, 114.6, 114.6, 111.6, 109.1, 105.0, 55.7, 56.0, 56.0; EI-MS: *m/z* (rel.int. %): 206 (M^{+}, 59), 191 (68), 164 (43), 149 (60), 82 (100).

4.3.29. (E)-2-methoxy-5-((2-(4-(4-methoyphenyl)thiazol-2-yl)hydrazono)methyl)-phenol ***(29)***

Yield: 52%; Solid m.p. 270 °C; ^{1}H-NMR: (DMSO-*d6*, 300 MHz): *δ* 7.8 (s, 1H, HC=N), 7.7(d, *J*2″,3″/6″,5″ = 6.6 Hz , 2H, H-2″/6″), 7.2 (d, *J*2,6 = 0.9 Hz , 1H, H-2), 7.1 (s, 1H, H-5′), 6.9 (m, 4H, H-3″/5″/5/6), 3.7 (s, 6H, 2OMe); IR (ATR): 3450, 3020, 2970, 1670, 1290, 690, 575 cm^{-1}; ^{13}C NMR (75 MHz, DMSO-*d6*): 171.6, 160.4, 152.1, 150.0, 147.0, 143.1, 131.2, 128.2, 128.2, 125.1, 122.6, 115.8, 114.7, 114.7, 112.2, 105.1, 56.2, 55.7; EI-MS: *m/z* (rel. int. %): 355 (M+, 30), 206 (100), 191 (27), 164 (54), 149 (43); MS: as calculated 355.0991and found 355.0999.

4.3.30. (E)-4-((2-(4-(4-Methoxyphenyl)thiazol-2-yl)hydrazon)methy)benzone-1,2-diol ***(30)***

Yield: 86%; Solid m.p. 287 °C; 1H-NMR: (DMSO-*d6*, 300 MHz): *δ* 7.8 (s, 1H, HC=N), 7.7 (d, *J*5,6 = 6.6 Hz , 1H, H-5), 7.2 (d, *J*6,5 = 6.6 Hz , 1H, H-6), 7.1(d, *J*2,6 = 1.5 Hz , 1H, H-2), 7.0 (s, 1H, H-5′), 6.9 (d, *J*2″,3″/6″,5″ = 6.6 Hz , 2H, H-2″/6″), 6.7 (d, *J*3″,2″/5″,6″ = 6 Hz, 2H, H-3″/5″), 3.7 (s, 3H, OMe); IR (ATR): 3430, 3010, 2975, 1680, 1275, 670, 595 cm^{-1}; 13C NMR (75 MHz, DMSO-*d6*): 171.5, 150.1, 149.4, 146.0, 143.2, 133.1, 131.1, 129.1, 129.1, 128.6, 127.4,

127.4, 123.1, 117.2, 116.2, 105.1, 46.5; EI-MS: *m/z* (rel. int. %): 341 (M+, 30), 217 (100), 191 (27), 164 (54), 149 (43); MS: as calculated 341.0834 and found 341.0823.

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Captions

Scheme-1: Synthesis of thiazoleanalogs (1-30)

Table-1: Butyrylcholinesterase activity of thiazole derivatives

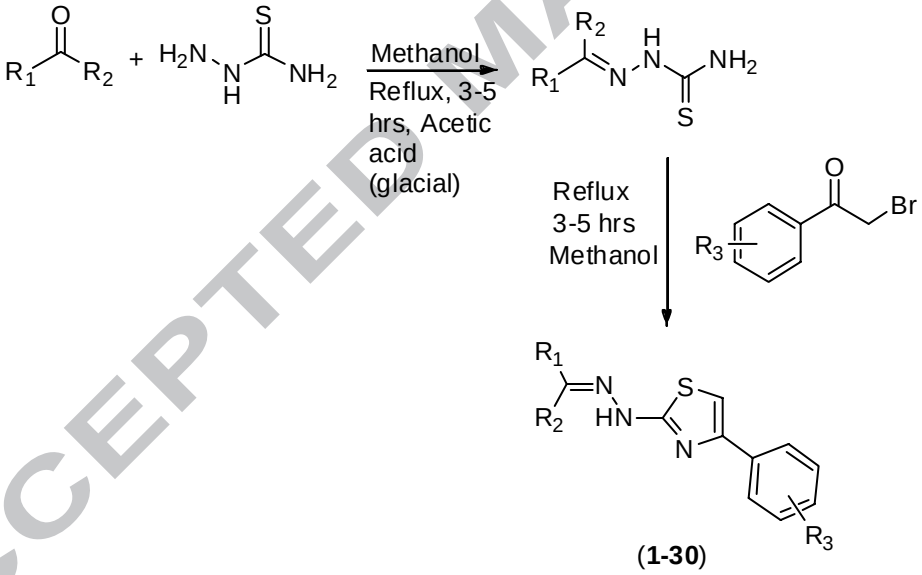
Table-2: Acetyl cholinesterase activity of thiazole derivatives

Figure-1: Putative binding mode of compound **15**

Figure-2: Putative binding mode of compound **07**

Figure-3: Putative binding mode of compound **12, 09, 14 and 01**

Scheme-1;



(**1-30**)

Figure-1:

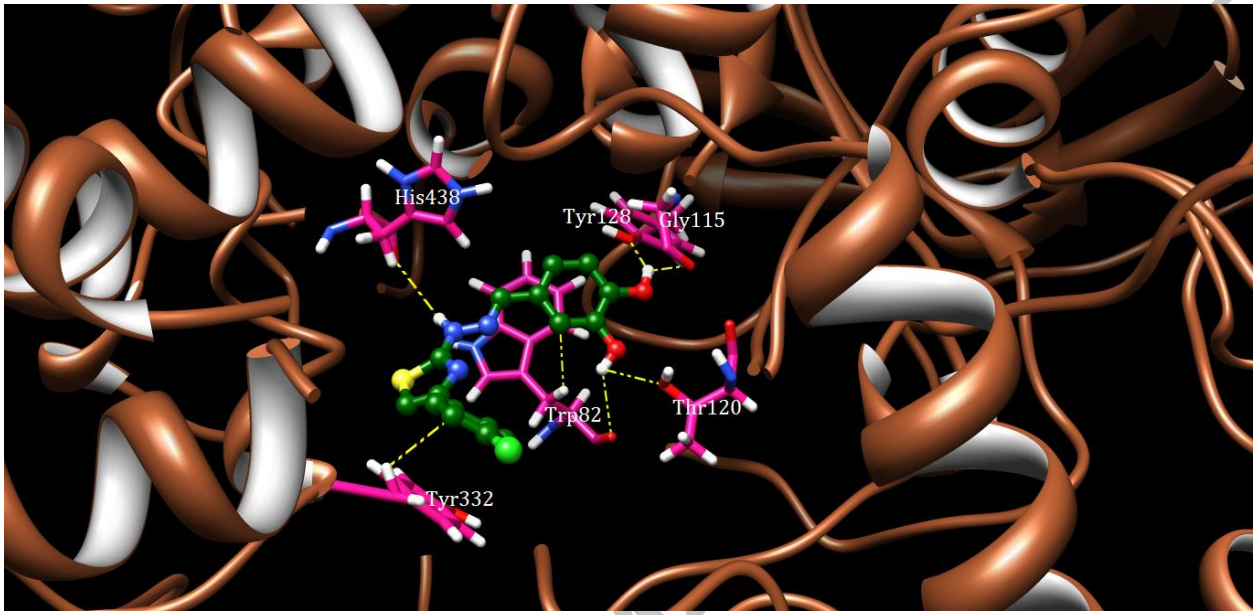


Figure -2:

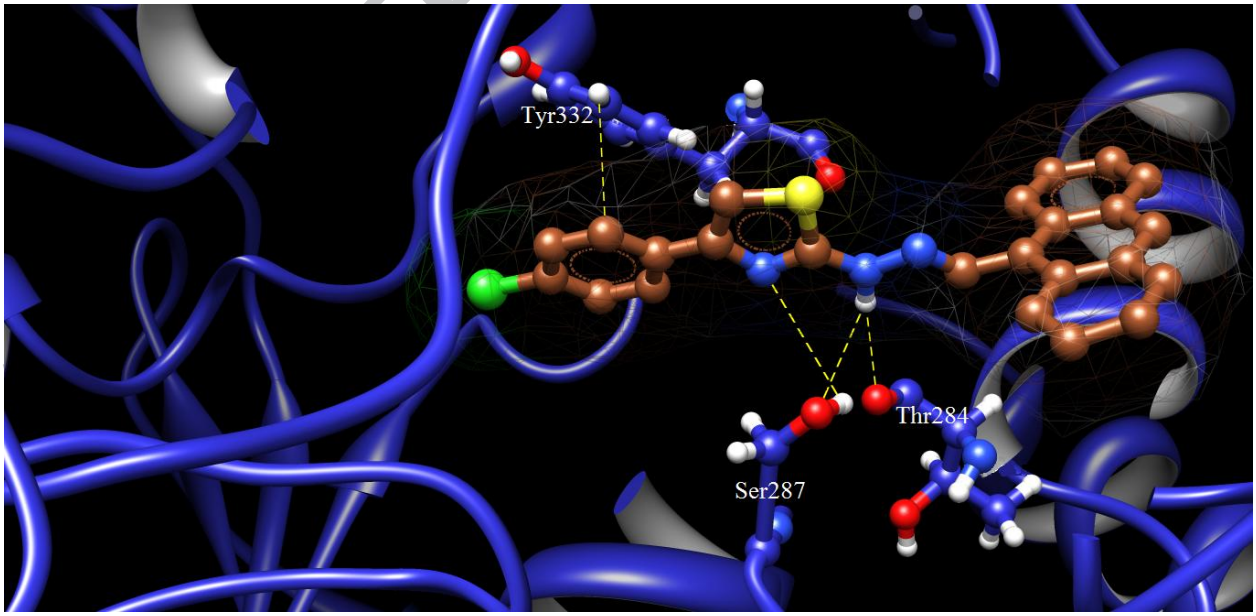


Figure -3:

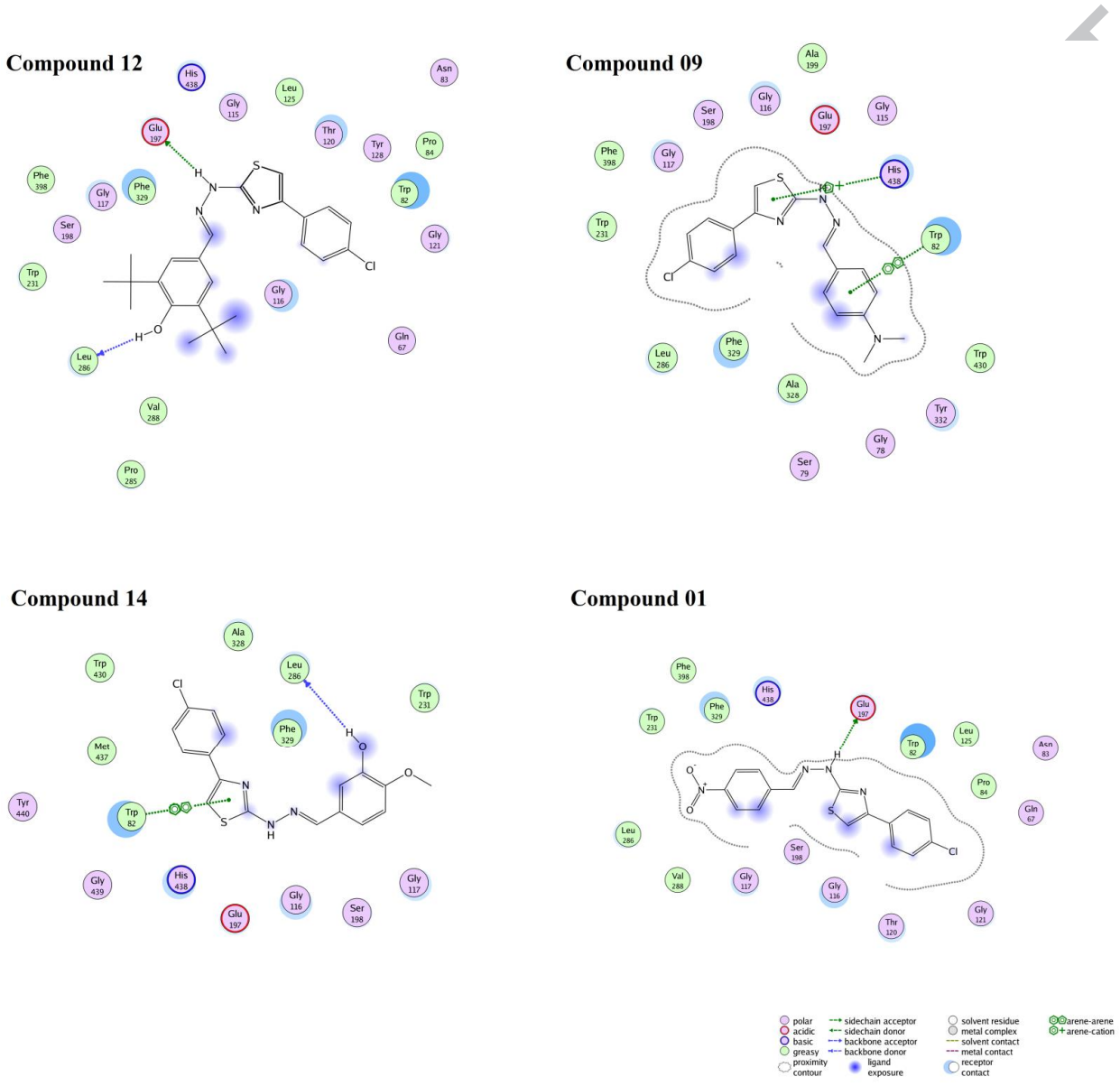
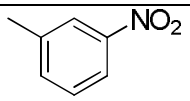
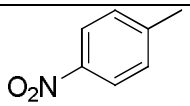
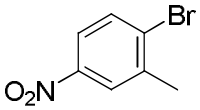
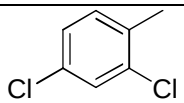
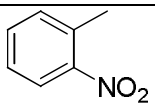
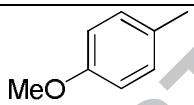
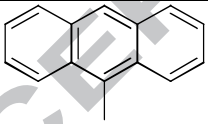
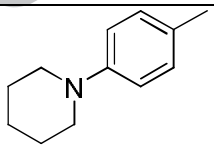
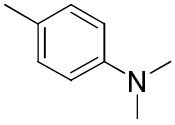
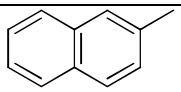
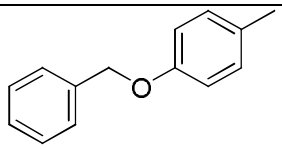
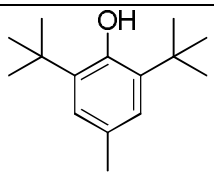
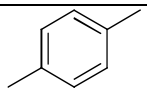
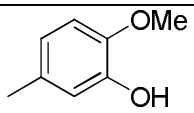
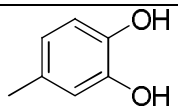
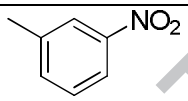
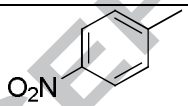
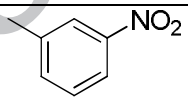
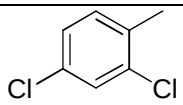
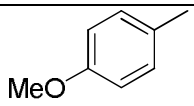
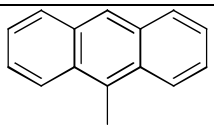
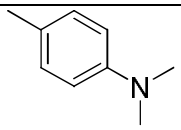
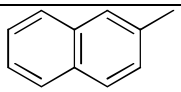
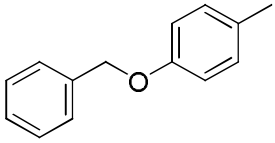
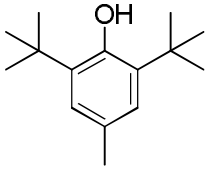
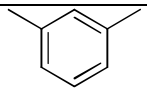
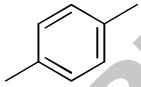
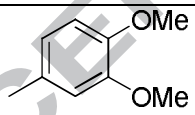
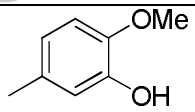
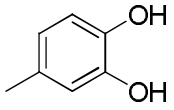

Compound 12
Compound 09
Compound 14
Compound 01
polar
acidic
basic
greasy
proximity contour
sidechain acceptor
sidechain donor
backbone acceptor
backbone donor
ligand exposure
solvent residue
metal complex
solvent contact
metal contact
receptor contact
arene-arene
arene-cation

Table-1:

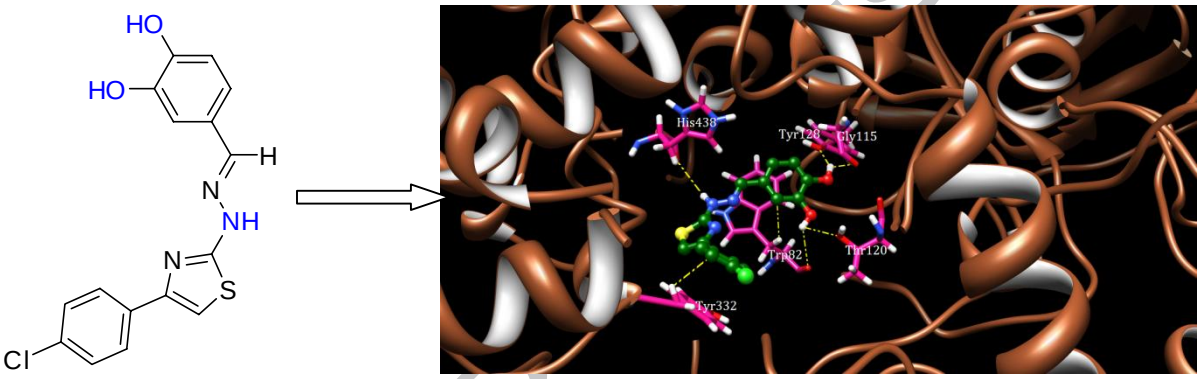
S.No	R_1	R_2	R_3	IC_{50} ± SEM[a] (μM) Buterycholinesterase	IC_{50} ± SEM[a] (μM) Acetylcholinesterase
1		CH_3	4-Cl	14.81 ± 0.32	344.8 ± 1.21
2		H	4-Cl	29.62 ± 0.40	306.5 ± 1.35
3		CH_3	4-Cl	35.56 ± 0.46	-
4		H	4-Cl	45.43 ± 0.45	452.1 ± 1 .27
5		H	4-Cl	37.18 ± 0.42	-
6		CH_3	4-Cl	112.73 ± 1.09	348.4 ± 1.36
7		H	4-Cl	1.77 ± 0.01	412.2 ± 1.22
8		CH_3	4-Cl	42.38 ± 0.47	159.3 ± 1.09
9		H	4-Cl	7.56 ± 0.01	355.2 ± 1.25

10		H	4-Cl	23.61 ± 0.24	321.4 ±1.23
11		H	4-Cl	30.28 ± 0.35	211.6 ± 1.12
12		H	4-Cl	6.21 ± 0.01	93.12 ± 1.05
13		H	4-Cl	113.18 ± 1.08	357.7 ± 1.63
14		H	4-Cl	8.46 ± 0.01	85.22 ± 1.21
15		H	4-Cl	1.59 ± 0.01	21.3 ± 0.50
16		CH_3	4-OMe	216.14 ± 1.53	379.9 ± 1.17
17		H	4-OMe	389.25 ± 1.75	376.8±0.31
18		H	4-OMe	318.92 ± 1.95	363.3 ± 1.16
19		H	4-OMe	263.97 ± 1 .84	36.6 ± 0.70
20		CH_3	4-OMe	389.24 ± 1.51	35.3 ± 0.64

21		H	4-OMe	87.25 ± 1.17	305.1 ± 1.25
22		H	4-OMe	151.59 ± 1.54	91.21 ± 1.23
23		H	4-OMe	49.17 ± 0.65	368.41 ± 0.81
24		H	4-OMe	161.85 ± 1.73	44.81 ± 0.84
25		H	4-OMe	96.57 ± 1.08	48.72 ± 1.09
26		H	4-OMe	99.24 ± 1.21	211.25 ± 1.25
27		H	4-OMe	219.93 ± 1.85	94.31 ± 1.17
28		H	4-OMe	144.37 ± 1.53	46.36 ± 1.02
29		H	4-OMe	99.85 ± 1.31	-
30		H	4-OMe	16.54 ± 0.21	48.2 ± 1.06

Eserine	-	-	-	0.85 ± 0.0001	0.04 ± 0.0001

Graphical



(Compound 15)

IC_{50} = 1.59 ± 0.003

Potent butyrylcholinesterase Inhibitor