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Research Article

Synthesis, Docking and Acetylcholinesterase Inhibitory Evaluation of 1,3,4-Thiadiazole Derivatives as Potential Anti-Alzheimer Agents

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Abstract

Background: According to the cholinergic hypothesis for Alzheimer's disease, potentiation of cholinergic neurotransmission is one of the best strategies for combating dementia.

Methods: A new series of benzamide derivatives bearing 1,3,4-thiadiazole nucleus were synthesized and subsequently, their anticholinesterase activity was evaluated. Molecular docking was carried out to explore the likely binding mode and interactions.

Results: Fortunately, some of the tested compounds exhibited more activity than donepezil as a reference drug (IC_{50} = 0.6 ± 0.05 µM). Some of the evaluated derivatives displayed potency in the nanomolar range. Compound 7e with fluorine atom on the meta position of the phenyl ring was the most active compound in this series (IC_{50} = 1.82 ± 0.6 nM).

Conclusion: The 1,3,4-thiadiazole derivatives that were synthesized and tested in the current manuscript demonstrated remarkable anticholinesterase activity. Therefore, these compounds could be suggested as potential anti-Alzheimer agents.

Introduction

Alzheimer's disease (AD) is the most well-known kind of dementia and the sixth most common reason for death in the US according to the Alzheimer's Association.[1,2] This age-related disease gradually spreads all overhead the brain and induces progressive memory decline, gradual drop in day-to-day natural functions, progressive cognitive drop, and finally dementia.[3,4] Despite the worries of the World Health Organization (WHO) owing to the remarkable increase in the population of Alzheimer's cases, the lack of an efficacious anti-AD drug discovery strategy chiefly because of the multifactorial nature of the disease has become a universal concern matter.[5-8] Various hypotheses have been offered to explain the mechanism of AD based on the effective role of different factors regarding the pathogenesis of this disease. Among the proposed pathogenesis theory, the well-known cholinergic hypothesis has received a great deal of consideration from many researchers. Based on the "cholinergic hypothesis", the drop in the level of the neurotransmitter acetylcholine (ACh) in the synaptic gap by acetylcholinesterase (AChE) compared with normal conditions, makes AD.[9-12] Therefore, improvement of the cholinergic neurotransmission *via* cholinesterase inhibition to decrease neurodegeneration symptoms and restoration of memory impairments is a potential and valuable therapeutic method to delay or ameliorate the progression of AD.[13] At the moment, the FDA (Food and Drug Administration)-approved four AChE inhibitors (tacrine, donepezil, galanthamine, and rivastigmine) (Figure 1) can just slow the progress of the disease and assist in stabilizing the symptoms of dementia.[14-16] In the structure of AD-induced AChE, two active sites comprise the catalytic anionic site (CAS) and the peripheral anionic site (PAS). Inhibitors interaction with AChE occurs through these sites. Given the positive pharmacological options, donepezil is a well-renowned cholinesterase inhibitor with highly selective, reversible, centrally function, and AChE-dual connectivity that improves neuronal function by delaying the breakdown of acetylcholine released into synaptic clefts.[16] Therefore, many researchers focused on the design and synthesis of donepezil-like compounds with the most potent activity and lower side effects as potential anti-Alzheimer's agents. Based on molecular modeling studies, in the dual-

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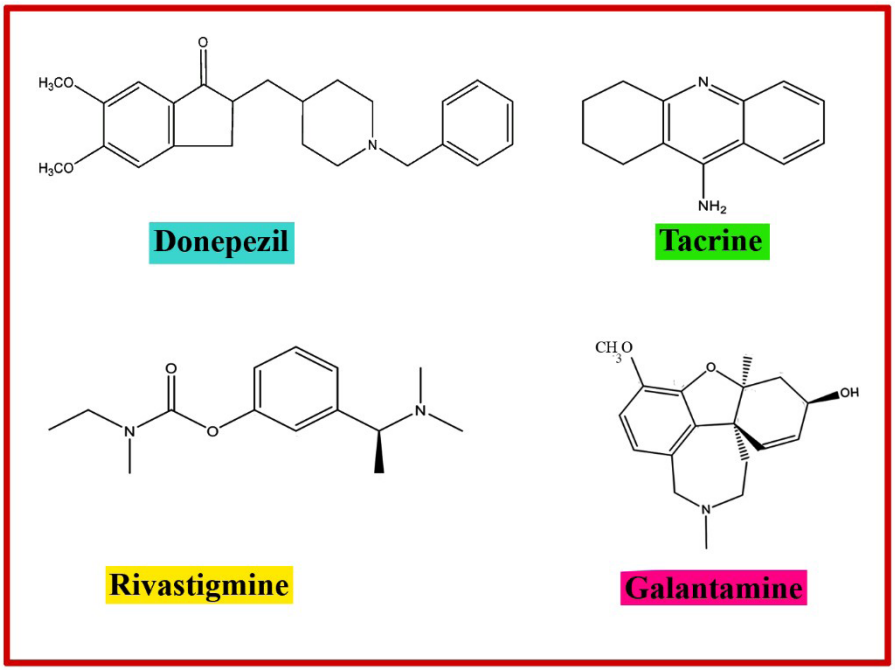

Figure 1. Chemical structures of FDA (Food and Drug Administration)-licensed AChE inhibitors for the symptomatic treatment of AD.

function of donepezil, the *N*-benzylpiperidine moiety of the molecule links with the CAS, while the indanone part binds to the PAS.[17] Also, Given that the anti-AD potential of compounds containing 1,3,4- thiadiazole and benzamide nucleus has been investigated and proven,[16] we were encouraged to focus on synthesis and design compounds bearing 1,3,4-thiadiazole ring and benzamide nuclei as prospect scaffolds. On the other hand, the presence of piperidine moiety in the designed compounds brings us closer to donepezil-like structures (Figure 2). Therefore, in the current work, we replaced the indanone ring of the donepezil bonded to *N*-benzylpiperidine with benzamide combined 1,3,4-thiadiazole nuclei and evaluated their anti-acetylcholinesterase ability using Ellman's spectrophotometric test. The resulting biological assessment along with molecular docking shows that these obtained new hybrids have potential inhibitory activity.

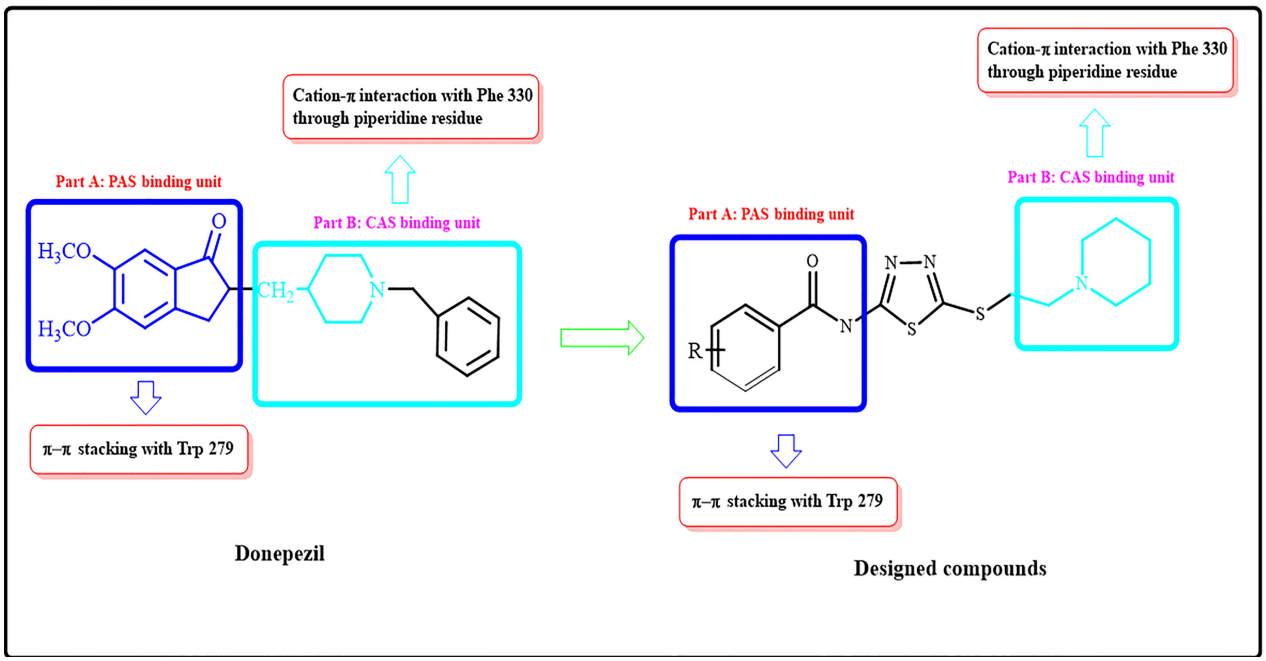

Figure 2. The pharmacophore of donepezil and designed compounds. The requisite parts have been considered in the structure of target compounds.

Methods

General Considerations

Commercial chemicals and solvents were all purchased from common commercial suppliers and utilized without additional purification. All experiments were performed in glassware that was thoroughly washed and dried in an oven with essential precautions. To dry products and remove solvents under reduced pressure, a rotary vacuum evaporator was used. ^{1}H (250 MHz) NMR spectra were run on a Bruker spectrophotometer in deuterated chloroform solvent using TMS as an internal standard. The Chemical bonding information of the synthesized compounds is extracted by recorded IR spectra on a Shimadzu 470 spectrophotometer in the range 600-4000 cm^{-1}. A TSQ-70 spectrometer (Finigan, USA) at 70 eV was used for recording mass spectra.

General Experimental Procedure for the Synthesis of N-(5-((2-(piperidin-1-yl)ethyl)thio)-1,3,4-thiadiazol-2-yl)benzamide derivatives (7a-7l)

The whole steps of the synthesis of *N*-(5-((2-(piperidin-1-yl)ethyl)thio)-1,3,4-thiadiazol-2-yl)benzamide derivatives **(7a-7l)** and demanded precursors to achieve these target compounds are illustrated in Figure 3. The structure of all the synthesized compounds was identified by ^{1}H-NMR, ^{13}C-NMR, mass spectroscopy, IR, and elemental analysis.

Synthesis of intermediate 3 (Route I)

As indicated in Figure 3, in a typical procedure to the synthesis of intermediate 3, molar ratios 1:1:2 of 5-amino-1,3,4-thiadiazole-2-thiol **(A)**, Phthalic anhydride **(B)/,** triethylamine (Et_3N) in 20 mL of toluene solvent were mixed in a 50 mL flat-bottom glass flask. Then, the resulting mixture was refluxed for 24 h, and the reaction progress to detect the completion was checked by TLC. After completion of the reaction, toluene was evaporated using rotary evaporator apparatus, and DI water was added to the remaining solid, which was extracted with ethyl acetate. Then, the extracted organic layer was washed several times by brine. The organic layer dried over anhydrous Na_2SO_4, filtered off, and corresponding intermediates in a high yield obtained after solvent removal.

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 7.81-7.92 (m, 4H, phthalimide), 11.56 (brs, 1H). IR (KBr, cm^{-1}): 3053 (stretch,

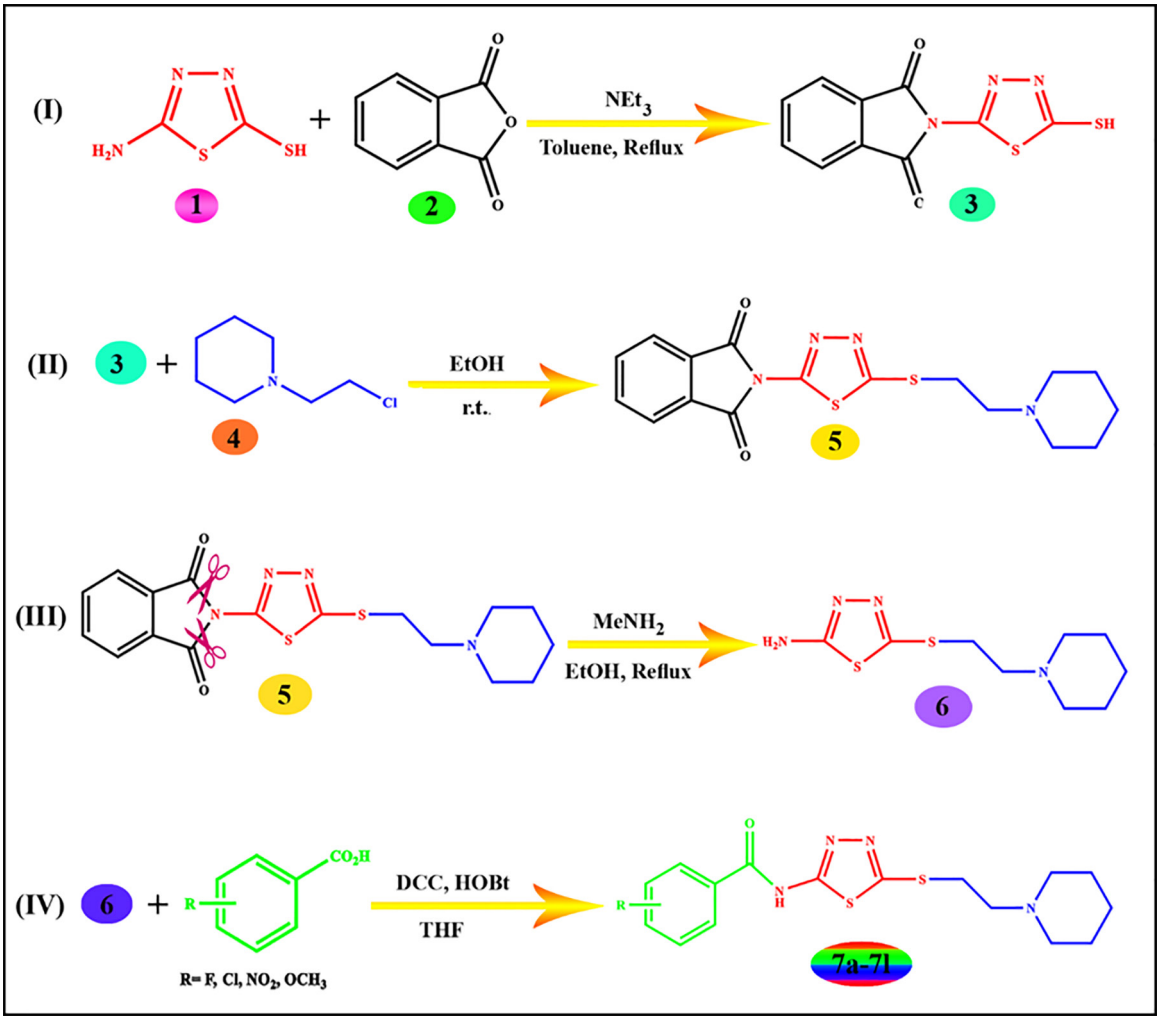

Figure 3. Total protocol for the synthesis of N-(5-((2-(piperidin-1-yl)ethyl)thio)-1,3,4-thiadiazol-2-yl)benzamide derivatives (7a-7l).

C-H, aromatic), 2976, 2856 (stretch, C-H, aliphatic), 1732 (stretch, C=O). MS (m/z, %): 263 (M^+, 100), 190 (20), 158 (90), 104 (25), 76 (40).

Synthesis of intermediate 5 (Route II)

To synthesize the intermediate compound 5, first, molar ratios 1:1 of 1-(2-chloroethyl)piperidinium chloride: triethylamine (Et_3N) were weighed and mixed into a flat-bottom flask containing 20 mL of CH_3CN solvent. Then, the reaction container was stirred at room temperature overnight with continuous stirring. After this time, the reaction solvent was evaporated using a rotary apparatus, and the residue was extracted with ethyl acetate and DI water. Then, the extracted organic layer was washed several times by brine. The organic layer dried over Na_2SO_4, filtered off, and the target product in a good yield obtained after solvent removal and used in the next step **(II)**. Then, equimolar ratios 1:1 of 1-(2-chloroethyl)piperidine and compound **3** were mixed into a flat-bottom flask containing 20 mL of EtOH solvent. Then, the reaction medium was stirred at room temperature overnight. After detecting the reaction completion by TLC, the reaction solvent was evaporated utilizing rotary under vacuum conditions, and the residue was extracted with ethyl acetate and DI water. Then, The extracted organic layer was washed several times with brine. The organic layer dried over Na_2SO_4, filtered off, and targeted intermediate **(5)** in a good yield obtained after solvent removal.

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 1.42 (m, 2H, H_4-piperidine), 2.38 (m, 4H, $H_{3,5}$-piperidine), 2.58 (t, 2H, -S-CH_2-C$\underline{H}_2$-N-), 3.02 (m, 4H, $H_{2,6}$-piperidine), 3.25 (t, 2H, -S-C$\underline{H}_2$-CH_2-N-), 7.86-7.92 (m, 4H, phthalimide). IR (KBr, cm^{-1}): 3066 (stretch, C-H, aromatic), 2929, 2854 (stretch, C-H, aliphatic), , 1716 (stretch, C=O). MS (m/z, %): 374 (M^+).

Synthesis of intermediate 6 (Route III)

In a typical procedure for the synthesis of intermediate 6, molar ratios 1:10 of intermediate 5 and methylamine were mixed into a flat-bottom flask containing 20 mL of EtOH solvent, and the reaction mixture was then refluxed for 24 h. After detecting the reaction completion by TLC, the reaction solvent was evaporated using a rotary. The obtained solid was washed using diethyl ether (Et_2O) to eliminate impurities.

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 1.43 (m, 2H, H_4-piperidine), 2.47 (m, 4H, $H_{3,5}$-piperidine), 2.61 (t, 2H, -S-CH_2-C$\underline{H}_2$-N-), 3.22 (m, 4H, $H_{2,6}$-piperidine), 3.24 (t, 2H, -S-C$\underline{H}_2$-CH_2-N-), 3.68 (brs, NH). IR (KBr, cm^{-1}): 3402 (stretch, NH), 3057 (stretch, C-H, aromatic), 2924, 2854 (stretch, C-H, aliphatic). MS (m/z, %): 244 (M^+).

Synthesis of final products (Route IV)

As clearly illustrated in Figure 3, various derivatives of the *N*-(5-((2-(piperidin-1-yl)ethyl)thio)-1,3,4-thiadiazol-2-yl)benzamide **(7a-7l)** were synthesized according to our previously reported similar methods.[18,19] In a typical procedure to synthesize these new analogs, equimolar amounts of the different derivatives of benzoic acid, dicyclohexylcarbodiimide (DCC), and hydroxybenzotriazole (HOBt), and compound 6 were poured together into a flat-bottom flask containing 20 mL of THF solvent. Then, the resulting mixture was stirred for 60 min in the ice bath. After this time, the resulting mixture was stirred again at room temperature for 24 hours. After making sure the reaction was completed using TLC, the reaction mixture was filtered off and the THF solvent under reduced pressure using a rotary evaporator apparatus was removed from the filtrate. The residue was diluted by ethyl acetate/water (20/20 mL). The organic phase was washed two times using brine and, the extracted organic layer dried over Na_2SO_4. The mixture was filtrated. The ethyl acetate was separated using a rotary apparatus and then the obtained solid was washed using diethyl ether (Et_2O) to eliminate impurities.

2-Fluoro-N-(5-((2-(piperidin-1-yl)ethyl)thio)-1,3,4-thiadiazol-2-yl)benzamide (7a)

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 1.19-1.27 (m, 2H, H_4-piperidine), 1.73 (brs, 4H, $H_{3,5}$-piperidine), 3.35 (brs, 4H, $H_{2,6}$-piperidine), 3.94 (t, 2H, -S-CH_2-C$\underline{H}_2$-N-), 4.23 (t, 2H, -S-C$\underline{H}_2$-CH_2-N-), 7.24 (dd, 1H, H_3-2-fluorophenyl), 7.47 (m, 1H, H_5-2-fluorophenyl), 7.68 (m, 1H, H_4-2-fluorophenyl), 7.82 (t, 1H, J = 7.75 Hz, H_6-2-fluorophenyl), 13.00 (brs, NH). IR (KBr, cm^{-1}): 3327 (stretch, NH), 3028 (stretch, C-H, aromatic), 2929, 2852 (stretch, C-H, aliphatic), 1720 (stretch, C=O, amide).

3-Fluoro-N-(5-((2-(piperidin-1-yl)ethyl)thio)-1,3,4-thiadiazol-2-yl)benzamide (7b)

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 1.03-1.24 (m, 2H, H_4-piperidine), 1.71 (brs, 4H, $H_{3,5}$-piperidine), 3.33 (t, 2H, -S-CH_2-C$\underline{H}_2$-N-), 3.70 (brs, 4H, $H_{2,6}$-piperidine), 4.23 (t, 2H, -S-C$\underline{H}_2$-CH_2-N-), 7.33-7.93 (m, 4H, 3-fluorophenyl). IR (KBr, cm^{-1}): 3327 (stretch, NH), 3064 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1720 (stretch, C=O, amide). MS (m/z, %): 366 (M^+, weak), 139 (15), 123 (15), 95 (15), 71 (100), 43 (30).

4-Fluoro-N-(5-((2-(piperidin-1-yl)ethyl)thio)-1,3,4-thiadiazol-2-yl)benzamide (7c)

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 1.03-1.24 (m, 2H, H_4-piperidine), 1.72 (brs, 4H, $H_{3,5}$-piperidine), 3.31 (t, 2H, -S-CH_2-C$\underline{H}_2$-N-), 3.70 (brs, 4H, $H_{2,6}$-piperidine), 4.10 (t, 2H, -S-C$\underline{H}_2$-CH_2-N-), 7.26 (dd, 2H, J = 17.25, 8.25 Hz, $H_{2,6}$-4-fluorophenyl), 8.07 (dd, 2H, J = 17.25, 8.25 Hz, $H_{3,5}$-4-fluorophenyl), 13.25 (brs, NH). IR (KBr, cm^{-1}): 3327 (stretch, NH), 3064 (stretch, C-H, aromatic), 2929, 2852 (stretch, C-H, aliphatic), 1716 (stretch, C=O, amide).

2-Chloro-N-(5-((2-(piperidin-1-yl)ethyl)thio)-1,3,4-thiadiazol-2-yl)benzamide (7d)

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 1.11-1.17 (m, 2H, H_4-piperidine), 1.72 (brs, 4H, $H_{3,5}$-piperidine), 3.02

(t, 2H, -S-CH_2-C$\underline{H}_2$-N-), 3.48 (brs, 4H, $H_{2,6}$-piperidine), 4.26 (t, 2H, -S-C$\underline{H}_2$-CH_2-N-), 7.21-7.39 (m, 2H, $H_{3,5}$-2-chlorophenyl), 7.55 (t, 1H, H_4-2-chlorophenyl), 7.79 (d, 1H, H_6-2-chlorophenyl). IR (KBr, cm^{-1}): 3331 (stretch, NH), 3061 (stretch, C-H, aromatic), 2927, 2854 (stretch, C-H, aliphatic), 1718 (stretch, C=O, amide).

3-Chloro-N-(5-((2-(piperidin-1-yl)ethyl)thio)-1,3,4-thiadiazol-2-yl)benzamide (7e)

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 1.17-1.27 (m, 2H, H_4-piperidine), 1.71 (brs, 4H, $H_{3,5}$-piperidine), 3.31 (brs, 4H, $H_{2,6}$-piperidine), 3.91 (t, 2H, -S-CH_2-C$\underline{H}_2$-N-), 4.23 (t, 2H, -S-C$\underline{H}_2$-CH_2-N-), 7.46 (t, 1H, *J* = 8 Hz, H_5-3-chlorophenyl), 7.65 (d, 1H, *J* = 8 Hz, H_3-3-chlorophenyl), 7.85 (s, 1H, H_2-3-chlorophenyl), 7.91 (d, 1H, *J* = 8 Hz, H_6-3-chlorophenyl). IR (KBr, cm^{-1}): 3323 (stretch, NH), 3066 (stretch, C-H, aromatic), 2929, 2854 (stretch, C-H, aliphatic), 1716 (stretch, C=O, amide). MS (m/z, %): 382 (M^+, weak), 158 (20), 156 (60), 141 (35), 139 (80), 111 (40), 71 (100), 41 (35).

4-Chloro-N-(5-((2-(piperidin-1-yl)ethyl)thio)-1,3,4-thiadiazol-2-yl)benzamide (7f)

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 1.10-1.24 (m, 2H, H_4-piperidine), 1.72 (brs, 4H, $H_{3,5}$-piperidine), 3.06 (t, 2H, -S-CH_2-C$\underline{H}_2$-N-), 3.92 (brs, 4H, $H_{2,6}$-piperidine), 4.23 (t, 2H, -S-C$\underline{H}_2$-CH_2-N-), 7.50 (d, 2H, *J* = 7 Hz, $H_{2,6}$-4-chlorophenyl), 7.90 (d, 2H, *J* = 7 Hz, $H_{3,5}$-4-chlorophenyl). IR (KBr, cm^{-1}): 3325 (stretch, NH), 3032 (stretch, C-H, aromatic), 2929, 2850 (stretch, C-H, aliphatic), 1712 (stretch, C=O, amide).

2-Nitro-N-(5-((2-(piperidin-1-yl)ethyl)thio)-1,3,4-thiadiazol-2-yl)benzamide (7g)

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 0.98-1.23 (m, 2H, H_4-piperidine), 1.70 (brs, 4H, $H_{3,5}$-piperidine), 3.05 (t, 2H, -S-CH_2-C$\underline{H}_2$-N-), 3.68 (brs, 4H, $H_{2,6}$-piperidine), 3.92 (t, 2H, -S-C$\underline{H}_2$-CH_2-N-), 7.36 (t, 1H, *J* = 7.25 Hz, H_5-2-nitrophenyl), 7.46 (t, 1H, *J* = 7.25 Hz, H_4-2-nitrophenyl), 7.71 (d, 1H, *J* = 7.25 Hz, H_6-2-nitrophenyl), 7.92 (d, 1H, *J* = 7.25 Hz, H_3-2-nitrophenyl). IR (KBr, cm^{-1}): 3327 (stretch, NH), 3034, 3061 (stretch, C-H, aromatic), 2929, 2850 (stretch, C-H, aliphatic), 1720 (stretch, C=O, amide).

3-Nitro-N-(5-((2-(piperidin-1-yl)ethyl)thio)-1,3,4-thiadiazol-2-yl)benzamide (7h)

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 1.17-1.23 (m, 2H, H_4-piperidine), 1.72 (brs, 4H, $H_{3,5}$-piperidine), 3.07 (brs, 4H, $H_{2,6}$-piperidine), 3.94 (t, 2H, -S-CH_2-C$\underline{H}_2$-N-), 4.23 (t, 2H, -S-C$\underline{H}_2$-CH_2-N-), 7.39 (t, 1H, H_5-2-nitrophenyl), 7.57 (d, 1H, H_4-2-nitrophenyl), 7.84 (d, 1H, H_4-2-nitrophenyl), 8.58 (s, 1H, H_2-2-nitrophenyl). IR (KBr, cm^{-1}): 3327 (stretch, NH), 3034 (stretch, C-H, aromatic), 2929, 2850 (stretch, C-H, aliphatic), 1716 (stretch, C=O, amide).

4-Nitro-N-(5-((2-(piperidin-1-yl)ethyl)thio)-1,3,4-thiadiazol-2-yl)benzamide (7i)

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 0.99-1.21 (m, 2H, H_4-piperidine), 1.71 (brs, 4H, $H_{3,5}$-piperidine), 3.10 (t, 2H, -S-CH_2-C$\underline{H}_2$-N-), 3.68 (brs, 4H, $H_{2,6}$-piperidine), 3.92 (t, 2H, -S-C$\underline{H}_2$-CH_2-N-), 8.07 (d, 2H, $H_{2,6}$-4-nitrophenyl), 8.21 (d, 2H, $H_{3,5}$-4-nitrophenyl). IR (KBr, cm^{-1}): 3327 (stretch, NH), 3034 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1625 (stretch, C=O, amide). MS (m/z, %): 393 (M^+, weak), 224 (30), 161 (35), 143 (20), 120 (20), 98 (85), 76 (30), 56 (100).

2-Methoxy-N-(5-((2-(piperidin-1-yl)ethyl)thio)-1,3,4-thiadiazol-2-yl)benzamide (7j)

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 0.99-1.20 (m, 2H, H_4-piperidine), 1.72 (brs, 4H, $H_{3,5}$-piperidine), 3.94 (t, 2H, -S-CH_2-C$\underline{H}_2$-N-), 3.33 (brs, 4H, $H_{2,6}$-piperidine), 3.82 (s, 3H, -OCH_3), 4.23 (t, 2H, -S-C$\underline{H}_2$-CH_2-N-), 6.99 (t, 1H, H_5-2-methoxyphenyl), 7.08 (d, 1H, H_3-2-methoxyphenyl), 7.51 (t, 1H, H_4-2-methoxyphenyl), 7.62 (d, 1H, H_3-2-methoxyphenyl), 12.60 (brs, NH). IR (KBr, cm^{-1}): 3325 (stretch, NH), 3066 (stretch, C-H, aromatic), 2929, 2856 (stretch, C-H, aliphatic), 1720 (stretch, C=O, amide).

3-Methoxy-N-(5-((2-(piperidin-1-yl)ethyl)thio)-1,3,4-thiadiazol-2-yl)benzamide (7k)

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 1.03-1.17 (m, 2H, H_4-piperidine), 1.71 (brs, 4H, $H_{3,5}$-piperidine), 3.56 (brs, 4H, $H_{2,6}$-piperidine), 3.76 (s, 3H, -OCH_3), 3.95 (t, 2H, -S-CH_2-C$\underline{H}_2$-N-), 4.22 (t, 2H, -S-C$\underline{H}_2$-CH_2-N-), 6.69 (s, 1H, H_2-3-methoxyphenyl), 7.15 (d, 1H, H_4-3-methoxtphenyl), 7.49 (d, 1H, H_6-3-methoxtphenyl), 7.79 (t, 1H, H_5-3-methoxtphenyl). IR (KBr, cm^{-1}): 3327 (stretch, NH), 3064 (stretch, C-H, aromatic), 2929, 2852 (stretch, C-H, aliphatic), 1662 (stretch, C=O, amide). MS (m/z, %): 378 (M^+, weak), 276 (5), 189 (7), 152 (12), 135 (25), 120 (20), 91 (20), 71 (100).

4-Methoxy-N-(5-((2-(piperidin-1-yl)ethyl)thio)-1,3,4-thiadiazol-2-yl)benzamide (7l)

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 0.99-1.20 (m, 2H, H_4-piperidine), 1.72 (brs, 4H, $H_{3,5}$-piperidine), 3.08 (t, 2H, -S-CH_2-C$\underline{H}_2$-N-), 3.34 (brs, 4H, $H_{2,6}$-piperidine), 3.79 (s, 3H, -OCH_3), 3.94 (t, 2H, -S-C$\underline{H}_2$-CH_2-N-), 6.95 (d, 2H, *J* = 8.75 Hz, $H_{3,5}$-4-methoxyphenyl), 7.90 (d, 2H, *J* = 8.75 Hz, $H_{2,6}$-4-methoxyphenyl). IR (KBr, cm^{-1}): 3334 (stretch, NH), 3032 (stretch, C-H, aromatic), 2927, 2852 (stretch, C-H, aliphatic), 1716 (stretch, C=O, amide).

Ellman's test

Ellman's test is used to evaluate the possible inhibitory effects of the compounds on the enzyme acetylcholinesterase.[20] The procedure was done according to Rahmani-Khajouei *et al.*[21] with slight modifications.Briefly, the agents were solubilized in ethanol and prepared in 8 different concentrations (0, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} µM). Ellman's test is based on the conversion of acetylthiocholine

to acetate and thiocholine. The thio compound then reacts with 5,5'-dithio-bis-[2-nitrobenzoic acid] (BTNB) to form 5-thio-2-nitrobenzoate, which is a chromophore with maximum absorption at 412 nM. The test was done in a 96-well microplate. Enzyme inhibition is measured when different concentrations of the drug are used with Ellman's reagents. The results are then normalized based on the protein content of each well which is determined by the method of Bradford.[22]

Docking

Molecular docking investigation was carried out for all ligands in ArgusLab 4.0 software.[23] All synthesized compounds were assumed as ligands and were built in Arguslab workspace and were minimized energetically using AM_1 as a semiempirical quantum mechanics method. The PDB files of acetylcholinesterase in complex with donepezil (PDB code: 1EVE) were downloaded from the Brookhaven protein databank.[24-26] Internal validation of the software was implemented by the performance of the docking process of the cocrystallized ligand. Namely, the cocrystalized donepezil molecule was supposed a ligand, and docking was done. Then, the root mean squared deviation (RMSD) between the cocrystalized molecule and the defined donepezil molecule was calculated. The obtained RMSD was 0.064 Å and the related binding free energy was -12.74 kcal/mol. In the beginning, geometry optimization was carried out by the UFF method. The corresponding dimensions of the bounding box (x, y, z) inside the active site of the AChE were calculated by the software automatically. The obtained results were 17.21, 24.78, and 21.38 angstrom for x, y, and z respectively. The rest of the parameters such as docking engine (ArgusDock), grid resolution (0.4 angstroms), and resumming of the ligands in flexible mode were considered according to the software defaults. A regular precision was applied for the docking precision. The maximum number of poses that were defined was 150. The corresponding ligands, as well as binding site groups, were defined. The binding site of donepezil was defined as the binding area for searching for the best pose and conformation related to all ligands. The binding mode and respective interactions of ligands with acetylcholinesterase enzyme were explored in Arguslab space (Figure 4) and Molegro molecular viewer software (Figure 5).[27]

Results and Discussion

Chemistry

The intended compounds **7a-7l** were prepared according to Figure 3 and related physicochemical properties were reported in Table 1. Imide formation (compound **3**) was done according to the Gabriel synthesis and was carried out in the presence of triethylamine as a proton acceptor. The optimum solvent was toluene to attain the highest yield. Trituration of the obtained product was performed in *n*-hexane. The incorporation of the phthalimide residue was utilized to protect the amine moiety and to impede its reaction in step **3** as an amidation reaction. Compound **4** (1-(2-chloroethyl)piperidinium chloride) was accessible as hydrochloride (HCl) salt. For the preparation of compound **5**, at first, it is necessary to liberate the 1-(2-chloroethyl) piperidinium chloride from hydrochloride salt. For this purpose, compound **4** was treated with triethylamine in acetonitrile according to the mentioned protocol in the

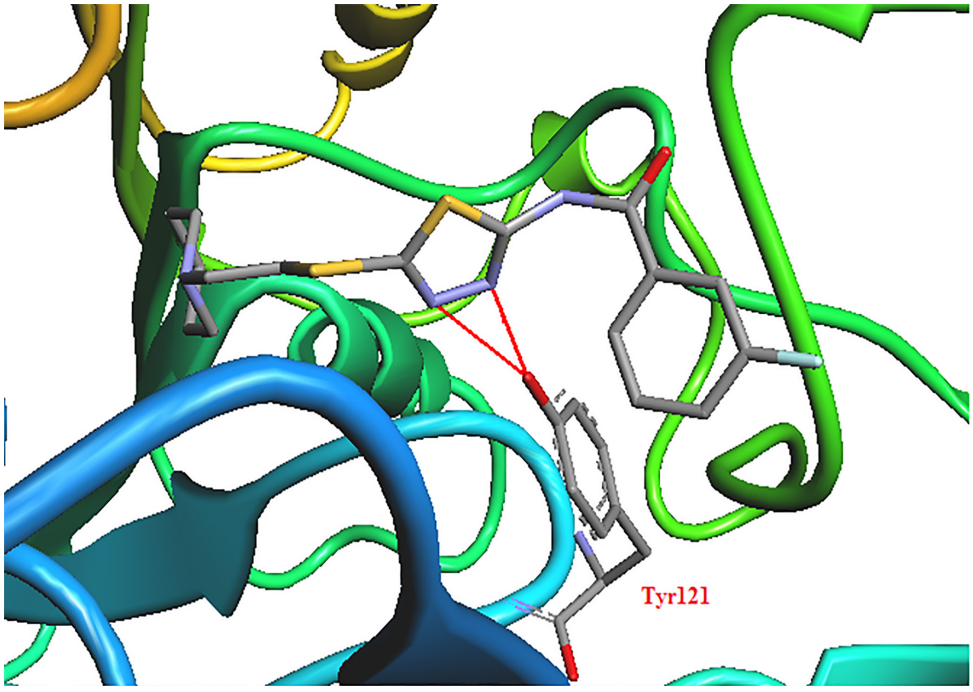

Figure 4. Docking results of compound 7e into the active site of acetylcholinesterase. Two hydrogen bonding interactions were observed with the nitrogen atoms of the 1,3,4-thiadiazole ring and hydroxyl group of Tyr121.

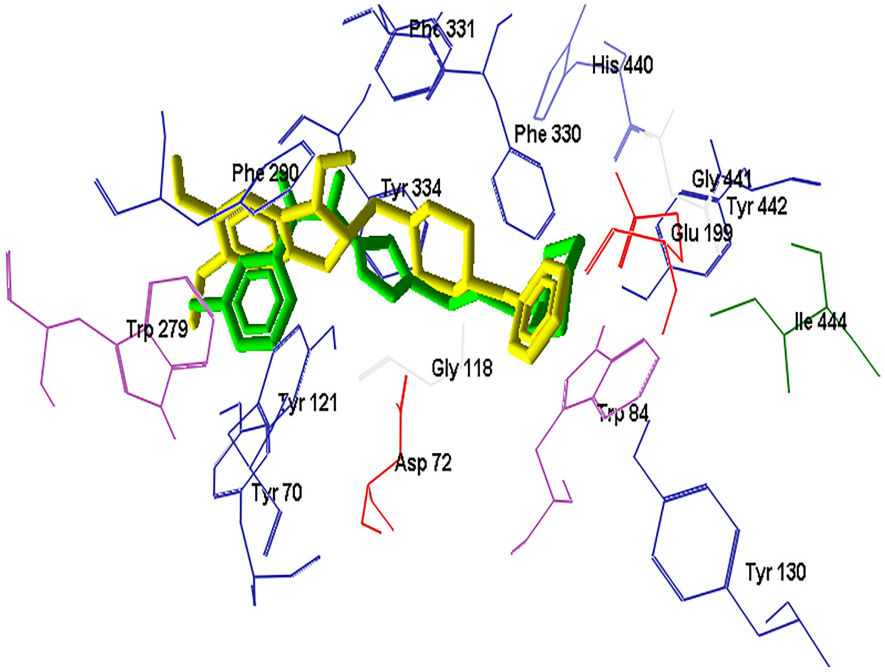

Figure 5. Superimposed visualization of compound 7e (green) with donepezil (yellow).

synthesis part and subsequently, the intended reaction was implemented to achieve compound **5**. The compound **5** was afforded after exposure of the thiol functional group of the compound **3** to potassium hydroxide to form the thiolate anion and then alkylation by 1-(2-chloroethyl) piperidine was done. Compound **5** was applied in the next step as a cleaving reaction to omit the phthalimide residue (protective group) to free the amine substituent again (compound **6**). Methylamine was used as a cleaving agent for this aim in absolute ethanol under refluxing conditions. All intermediate and final compounds were afforded an acceptable yield (Table 1). Melting points were measured and spectroscopic data were also obtained.

Anti-acetylcholinesterase evaluation

Various substituents such as chlorine, fluorine, nitro, and methoxy were introduced on the phenyl residue. The presence of these moieties caused us to explore the impact of electronic and steric parameters on enzyme inhibition. Fortunately, the tested compounds exhibited remarkable inhibitory effects against AChE in the nanomolar range (Table 2). Compound **7e** (IC_{50} = 1.82±0.6 nM) as fluorinated derivatives at position *meta* of the phenyl ring, was the most active compound in this series. This compound showed more activity than donepezil (IC_{50} = 0.6±0.05 nM) as a standard anti-cholinesterase drug. Utilization of an electron-withdrawing substituent at position *meta* of the phenyl ring has a beneficial effect on enzyme inhibition. But, this event was not observed while testing the chlorine and nitro moieties. Probably, the steric impact of the chlorine and nitro groups is an interrupting factor for enzyme interaction. In addition, compound **7a** (IC_{50} = 2.03±0.1 nM) which bears a chlorine atom at position *ortho* was also the second rank in this series as inhibitory potential viewpoint. The mentioned derivative also rendered more activity than compound **7d** as an *ortho*-fluorinated derivative. In conclusion, as we know fluorine has more electron-receiving effect than chlorine, but steric and lipophilic features of the chlorine atom are probable effective parameters for increasing the inhibitory effect in compound **7a**. Compounds **7d** (2-F) and **7f** (4-F) were also potent AChE inhibitors and possessed inhibitory effects in nanomolar concentration (Table 2). Amongst the nitrated derivatives, compound **7i** (*p*-nitro), was the most active compound (IC_{50} = 0.35±0.04 μM). Electron withdrawing effect, electrostatic interaction, and hydrophilicity are favorable factors in nitro moiety to enhance the enzyme inhibitory effect. As mentioned above, steric and lipophilicity were effective parameters for chlorine atoms while introduced at position *ortho* of the phenyl residue. The same result was also seen for *O*-methoxylated derivative **7j**. This compound was the third rank (IC_{50} = 18.2±5.3 nM) in potency among the evaluated compounds.

The synthesized 1,3,4-thiadiazole compounds according to the donepezil pharmacophore presented herein displayed a favorable inhibitory effect toward AChE. Comparison with previously reported derivatives (phthalimide analogs) rendered better inhibitory potency towards AChE.[28] Introduction of the 1,3,4-thiadiazole nucleus enhanced enzyme inhibitory potency of these series. In addition, the replacement of the phthalimide substructure with benzamide residue also caused a beneficial impact on activity. Probably, hydrogen bonding of the 1,3,4-thiadiazole heterocycle *via* its nitrogen atoms with Tyr121 as well as π-interaction with Tyr334

Table 1. Properties of intermediate and final compounds.

Compound	R	Chemical Formula	MW (g/mol)	mp (°C)	Yield (%)
3	_	C11H6N2O2S2	262.00	95.3	65
5	_	C18H19N3O2S2	373.09	246	35
6	_	C9H16N4S2	244.08	ND*	43
7a	2-Cl	C16H19ClN5OS	364.84	ND	94
7b	3-Cl	C16H19ClN5OS	364.84	ND	42
7c	4-Cl	C16H19ClN5OS	364.84	ND	61.2
7d	2-F	C16H19FN5OS	348.42	ND	75
7e	3-F	C16H19FN5OS	348.42	157	67
7f	4-F	C16H19FN5OS	348.42	ND	53.3
7g	2-NO2	C16H19N6O3S	375.12	137	57
7h	3-NO2	C16H19N6O3S	375.12	ND	69.5
7i	4-NO2	C16H19N6O3S	375.12	181	84.3
7j	2-OCH3	C17H22N5O2S	360.46	ND	38
7k	3-OCH3	C17H22N5O2S	360.46	ND	45
7l	4-OCH3	C17H22N5O2S	360.46	ND	48.8

*ND: Not determined, obtained as liquid.

and Phe331 are fortifying factors for anti-cholinesterase activity (Figure 4). On the other hand, the replacement of the piperazine moiety with piperidine congener also enhanced the anti-acetylcholinesterase activity. As reported previously, the piperazinyl derivatives demonstrated inferior enzyme inhibitory toward AChE.[29] It could be supposed that the more lipophilic nature of the piperidine heterocycle compared to piperazine is responsible for the activity enhancement. Furthermore, the tested piperidinyl compounds displayed higher potency than acyclic analogs (diethylamine derivatives) that were presented former.[30] It seems the reduction of the polarity in the site of interaction of the amine-containing heterocycle (piperazine/piperidine) is a favorable parameter for enzyme inhibition. Surely, one of the nitrogen atoms of the amino heterocyclic ring is necessary for keeping the polarity and suitable interactions. This nitrogen atom increases the ligand polarity by electron receiving feature. Besides, nitrogen has also the capability to be protonated to form a quaternary ammonium ion. This ionic form of the nitrogen also potentiates the polarity and potential for electrostatic (ion-ion) interaction.

Benzamidic derivatives that were presented in the current project, possessed more activity than phthalimide and chalcone derivatives.[28-30] It's probably the more hydrophilic character of the amidic functional group compared to rigid imide moiety and α,β-unsaturated system that are present in the phthalimide and chalcone respectively, is more efficacious for inhibition of the AChE. More flexibility of the amide bond in comparison with phthalimide and chalcone derivatives has a critical impact on the optimal interacting state.

Table 2. Results of Acetylcholinesterase inhibitory test (IC_{50} ± SEM).

Compound	R	IC_{50}
7a	2-Cl	2.03 ± 0.1 nM
7b	3-Cl	8.37 ± 1.4 μM
7c	4-Cl	1.38 ± 0.16 μM
	2-F	28 ± 11 nM
7e	3-F	1.82 ± 0.6 nM
7f	4-F	29.5 ± 3 nM
7g	2-NO2	19 ± 4 μM
7h	3-NO2	5.7± 0.4 μM
7i	4-NO2	0.35 ± 0.04 μM
7j	2-OCH3	18.2 ± 5.3 nM
7k	3-OCH3	2.01 ± 0.84 μM
7l	4-OCH3	5.07 ± 1.4 μM
Donepezil	_	0.6 ± 0.05 μM

Docking

A docking study of compound **7e** (3-F) as the most active compound in Ellman's test was done (Figure 4). Interestingly, two hydrogen bondings were observed by nitrogen atoms of the 1,3,4-thiadiazole ring and hydroxyl group of Tyr121. Nitrogen atoms of this heterocycle act as hydrogen acceptors to form effective hydrogen bonds. One of the nitrogen atoms also interacts with a water molecule that is associated with the enzyme's active site. Neighboring the 1,3,4-thiadiazole ring to the aromatic amino acids such as Tyr334 and Phe331 facilitates the π-π interaction and could be an influencing factor for increasing the potency (Figure 5). As seen in Figure 5, other important amino acids such as Trp84, Phe330, and Trp279 that played a pivotal and important role in the interaction of the donepezil drug into the AChE active site are also present herein.[31] Surely, all of the mentioned interactions especially hydrogen bonds that form by 1,3,4-thiadiazole are effective factors for potent interaction with the enzyme active site and consequently demonstrate the potency in the nanomolar range.

Conclusion

The 1,3,4-thiadiazole derivatives that are presented currently were synthesized in a multistep process. Prepared final derivatives tested in the current manuscript demonstrated remarkable anticholinesterase activity. Using 1,3,4-thiadiazole nucleus as the main core of the pharmacophore with an amidic linkage caused a significant potency for AChE inhibition. Improvement of the biological activity with the presence of 1,3,4-thiadiazole could be a positive point in the study of the structure-activity relationship (SAR) of this chemical structure as an AChE inhibitor. 1,3,4-Thiadiazole as a beneficial substructure linked to an amide functional group could be utilized in the design of novel AChE inhibitors in the future. Six out of twelve synthesized compounds demonstrated more enzyme inhibitory potency than donepezil as a reference drug. Compound **7e** as the most active one (IC_{50} = 1.82 ± 0.6 nM) could be introduced as a promising anti-Alzheimer agent. In conclusion, these compounds could be suggested as potential anti-Alzheimer agents and more experimental tests such as *in vivo* assay would be performed in the future.

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Author Contributions

Ahmad Mohammadi-Farani: Investigation, Formal Analysis, Resources. Milad Takesh: Data curation, Investigation. Mahsa Mohammadi: Supervision, Formal analysis. Amin Hosseini: Writing - Original Draft, Formal Analysis. Amin Aliabadi: Writing - Review & Editing. Alireza Aliabadi: Conceptualization, Supervision, Funding Acquisition, Project administration.

Conflict of Interest

There was no conflict of interest.

Supplementary Data

The spectra of synthsized compounds are available at: https://doi.org/10.34172/PS.2024.7.

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