Synthesis and Evaluation of A New Series of Thiazolyl-Pyrazoline Derivatives as Cholinesterase Inhibitors

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INTRODUCTION

Alzheimer's disease (AD), a progressive multifarious neurodegenerative disorder, is the leading cause of dementia in elderly people all over the world. The incidence of AD is predicted to increase dramatically in the future, as the average age of the population increases. Although extensive efforts have been devoted to the discovery of anti-AD drugs for almost a century, donepezil, galantamine, rivastigmine (cholinesterase inhibitors) and memantine (*N*-methyl-D-aspartate receptor antagonist) are the only drugs currently used for the management of AD. These agents only provide symptomatic relief but do not halt the progression of the disease.[1-5]

Development of new potent anti-AD drugs is a difficult and time-consuming process, and many molecules reaching clinical trials simply fail. Most phase II clinical trials ending with a positive outcome do not succeed in phase III, often due to serious adverse effects or lack of therapeutic efficacy.[6,7]

Acetylcholinesterase (AChE) is a highly viable target for the design and development of potent anti-AD agents due to its role in the pathogenesis of AD.[1-8] On the other hand, in progressed AD, the level of AChE in brain declines to 55-67% of normal values, whereas butyrylcholinesterase (BuChE) increases to 120% of normal levels, indicating that BuChE plays a pivotal role for acetylcholine (ACh) hydrolysis in the late stage of AD. As a result, selective inhibition of BuChE has emerged as a promising therapeutic approach to elevate ACh level in progressed AD.[9,10]

Thiazole has been recognized as a promising scaffold for the design and development for central nervous system (CNS) active agents. There are thiazole-based CNS drugs currently used as therapeutic agents for the treatment of various CNS disorders and a number of thiazole derivatives are in clinical trials.[11] Diverse modifications of the thiazole ring at various positions have led to a variety of thiazole-based CNS agents as AChE and BuChE inhibitors, secretase inhibitors, monoamine oxidase (MAO)

inhibitors, neuronal nitric oxide synthase (nNOS) inhibitors, ACh receptor ligands, adenosine receptor ligands, dopamine receptor ligands, serotonin receptor ligands, glutamate receptor ligands, ɣ-aminobutyric acid (GABA) receptor ligands, opioid receptor ligands, neuroprotective and anticonvulsant agents.[11-14] Acotiamide has been reported to be a promising thiazole-based agent for the treatment of functional dyspepsia in clinical trials. Acotiamide enhances ACh release in the enteric nervous system through AChE inhibition and M1/M2 muscarinic receptor antagonism.[15]

Pyrazoline has also attracted a great deal of interest as an indispensable scaffold due to its diverse therapeutic applications extending from CNS applications to antimicrobial therapy.[16,17] Diversely substituted pyrazolines embedded with a variety of functional groups have been reported to inhibit MAOs and cholinesterases, molecular targets important for the treatment of neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease.[16-23]

Prompted by the aforementioned findings and in the continuation of our ongoing research on thiazoles[24] and pyrazolines[25] as cholinesterase inhibitors, herein we designed a new series of thiazolyl-pyrazoline derivatives based on molecular hybridization of thiazole and pyrazoline scaffolds[26]. A facile and versatile synthetic route was used to prepare the title compounds and their inhibitory effects on AChE and BuChE were investigated. A computational study for the prediction of ADME properties of all compounds was also performed.

MATERIALS AND METHODS

Chemistry

All reagents were purchased from commercial suppliers and were used without further purification. Melting points (M.p.) were determined on an Electrothermal 9100 melting point apparatus (Weiss-Gallenkamp, Loughborough, UK). ^{1}H NMR spectra were recorded on a Bruker spectrometer (Bruker, Billerica, MA, USA). Mass spectra were recorded on an Agilent LC-MSD-Trap-SL Mass spectrometer (Agilent, Minnesota, USA). Elemental analyses (C, H, N) were performed on a Perkin Elmer EAL 240 elemental analyzer (Perkin-Elmer, Norwalk, CT, USA) and the results were within ±0.4% of the theoretical values. Thin Layer Chromatography (TLC) was performed on TLC Silica gel 60 F$_{254}$ aluminium sheets (Merck, Darmstadt, Germany) to check the purity of the compounds.

General procedure for the synthesis of the compounds

*1-(2-Furanyl)-3-(1,3-benzodioxol-5-yl)-2-propen-1-one (**1**)*

A mixture of 2-acetylfuran (0.06 mol), 1,3-benzodioxole-5-carboxaldehyde (0.06 mol) and 40% (w/v) sodium hydroxide (10 mL) in ethanol (30 mL) was stirred at room temperature for about 24 h. The resulting solid was washed, dried, and crystallized from ethanol.[27,28]

*3-(2-Furanyl)-5-(1,3-benzodioxol-5-yl)-1-thiocarbamoyl-4,5-dihydro-1H-pyrazole (**2**)*

A mixture of compound **1** (0.03 mol), thiosemicarbazide (0.036 mol) and sodium hydroxide (0.075 mol) was refluxed in ethanol (30 mL) for 12 h. The product was poured into crushed ice. The precipitate was filtered and crystallized from ethanol.[29]

*2-[5-(1,3-benzodioxol-5-yl)-3-(furan-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(aryl)thiazole (**3a-g**)*

A mixture of compound **2** (0.01 mol), substituted aryl acyl bromide (0.01 mol) in ethanol (20 mL) was refluxed for 10 h. After cooling, the precipitate was collected by suction filtration. The product was crystallized from ethanol.[29]

*2-[5-(1,3-Benzodioxol-5-yl)-3-(furan-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-(methylsulfonyl)phenyl)thiazole (**3a**)*

Yield: 76%; M.p. 178-179 °C.

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.99-3.00 (1H, m), 3.22 (3H, s), 3.92 (1H, dd, J= 17.36, 11.16 Hz), 5.57 (1H, dd, J= 11.52, 5.96 Hz), 5.93 (2H, s), 6.65 (1H, s), 6.87-6.95 (4H, m), 7.59 (1H, s), 7.86 (3H, d, J= 8.72 Hz), 7.94 (2H, d, J= 7.44 Hz).

Anal. calcd for $C_{24}H_{19}N_3O_5S_2$ (%): C, 58.40; H, 3.88; N, 8.51. Found (%): C, 58.45; H, 3.89; N, 8.58.

MS (ESI) *m/z*: 494 [M+H]$^+$.

*2-[5-(1,3-Benzodioxol-5-yl)-3-(furan-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-(trifluoromethyl)phenyl)thiazole (**3b**)*

Yield: 80%; M.p. 77-78 °C.

¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 3.12-3.13 (1H, m), 3.90 (1H, dd, *J*= 18.32, 12.20 Hz), 5.57 (1H, dd, *J*= 11.48, 5.96 Hz), 5.94 (2H, s), 6.65 (1H, s), 6.86-6.96 (4H, m), 7.55 (1H, s), 7.69 (2H, d, *J*= 8.12 Hz), 7.88-7.92 (3H, m).

Anal. calcd for $C_{24}H_{16}F_3N_3O_3S$ (%): C, 59.62; H, 3.34; N, 8.69. Found (%): C, 59.57; H, 3.39; N, 8.78.

MS (ESI) *m/z*: 484 [M+H]⁺.

2-[5-(1,3-Benzodioxol-5-yl)-3-(furan-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-fluorophenyl)thiazole (3c)

Yield: 81%; M.p. 89-90 °C.

¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 3.10-3.11 (1H, m), 3.89 (1H, dd, *J*= 17.76, 11.80 Hz), 5.55 (1H, dd, *J*= 11.52, 5.92 Hz), 5.94 (2H, s), 6.64-6.65 (1H, m), 6.85-6.94 (4H, m), 7.14-7.28 (3H, m), 7.71-7.75 (2H, m), 7.86-7.87 (1H, m).

Anal. calcd for $C_{23}H_{16}FN_3O_3S$ (%): C, 63.73; H, 3.72; N, 9.69. Found (%): C, 63.69; H, 3.77; N, 9.63.

MS (ESI) *m/z*: 434 [M+H]⁺.

2-[5-(1,3-Benzodioxol-5-yl)-3-(furan-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(2,6-difluorophenyl)thiazole (3d)

Yield: 78%; M.p. 137-138 °C.

¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 3.13-3.14 (1H, m), 3.93 (1H, dd, *J*= 17.73, 11.82 Hz), 5.58 (1H, dd, *J*= 11.70, 6.30 Hz), 5.98 (2H, s), 6.67-6.69 (1H, m), 6.88-6.98 (4H, m), 7.10-7.21 (2H, m), 7.26-7.34 (1H, m), 7.83-7.90 (2H, m).

Anal. calcd for $C_{23}H_{15}F_2N_3O_3S$ (%): C, 61.19; H, 3.35; N, 9.31. Found (%): C, 61.13; H, 3.42; N, 9.33.

MS (ESI) *m/z*: 452 [M+H]⁺.

2-[5-(1,3-Benzodioxol-5-yl)-3-(furan-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(2,5-dimethoxyphenyl)thiazole (3e)

Yield: 77%; M.p. 113-114 °C.

¹H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.03-3.04 (1H, m), 3.68 (3H, s), 3.76 (3H, m), 3.88 (1H, dd, $J=$ 17.84, 12.24 Hz), 5.50 (1H, dd, $J=$ 11.52, 7.08 Hz), 5.94 (2H, s), 6.64-6.65 (1H, m), 6.75-6.94 (5H, m), 7.07-7.19 (2H, m), 7.38-7.41 (1H, m), 7.87 (1H, s).

Anal. calcd for $C_{25}H_{21}N_3O_5S$ (%): C, 63.15; H, 4.45; N, 8.84. Found (%): C, 63.11; H, 4.42; N, 8.85.

MS (ESI) *m/z*: 476 [M+H]⁺.

2-[5-(1,3-Benzodioxol-5-yl)-3-(furan-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-[4-(morpholin-4-yl)phenyl]thiazole (3f)

Yield: 78%; M.p. 210-211 °C.

¹H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.01-3.02 (1H, m), 3.08 (4H, bs), 3.69 (4H, s), 3.87 (1H, dd, $J=$ 17.60, 12.72 Hz), 5.54 (1H, dd, $J=$ 12.44, 5.20 Hz), 5.93 (2H, s), 6.59-6.64 (3H, m), 6.80-6.94 (6H, m), 7.55 (1H, s), 7.86 (1H, s).

Anal. calcd for $C_{27}H_{24}N_4O_4S$ (%): C, 64.78; H, 4.83; N, 11.19. Found (%): C, 64.72; H, 4.88; N, 11.16.

MS (ESI) *m/z*: 501 [M+H]⁺.

2-[5-(1,3-Benzodioxol-5-yl)-3-(furan-2-yl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(naphthalen-2-yl)thiazole (3g)

Yield: 83%; M.p. 153-154 °C.

¹H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.09 (1H, dd, $J=$ 17.76, 2.72 Hz), 3.78 (1H, dd, $J=$ 17.96, 11.48 Hz), 5.80 (1H, dd, $J=$ 11.12, 2.68 Hz), 5.94 (2H, s), 6.59-6.87 (6H, m), 7.06 (1H, s), 7.19 (1H, s), 7.69 (1H, s), 7.81 (1H, s), 7.88 (1H, s), 7.95 (2H, d, $J=$ 8.80 Hz), 8.16-8.19 (1H, m)

Anal. calcd for $C_{27}H_{19}N_3O_3S$ (%): C, 69.66; H, 4.11; N, 9.03. Found (%): C, 69.75; H, 4.29; N, 9.06.

MS (ESI) *m/z*: 466 [M+H]⁺.

Determination of AChE and BuChE Inhibitory Activity

The inhibitory effects of compounds **3a-g** on AChE and BuChE were determined by Ellman's method[30] with minor modifications (Electric eel acetylcholinesterase enzyme was used instead of bovine acetylcholinesterase enzyme and buffer was added 2.4

mL instead of 3 mL).[31] The compounds were dissolved in DMSO and tested at final concentration range 5–80 μg/mL. 20 μL of enzyme (AChE or BuChE, 1 U/mL), 10 μL sample added to 2.4 mL buffer, the mixture was incubated at 37 °C for 15 min. After 15 min incubation, 50 μL of 0.01 M 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and 20 μL of 75 mM acetylthiocholine iodide (ATCI) or 25 mM butyrylthiocholine iodide (BTCI) were added, and the final mixture was incubated at room temperature for 30 min. Blank was prepared using 10 μL of DMSO instead of the test sample, with all other procedures similar to those used in the case of the sample mixture. Absorbances were measured at 412 nm and 37 °C using polystyrene cuvettes with spectrophotometer (UV-1700, Shimadzu). Experiment was done in triplicate. Galantamine was used as a positive control. Data are expressed as mean ± standard deviation (SD). The inhibition (percent) of AChE or BuChE was calculated using the following equation:

$$I\ (\%) = 100 - (OD_{sample} / OD_{control}) \times 100$$

In silico Prediction of ADME Parameters

The physicochemical parameters (log P, TPSA, nrotb, molecular weight, number of hydrogen bond donors and acceptors, molecular volume) of compounds **3a-g** were calculated using Molinspiration software.[32-35]

RESULTS AND DISCUSSION

The synthesis of thiazole derivatives (**3a-g**) followed the general pathway outlined in Scheme 1. The base-catalyzed Claisen-Schmidt condensation of 2-acetylfuran with 1,3-benzodioxole-5-carboxaldehyde gave 1-(2-furanyl)-3-(1,3-benzodioxol-5-yl)-2-propen-1-one (**1**), which underwent a subsequent cyclization reaction with thiosemicarbazide in the presence of sodium hydroxide affording 3-(2-furanyl)-5-(1,3-benzodioxol-5-yl)-1-thiocarbamoyl-4,5-dihydro-1H-pyrazole (**2**). Finally, new thiazolyl-pyrazoline derivatives (**3a-g**) were synthesized *via* the ring closure reaction of compound **2** with substituted aryl acyl bromides.
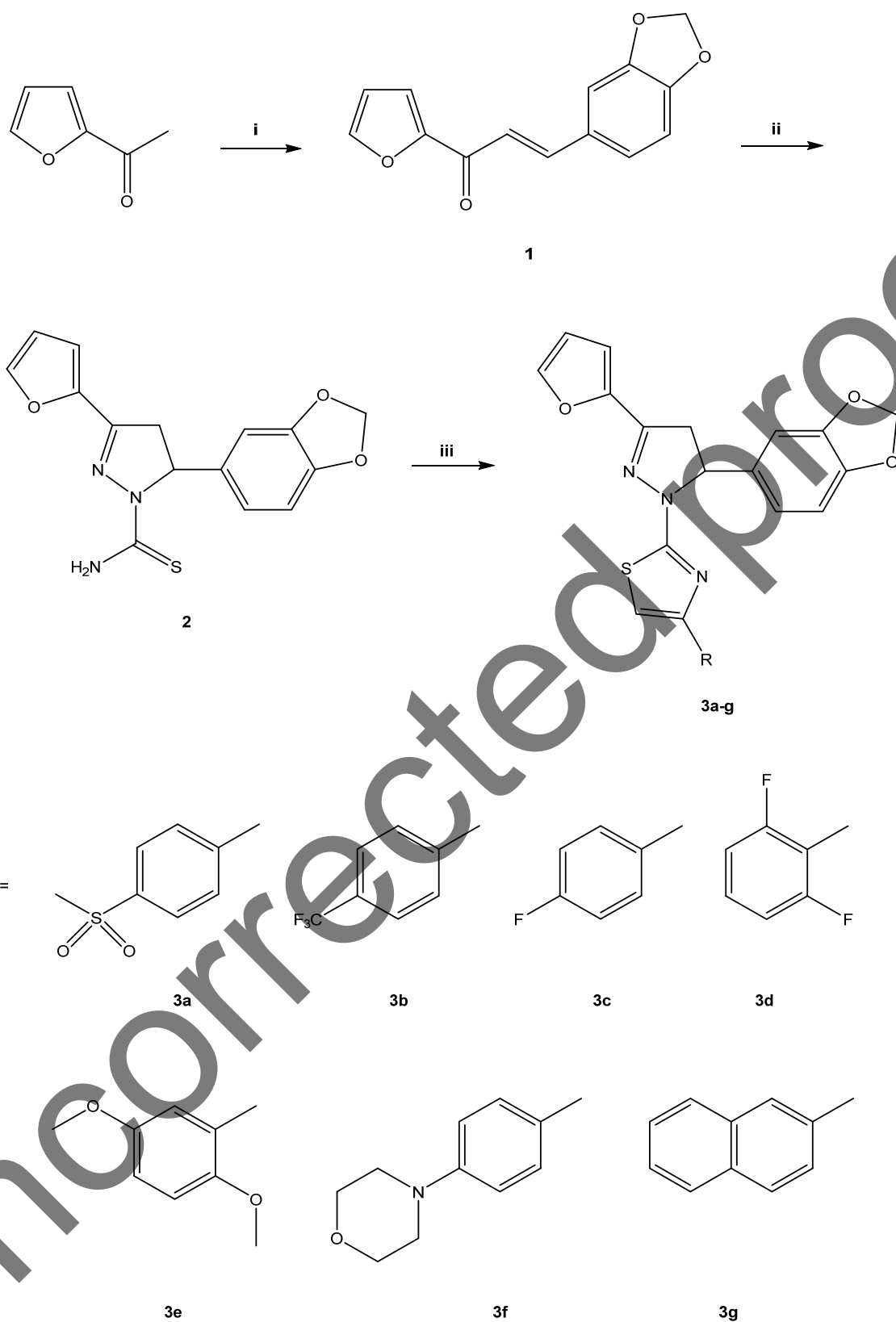
Scheme 1. The synthetic route for the preparation of compounds **3a-g**. Reagents and conditions: (i) 1,3-Benzodioxole-5-carboxaldehyde, 40% (w/v) NaOH, ethanol, rt, 24 h; (ii) thiosemicarbazide, NaOH, ethanol, reflux, 12 h; (iii) substituted aryl acyl bromide, ethanol, reflux, 10 h.

The inhibitory effects of compounds **3a-g** on AChE and BuChE were determined by a modification of Ellman's spectrophotometric method (Table 1). Galantamine was used as a positive control. According to the results, compounds **3a-g** showed less ChE inhibitory activity than galantamine.

Compounds **3a**, **3b**, **3e**, **3f** and **3g** showed less than 50% AChE inhibition at 80 µg/mL, whereas compounds **3c** and **3d** did not show any inhibitory activity against AChE. Compound **3g** was identified as the most potent AChE inhibitor (38.5±2.85%) in this series. This outcome indicated that naphthalene ring enhanced the inhibitory activity against AChE. The increased activity can be attributed to its high lipophilicity due to the presence of naphthalene moiety.

Compounds **3a**, **3f** and **3g** did not show any inhibitory activity against BuChE, whilst the other compounds showed BuChE inhibitory activity ranging from 43.02 to 21.44%. The most selective BuChE inhibitor was found as compound **3c** (43.02±2.71%) followed by compound **3d** (38.52±3.33%). This outcome pointed out the importance of fluoro substituent for BuChE inhibitory activity.

Table 1. The inhibitory effects of compounds **3a-g** on AChE and BuChE

Compound	Inhibition% (80 µg/mL)	
	AChE	**BuChE**
3a	8.60±1.29	---
3b	29.57±2.31	24.24±2.62
3c	---	43.02±2.71
3d	---	38.52±3.33
3e	25.62±3.23	21.44±2.85
3f	19.79±0.41	---
3g	38.5±2.85	---
Galantamine	(97.17±0.48)[a]	(80.98±0.22)[a]

---: No inhibition.

[a] Inhibition% at 16 µg/mL.

As a part of this study, Molinspiration software was used to determine their physicochemical parameters (log P, TPSA, nrotb, molecular weight, number of

hydrogen bond donors and acceptors, molecular volume) for the evaluation of the compliance of the compounds to Lipinski's rule of five.[32] This rule states that most "drug like" molecules have log P ≤ 5, molecular weight ≤ 500, number of hydrogen bond acceptors ≤ 10, and number of hydrogen bond donors ≤ 5. Compounds violating more than one of these rules may have bioavailability problems.[32-35] According to *in silico* studies, compound **3b**, **3c**, **3d**, **3e** and **3g** only violated one parameter of Lipinski's rule of five, whereas compound **3a** did not violate Lipinski's rule (Table 2). On the basis of Lipinski's rule of five, they were expected to have good oral bioavailability. On the other hand, compound **3f** violated two parameters of Lipinski's rule of five, and therefore it can be concluded that compound **3f** may have bioavailability problems.

Table 2. Pharmacokinetic parameters important for bioavailability of compounds **3a-g**

Compound	Molecular properties [a]							
	MW	logP	TPSA	nrotb	HBA	HBD	Volume	Violations
3a	493.57	4.00	94.24	5	8	0	397.15	0
3b	483.47	6.03	60.10	5	6	0	380.45	1
3c	433.46	5.30	60.10	4	6	0	354.08	1
3d	451.45	5.37	60.10	4	6	0	359.02	1
3e	475.53	5.17	78.57	6	8	0	400.25	1
3f	500.58	5.08	72.57	5	8	0	427.29	2
3g	465.53	6.32	60.10	4	6	0	393.15	1

[a] Molecular properties were calculated using Molinspiration software. MW: Molecular weight; logP: The logarithm of octanol/water partition coefficient, TPSA: Topological polar surface area; nrotb: Number of rotatable bonds, HBA: Number of hydrogen bond acceptors, HBD: Number of hydrogen bond donors.

CONCLUSION

In the current work, new thiazolyl-pyrazoline derivatives were synthesized and investigated for their *in vitro* inhibitory effects on AChE and BuChE. Moreover, the compliance of the compounds to the Lipinski's rule of five was evaluated. Naphthalene-substituted compound **3g** was the most potent AChE inhibitor (38.5±2.85%), whereas

fluoro-substituted compound **3c** was the most effective BuChE inhibitor (43.02±2.71%) in this series. In the view of this work, the structural modification of the identified compounds are on-going for the generation of new anticholinesterase agents with enhanced efficacy and selectivity.

Conflict of Interest: No conflict of interest was declared by the authors.

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