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ORIGINAL ARTICLE

New benzamide derivatives and their nicotinamide/cinnamamide analogs as cholinesterase inhibitors

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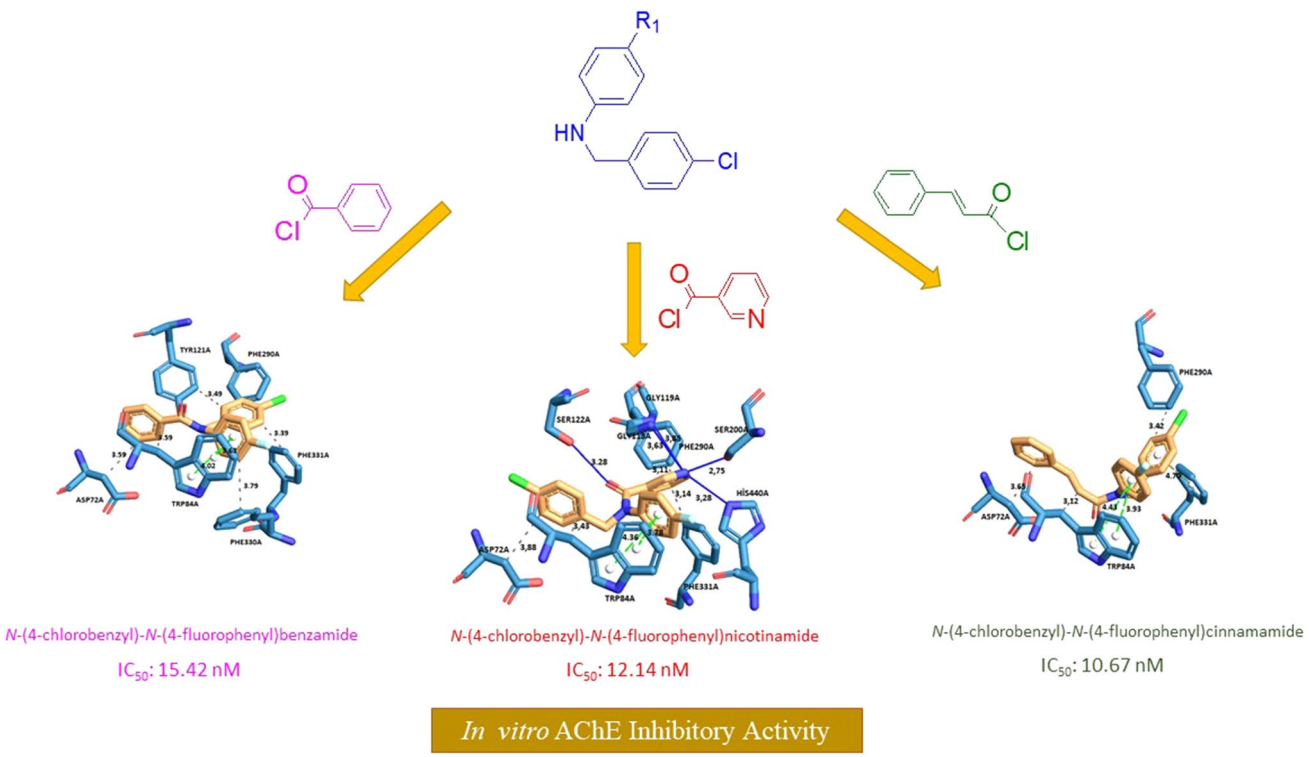
Abstract

In this study, a total of 18 new benzamide/ nicotinamide/ cinnamamide derivative compounds were designed and synthesized for the first time (except B1 and B5) by conventional and microwave irradiation methods. The chemical structures of the synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS spectra. In vitro acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibition effects of the compounds were evaluated to find out new possible drug candidate molecule/s. According to the inhibition results, the IC50 values of the compounds synthesized were in the range of 10.66–83.03 nM towards AChE, while they were in the range of 32.74–66.68 nM towards BuChE. Tacrine was used as the reference drug and its IC50 values were 20.85 nM and 15.66 nM towards AChE and BuChE, respectively. The most active compounds B4 (IC50: 15.42 nM), N4 (IC50: 12.14 nM), and C4 (IC50: 10.67 nM) in each series towards AChE were docked at the binding site of AChE enzyme to explain the inhibitory activities of each series. On the other hand, the compounds B4, N4, and C4 showed satisfactory pharmacokinetic properties via the prediction of ADME profiles.

Graphic abstract



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Keywords Benzamide · Nicotinamide · Cinnamamide · Cholinesterase inhibition · Molecular docking

Introduction

Alzheimer's disease (AD), which is generally observed especially in the elderly, has become an important health problem worldwide [1]. One of the most important findings in patients with AD is a decrease in the level of acetylcholine in the cerebral cortex [2]. Thus, one of the most important methods to slow down the progression of AD is by increasing the level of acetylcholine in the cerebral cortex via cholinesterase enzymes inhibition [3]. There are two types of cholinesterase enzymes which are known as acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Both of these enzymes play an important role in the regulation of the level of acetylcholine [4, 5]. It was reported that inhibition of cholinesterase enzymes reduces the progression of the disease and improves perception in patients with AD [6, 7]. Tacrine, rivastigmine, and donepezil are the main cholinesterase inhibitors used in clinics for the treatment of AD [8, 9]. On the other hand, ambenonium, a diamide derivative, is used in myasthenia gravis as an AChE inhibitor [10].

In order to reduce the hepatotoxicity of tacrine and increase its bioavailability, Ragab et al. made some modifications in the tacrine skeleton[11]. Among these modified compounds, chlorinated derivatives showed higher cholinesterase inhibitory activity and lower hepatotoxicity than non-chlorinated derivatives. In the same study, the derivative of tacrine with 1-benzylamino substituent was reported to have high cholinesterase inhibitory activity. There are also other studies in the literature reporting the cholinesterase inhibitory activities of various derivatives in which substituted benzene/benzyl structures are bound to the nitrogen atom [12–14].

There are many studies in the literature on the antimicrobial [15], antioxidant [16], antiasthmatic [17], and cholinesterase inhibitory [18, 19] activities of benzamide derivatives. Some compounds in the arylalkylaminoketone scaffold, in which structure of benzoyl/naphthoyl is linked to a secondary amine via the intermediate carbon chain, were reported to be cholinesterase inhibitors with an ADME profile that can pass into the central nervous system [20].

Nicotinamide is an amide derivative of nicotinic acid, also known as vitamin B3, found in many natural foods such as meat, milk, fish, and grains [21]. In humans, nicotinamide is converted to Nicotinamide Adenine Dinucleotide (NAD) which plays an important role in cellular functions in the body [22, 23]. Various nicotinamide derivatives have been reported with many biological activities such as antituberculosis [24, 25], antifungal [26] antitumor [27–29] antidiabetic [30–32], antioxidative [33] and neuroprotective [34, 35] activities. The effects of nicotinamide on AD and other neurodegenerative disorders have been investigated, and it was reported that nicotinamide can cross the blood and brain barrier [36] and thus, this may have some positive effects against neurodegenerative diseases [37–40]. Żurek et al. synthesized hybrid compounds in hydrazide structure containing donepezil and nicotinamide structures and these compounds had cholinesterase inhibitory activities with nanomolar enzyme inhibition constants [41].

Cinnamamide is a natural compound that has a phenyl ring at the 3rd position of the acrylamide structure and is found in many plants. In the literature, many biological activities of cinnamamide derivatives have been reported, such as antibacterial [42], anti-inflammatory [43], antimalarial [44], anticancer [45], tyrosinase inhibitory [46], antiviral [47], anticonvulsant [48], antifungal [49], anti-Alzheimer [50] activities. On the other hand, cinnamamide derivative some compounds and tacrine-cinnamic acid hybrids were reported with high cholinesterase inhibition effects with low nanomolar inhibition constant [51, 52]. Besides, in our previous study, new butendiamide and oxalamide derivative compounds were synthesized and some of them showed strong cholinesterase inhibition effects [53, 54].

In this study, we aimed to synthesize novel benzamide/nicotinamide/cinnamamide derivatives which have 4-chlorobenzyl and 4-substituted phenyl on their nitrogen atoms, to evaluate their in vitro cholinesterase inhibitory activities to find out new possible drug candidate molecule/s. Besides, we planned to identify the binding profiles of the most active compounds (**B4, N4, C4**) of the series with AChE enzyme by molecular docking studies to determine their possible mechanism of cholinesterase inhibitory activities.

Materials and methods

Chemistry

General procedure of the synthesis of the secondary amines

By conventional method para-Substituted aniline (20 mmol) and sodium bicarbonate (6 mmol) in 15 ml water were heated to 90–95 ºC. To this mixture, p-chlorobenzyl chloride (5 mmol) was added and stirred for 1.5–2 h, give of $PhCH_2NHPh$. After completion of the reaction, the reaction mixture was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layer was dried over with $MgSO_4$, filtered, and concentrated by rotary evaporation and the crude product was purified by silica gel column chromatography using hexane:ethylacetate (2:1).

By microwave irradiation A mixture of para-substituted aniline (20 mmol), p-chlorobenzyl chloride (10 mmol) was adsorbed on a mixture of potassium carbonate (12 mmol) and tetrabutylammonium bromide (1 mmol). The resulting fine powder was irradiated with microwave irradiation (150 W, 120 °C) for 5 min to give of $PhCH_2NHPh$. After completion of the reaction, the reaction mixture was quenched with 15 mL of distilled water and the aqueous phase was extracted with two parts of CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried over with $MgSO_4$, filtered, and concentrated. The crude product was purified by silica gel column chromatography using hexane:ethylacetate (2:1) [55].

General procedure of the synthesis of final compounds

By conventional method A mixture of the para-substituted benzaniline (1.7 mmol), triethylamine (TEA) (1.4 mmol), and benzoyl/nicotinoyl/cinnamoyl chloride (1.4 mmol) in dichloromethane (5 mL) were stirred at room temperature for 12 h. After completion of the reaction, the reaction mixture was quenched with 15 mL of distilled water and the aqueous phase was extracted of CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried over with $MgSO_4$, filtered, and concentrated. Finally, the crude product was purified by silica gel column chromatography hexane:ethylacetate (6:4) or crystallized from ethanol.

By microwave irradiation A mixture of para-substituted benzaniline (1.7 mmol), benzoyl/nicotinoyl/cinnamoyl chloride (1,7 mmol) in dioxane (0.6 mL) as solvent was reacted under microwave irradiation at 100 ºC for 10 min. After completion of the reaction, the reaction mixture was concentrated by rotary evaporation. Finally, the crude product was purified by silica gel column chromatography hexane:ethylacetate (6:4) or crystallized from ethanol.

^{1} H- and ^{13} C-NMR spectra were recorded with 400 (100) MHz Bruker and Varian instruments. Interchangeable hydrogens or carbons were shown with the same letters. Elemental analyses were performed with a LECO CHNS-932. HRMS spectra were recorded with an Agilent 6530 LC–MS QTOF.

N-(4-chlorobenzyl)-N-phenylbenzamide (B1) Yield: 76% (80% by MW), mp: 83–85 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 5.08 (s, 2H, N-CH_2), 6.88 (d, 2H, *J*:8.5 Hz, Ar–H), 7.16 -7.09 (m, 5 H, Ar–H), 7.25 -7.20 (m, 5 H, Ar–H) 7.32–7.30 (m, 2H, Ar–H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 53.24 (Ar-CH_2-N), 126.83 (CH_{Ar}), 127.74 (2xCH_{Ar}), 127.75 (2xCH_{Ar}), 128.64 (2xCH_{Ar}), 128.79 (2xCH_{Ar}), 129.13 (2xCH_{Ar}), 129.78 (CH_{Ar}), 129.95 (2xCH_{Ar}), 133.24 (C_{Ar}), 135.71 (C_{Ar}), 136.04 (C_{Ar}), 143.27 (C_{Ar}), 170.55 (C = O). Anal. Calcd. for $C_{20}H_{16}ClNO$ (MW 321.09): C, 74.65; H, 5.01%. Found: C, 74.32; H, 4.99%. HRMS (Q-TOF) m/z Calcd for $[M+H]^+$ 322.0920, found. 322.099.

N-(4-chlorobenzyl)-N-(p-tolyl)benzamide (B2) Yield: 77% (79% by MW), mp: 85–87 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 2.21(s, 3H, CH_3-Ar), 5.05 (s, 2H, N-CH_2), 6.75 (d, 2H, *J*:8.20 Hz, Ar–H), 6.92 (d, 2H, *J*:8.12 Hz, Ar–H), 7.28–7.12 (m, 8H, Ar–H), 7.32–7.30 (m, 2H, Ar–H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 20.95 (CH_3-Ar), 53.29 (Ar-CH_2-N), 127.49 (2xCH_{Ar}), 127.72 (2xCH_{Ar}), 128.59 (2xCH_{Ar}), 128.75 (2xCH_{Ar}), 129.65 (CH_{Ar}), 129.75 (2xCH_{Ar}), 130.00 (2xCH_{Ar}), 133.17 (C_{Ar}), 135.87 (C_{Ar}), 136.14 (C_{Ar}), 136.65 (C_{Ar}), 140.59 (C_{Ar}), 170.58 (C = O). Anal. Calcd. for $C_{21}H_{18}ClNO$ (MW 335.11): C, 75.11; H, 5.4%. Found: C, 74.48; H, 5.51%. HRMS (Q-TOF) m/z Calcd for $[M+H]^+$ 336,1077, found. 336.1148.

N-(4-chlorobenzyl)-N-(4-methoxyphenyl)benzamide (B3) Yield: 80% (85% by MW), mp: 93–95 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 3.68(s, 3H, Ar-OCH_3), 5.03 (s, 2H, N-CH_2), 6.65–6.63 (m, 2H, Ar–H), 6.77 (d, 2H, *J*:8.72 Hz, Ar–H), 7.20–7.14 (m, 5H, Ar–H), 7.25–7.22 (m, 2H, Ar–H), 7.31–7.29 (m, 2H, Ar–H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 53.35 (Ar-CH_2-N), 55.28 (OCH_3-Ar), 114.26 (2xCH_{Ar}), 127.74 (2xCH_{Ar}), 128.60 (4xCH_{Ar}), 128.67 (2xCH_{Ar}), 128.95 (2xCH_{Ar}), 129.56 (C_{Ar}), 130.13 (CH_{Ar}), 133.21 (C_{Ar}), 135.91 (C_{Ar}), 136.09 (C_{Ar}), 158.07 (C_{Ar}), 170.58 (C = O). Anal. Calcd. for $C_{21}H_{18}ClNO_2$ (MW 351.10): C, 71.69; H, 5.16%. Found: C, 74.44; H, 5.18%. HRMS (Q-TOF) m/z Calcd for $[M+H]^+$ 352.1026, found. 352.1099.

N-(4-chlorobenzyl)-N-(4-fluorophenyl)benzamide (B4) Yield: 72% (73% by MW), mp: 132–134 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 5.04 (s, 2H, N-CH_2), 6.83 (d, 2H, *J*: 6.48 Hz, Ar–H), 7.30 -7.15 (m, 11H, Ar–H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 53.26 (Ar-CH_2-N), 115.96–116.19 (J_{CF}:22.7 2xCH_{Ar}), 127.88 (2xCH_{Ar}), 128.64 (2xCH_{Ar}), 128.72 (2xCH_{Ar}), 129.52 -129.43 (J_{CF}:8.7 2xCH_{Ar}), 129.85 (CH_{Ar}), 130.04 (2xCH_{Ar}), 133.43 (C_{Ar}), 135.52 (C_{Ar}), 135.74 (C_{Ar}), 139.18 (C_{Ar}), 159.74–162.20 (J_{CF}:247.6 C_{Ar}), 170.56 (C = O). Anal. Calcd. for $C_{20}H_{15}ClFNO$ (MW 339.08): C, 70.7; H, 4.45%. Found: C, 69.06; H, 4.29%. HRMS (Q-TOF) m/z Calcd for $[M+H]^+$ 340.0826, found. 340.0899.

N-(4-chlorobenzyl)-N-(4-chlorophenyl)benzamide (B5) Yield: 75% (78% by MW), mp: 113–114 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 5.05 (s, 2H, CH_2N), 6.82–6.80 (m, 2H, Ar–H), 7.31–7.10 (m, 11H, Ar–H) ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 53.15 (Ar-CH_2-N), 127.96 (2xCH_{Ar}), 128.72 (2xCH_{Ar}), 128.75 (2xCH_{Ar}), 128.93 (2xCH_{Ar}), 129.34 (2xCH_{Ar}), 129.94 (2xCH_{Ar}), 130.03 (CH_{Ar}), 132.51 (C_{Ar}), 133.46 (C_{Ar}), 135.34 (C_{Ar}), 135.66(C_{Ar}), 141.78 (C_{Ar}), 170.46 (C = O). Anal. Calcd. for $C_{20}H_{15}Cl_2NO$ (MW 355.05): C, 67.43; H, 4.24%. Found: C, 66.89; H, 4.28%. HRMS (Q-TOF) m/z Calcd for $[M+H]^+$ 356.0530, found. 356.0603.

N-(4-bromophenyl)-N-(4-chlorobenzyl)benzamide (B6) Yield: 73% (75% by MW), mp: 91–93 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 5.05 (s, 2H, N-CH_2), 6.76–6.74 (m, 2H, Ar–H), 7.31–7.18 (m, 11H, Ar–H) ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 53.12 (Ar-CH_2-N), 120.45 (C_{Ar}), 127.99 (2×CH_{Ar}), 128.74 (2×CH_{Ar}), 128.76 (2×CH_{Ar}), 129.24 (2×CH_{Ar}), 129.92 (2×CH_{Ar}), 130.07 (CH_{Ar}), 132.33 (2×CH_{Ar}), 133.46 (C_{Ar}), 135.31 (C_{Ar}), 135.65 (C_{Ar}), 142.32 (C_{Ar}), 170.41 (C=O). Anal. Calcd. for $C_{20}H_{15}BrClNO$ (MW 399.00): C, 59.95; H, 3.77%. Found: C, 59.82; H, 3.77%. HRMS (Q-TOF) m/z Calcd for $[M+H]^+$ 400.0025, found. 400.0097.

N-(4-chlorobenzyl)-N-phenylnicotinamide (N1) Yield: 78% (83% by MW), mp: 112–114 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 5.09 (s, 2H, N-CH_2), 6.9 (d, 2H, *J*:6.7 Hz, Ar–H), 7.11 (dd, 1H, *J*: 4.9 Hz, *J*:5.0 Hz, Ar–H), 7.22–7.15 (m, 4H, Ar–H), 7.27–7.24 (m, 4H, Ar–H), 7.64–7.61 (m, 1H, Ar–H), 8.45–8.44 (m, 1H, Ar–H), 8.51 (d, 1H, *J*:2.6 Hz, Ar–H), ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 53.32 (Ar-CH_2-N), 122.71 (CH_{Ar}), 127.57 (CH_{Ar}), 127.97 (2xCH_{Ar}), 128.72 (2xCH_{Ar}), 129.54 (2xCH_{Ar}), 130.12 (2xCH_{Ar}), 131.61 (C_{Ar}), 133.49 (C_{Ar}), 135.42 (C_{Ar}), 136.21 (CH_{Ar}), 142.37 (C_{Ar}), 149.65 (CH_{Ar}), 150.47 (CH_{Ar}), 168.07 (C=O). Anal. Calcd. for $C_{19}H_{15}ClN_2O$ (MW 322.09): C, 70.7; H, 4.68%. Found: C, 69.31; H, 4.88%. HRMS (Q-TOF) m/z Calcd for $[M+H]^+$ 323.0872, found. 323.0945.

N-(4-chlorobenzyl)-N-(p-tolyl)nicotinamide (N2) Yield: 80% (80% by MW), mp: 126–128 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 2.24 (s, 3H, CH_3-Ar), 5.06 (s, 2H, N-CH_2), 6.77 (d, 2H, *J*: 8.1 Hz, Ar–H), 6.9 (d, 2H, *J*: 7.9 Hz, Ar–H), 7.13 (dd, 1H, *J*: 4.8 Hz, *J*:4.8 Hz,, Ar–H), 7.27–7.22 (m, 4H, Ar–H), 7.65 (d, 1H, *J*: 7.8 Hz, Ar–H), 8.45 (d, 1H, *J*: 3.2 Hz, Ar–H), 8.49 (s, 1H, Ar–H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 21.05 (CH_3-Ar), 53.32 (Ar-CH_2-N), 122.73 (CH_{Ar}), 127.72 (2xCH_{Ar}), 128.68 (2xCH_{Ar}), 130.15 (4xCH_{Ar}), 131.74 (C_{Ar}), 133.43 (C_{Ar}), 135.52 (C_{Ar}), 136.24 (CH_{Ar}), 137.49 (C_{Ar}), 139.70 (C_{Ar}), 149.64 (CH_{Ar}), 150.36 (CH_{Ar}), 168.07 (C=O). Anal. Calcd. for $C_{20}H_{17}ClN_2O$ (MW 336.10): C, 71.32; H, 5.09%. Found: C, 70.71; H, 5.10%. HRMS (Q-TOF) m/z Calcd for $[M+H]^+$ 337.1029, found. 337.1102.

N-(4-chlorobenzyl)-N-(4-methoxyphenyl)nicotinamide (N3) Yield: 81%, (85% by MW), mp: 133–135 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 3.71 (s, 3H, Ar-OCH_3), 5.03 (s, 2H, N-CH_2), 6.68 (d, 2H, *J*: 8.7 Hz, Ar–H), 6.79 (d, 2H, *J*: 8.6 Hz Ar–H), 7.12 (dd, 1H, *J*: 5.0 Hz, *J*:4.9 Hz, Ar–H), 7.27–7.14 (m, 4H, Ar–H), 7.63 (d, 1H, *J*: 7.8 Hz, Ar–H), 8.44 (d, 1H, *J*: 4.6 Hz, Ar–H), 8.5 (s, 1H, Ar–H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 53.39 (Ar-CH_2-N), 55.32 (OCH_3-Ar), 114.61 (2xCH_{Ar}), 122.75 (CH_{Ar}), 128.69 (2xCH_{Ar}), 129.18 (2xCH_{Ar}), 130.29 (2xCH_{Ar}), 131.83 (C_{Ar}), 133.47 (C_{Ar}), 134.92 (C_{Ar}), 135.49 (C_{Ar}), 136.20 (CH_{Ar}), 149.51 (CH_{Ar}), 150.22 (CH_{Ar}), 158.53 (C_{Ar}), 168.08 (C=O). Anal. Calcd. for $C_{20}H_{17}ClN_2O_2$ (MW 352.10): C, 68.09; H, 4.86%. Found: C, 67.18; H, 4.80%. HRMS (Q-TOF) m/z Calcd for $[M+H]^+$ 353.0978, found. 353.1051.

N-(4-chlorobenzyl)-N-(4-fluorophenyl)nicotinamide (N4) Yield: 76% (79% by MW), mp: 116–118 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 5.05 (s, 2H, N-CH_2), 6.90–6.86 (m, 4H, Ar–H), 7.14 (dd, 1H, *J*: 5.0 Hz, *J*:4.9 Hz, Ar–H), 7.27–7.20 (m, 4H, Ar–H), 7.62 (d, 1H, *J*: 7.8 Hz, Ar–H), 8.47 (d, 1H, *J*: 4.7 Hz, Ar–H), 8.5 (s, 1H, Ar–H) ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 53.39 (Ar-CH_2-N), 116.44–116.67 (J_{CF}:22.6 2xCH_{Ar}), 122.84 (CH_{Ar}), 128.81 (2xCH_{Ar}), 129.71 -129.80 (J_{CF}:10.7 2xCH_{Ar}), 130.20 (2xCH_{Ar}), 131.45 (C_{Ar}), 133.68 (C_{Ar}), 135.13 (CH_{Ar}), 136.15 (CH_{Ar}), 138.31 (C_{Ar}), 149.45 (CH_{Ar}), 150.53 (CH_{Ar}), 160.07–162.54 (J_{CF}:248.6 C_{Ar}), 168.08 (C=O). Anal. Calcd. for $C_{19}H_{14}ClFN_2O$ (MW 340.08): C, 66.97; H, 4.14%. Found: C, 66.99; H, 4.22%. HRMS (Q-TOF) m/z Calcd for $[M+H]^+$ 340.0778, found. 341.0851.

N-(4-chlorobenzyl)-N-(4-chlorophenyl)nicotinamide (N5) Yield: 78% (80% by MW), mp: 144–146 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 5.05 (s, 2H, N-CH_2), 6.82 (d, 2H, *J*: 8.3 Hz, Ar–H), 7.27–7.14 (m, 8H, Ar–H), 7.63 (d, 1H, *J*: 7.8 Hz, Ar–H), 8.50–8.47 (m, 1H, Ar–H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 53.26 (Ar-CH_2-N), 122.92 (CH_{Ar}), 128.84 (2xCH_{Ar}), 129.18 (2xCH_{Ar}), 129.78 (2xCH_{Ar}), 130.12 (2xCH_{Ar}), 131.27 (C_{Ar}), 133.36 (C_{Ar}), 133.71 (CH_{Ar}), 135.05 (C_{Ar}), 136.21 (CH_{Ar}), 140.87 (C_{Ar}), 149.54 (CH_{Ar}), 150.72 (CH_{Ar}), 167.96 (C=O). Anal. Calcd. for $C_{19}H_{14}Cl_2N_2O$ (MW 356.05): C, 63.88; H, 3.95%. Found: C, 62.71; H, 4.05%. HRMS (Q-TOF) m/z Calcd for $[M+H]^+$ 357.0483, found. 357.0555.

N-(4-bromophenyl)-N-(4-chlorobenzyl)nicotinamide (N6) Yield: 72% (72% by MW), mp: 156–158 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 5.06 (s, 2H, N-CH_2), 6.77 (d, 2H, *J*: 8.6 Hz, Ar–H), 7.33–7.15 (m, 8H, Ar–H), 7.65–7.62 (m, 1H, Ar–H), 8.51–8.49 (m, 1H, Ar–H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 53.24 (Ar-CH_2-N), 121.34 (C_{Ar}), 122.92 (CH_{Ar}), 128.90 (2xCH_{Ar}), 129.48 (2xCH_{Ar}), 130.10 (2xCH_{Ar}), 131.23 (C_{Ar}), 132.77 (2xCH_{Ar}), 133.73 (C_{Ar}), 135.04 (CH_{Ar}), 136.21 (CH_{Ar}), 141.42 (C_{Ar}), 149.58 (CH_{Ar}), 150.77 (CH_{Ar}), 167.96 (C=O). Anal. Calcd. for $C_{19}H_{14}BrClN_2O$ (MW 400.00): C, 56.81; H, 3.51%. Found: C, 57.20; H, 3.43%. HRMS (Q-TOF) m/z Calcd for $[M+H]^+$ 400.9978, found. 401.0050.

N-(4-chlorobenzyl)-N-phenylcinnamamide (C1) Yield: 80% (82% by MW), mp: 133–135 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 5.01 (s, 2H, N-CH_2), 6.33 (d, H, J_{trans}: 15.5 COCH=), 7.08 (d, 2H, *J*: 5.0 Hz, Ar–H), 7.28–7.20 (m, 8H, Ar–H), 7.41–7.30 (m, 4H, Ar–H), 7.76 (d, H, J_{trans}: 15.5 Ar–CH=). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 52.15 (Ar-CH_2-N), 118.06 (COCH=), 127.40 (2xCH_{Ar}), 127.49 (CH_{Ar}), 127.83 (2xCH_{Ar}), 128.08 (2xCH_{Ar}), 128.19 (2xCH_{Ar}), 129.11 (2xCH_{Ar}), 129.15 (CH_{Ar}), 129.64

(2xCH_{Ar}), 132.73 (C_{Ar}), 134.57 (C_{Ar}), 135.57 (C_{Ar}), 141.33 (C_{Ar}), 142.06 (CH = C_{Ar}), 165.55 (C = O). Anal. Calcd. for $C_{22}H_{18}ClNO$ (MW 347.11): C, 75.97; H, 5.22%. Found: C, 75.71; H, 5.42%. HRMS (Q-TOF) m/z Calcd for $[M+H]^+$ 348.1076, found. 348.1149.

N-(4-chlorobenzyl)-N-(p-tolyl)cinnamamide (C2) Yield: 78% (84% by MW), mp: 109–111 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 2.36 (s, 3H, CH_3Ar), 4.95 (s, 2H, N-CH_2), 6.33 (d, H, J_{trans}: 15.5 COCH=), 7.38–7.13 (m, 12H, Ar–H), 7.73 (d, H, J_{trans}: 15.5 Ar–CH=).^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 21.15 (CH_3-Ar), 52.69 (Ar-CH_2-N), 118.64 (COCH=), 127.92 (2xCH_{Ar}), 128.06 (2xCH_{Ar}), 128.55 (2xCH_{Ar}), 128.68 (2xCH_{Ar}), 129.60 (CH_{Ar}), 129.19 (2xCH_{Ar}), 130.23 (2xCH_{Ar}), 133.16 (C_{Ar}), 135.15 (C_{Ar}), 136.17 (C_{Ar}), 137.91 (C_{Ar}), 139.12 (C_{Ar}), 142.43 (CH = C_{Ar}), 166.18 (C = O). Anal. Calcd. for $C_{20}H_{15}ClFNO$ (MW 361.12): C, 76.34; H, 5.57%. Found: C, 74.49; H, 5.36%. HRMS (Q-TOF) m/z Calcd for $[M+H]^+$ 362.1233, found. 362.1306.

N-(4-chlorobenzyl)-N-(4-methoxyphenyl)cinnamamide (C3) Yield: 85% (85% by MW), mp: 84–87 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 3.84 (s, 3H, Ar-OCH_3), 4.96 (s, 2H, N-CH_2), 6.34 (d, H, J_{trans}: 15.5 COCH=), 6.88 (d, 2H, *J*: 8.7 Hz, Ar–H), 6.96 (d, 2H, *J*: 8.6 Hz, Ar–H), 7.32–7.20 (m, 8H, Ar–H), 7.74 (d, H, J_{trans}: 15.5 Hz, Ar–CH=).^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 52.23 (Ar-CH_2-N), 54.95 (OCH_3-Ar), 114.20 (2xCH_{Ar}), 118.06 (COCH=), 127.39 (2xCH_{Ar}), 128.05 (2xCH_{Ar}), 128.18 (2xCH_{Ar}), 128.99 (2xCH_{Ar}), 129.09 (CH_{Ar}), 129.79 (2xCH_{Ar}), 132.70 (C_{Ar}), 133.90 (C_{Ar}), 134.64 (C_{Ar}), 135.65 (C_{Ar}), 141.90(C_{Ar}), 158.49 (CH = C_{Ar}), 165.78 (C = O). Anal. Calcd. for $C_{23}H_{20}ClNO_2$ (MW 377.12): C, 73.11; H, 5.34%. Found: C, 73.01; H, 5.28%. HRMS (Q-TOF) m/z Calcd for $[M+H]^+$ 378.1182, found. 378.1255.

N-(4-chlorobenzyl)-N-(4-fluorophenyl)cinnamamide (C4) Yield: 73% (75% by MW), mp: 111–113 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 4.98 (s, 2H, N-CH_2), 6.29 (d, H, J_{trans}: 15.4 COCH=), 7.10–7.04 (m, 4H, Ar–H), 7.32–7.19 (m, 8H, Ar–H), 7.76 (d, H, J_{trans}: 15.4 Ar–CH=). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 52.17 (Ar-CH_2-N), 115.98–116.20 (J_{CF}:22.6 2xCH_{Ar}), 117.61 (COCH=), 127.41 (2xCH_{Ar}), 128.18 (2xCH_{Ar}), 128.21 (CH_{Ar}), 128.25 (2xCH_{Ar}), 129.30 (2xCH_{Ar}), 129.71 -129.61 (J_{CF}:10.7 2xCH_{Ar}), 132.92 (C_{Ar}), 134.43 (C_{Ar}), 135.31 (C_{Ar}), 137.26 (C_{Ar}), 142.50 (CH = C_{Ar}), 160.13–162.61 (J_{CF}:248.6 C_{Ar}), 165.78 (C = O). Anal. Calcd. for $C_{22}H_{17}ClFNO$ (MW 365.10): C, 72.23; H, 4.68%. Found: C, 71.25; H, 4.61%. HRMS (Q-TOF) m/z Calcd for $[M+H]^+$ 366.0982, found. 366.1055.

N-(4-chlorobenzyl)-N-(4-chlorophenyl)cinnamamide (C5) Yield: 81% (84% by MW), mp: 151–153 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 4.95 (s, 2H, N-CH_2), 6.28 (d, H, J_{trans}: 15.3 COCH=), 6.99–6.97 (m, 2H, Ar–H), 7.35–7.16 (m, 10H, Ar–H), 7.75 (d, H, J_{trans}: 15.7 Ar–CH=). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 52.79 (Ar-CH_2-N), 118.28 (COCH=), 128.18 (2xCH_{Ar}), 128.92 (2xCH_{Ar}), 128.99 (2xCH_{Ar}), 129.86 (2xCH_{Ar}), 130.08 (4xCH_{Ar}), 130.35 (CH_{Ar}), 133.66 (C_{Ar}), 134.07 (C_{Ar}), 135.09 (C_{Ar}), 135.96 (C_{Ar}), 140.55 (C_{Ar}), 143.40 (CH = C_{Ar}), 166.11 (C = O). Anal. Calcd. for $C_{22}H_{17}Cl_2NO$ (MW 381.07): C, 69.12; H, 4.48%. Found: C, 70.43; H, 4.62%. HRMS (Q-TOF) m/z Calcd for $[M+H]^+$ 382.0687, found. 382.0760.

N-(4-bromophenyl)-N-(4-chlorobenzyl)cinnamamide (C6) Yield: 74% (76% by MW), mp: 161–163 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 4.98 (s, 2H, N-CH_2), 6.30 (d, H, J_{trans}: 15.3 COCH=), 6.95 (d, 2H, *J*: 8.4 Hz, Ar–H), 7.19 (d, 2H, *J*: 8.3 Hz, Ar–H), 7.33–7.26 (m, 6H, Ar–H), 7.52 (d, 2H, *J*: 8.5 Hz, Ar–H), 7.77 (d, H, J_{trans}: 15.4 Hz, Ar–CH=). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 52.04 (Ar-CH_2-N), 117.52 (COCH=), 121.34 (C_{Ar}), 127.47 (2xCH_{Ar}), 128.21 (2xCH_{Ar}), 128.27 (2xCH_{Ar}), 129.38 (CH_{Ar}), 129.45 (2xCH_{Ar}), 129.61 (2xCH_{Ar}), 132.35 (2xCH_{Ar}), 132.95 (C_{Ar}), 134.35 (C_{Ar}), 135.21 (C_{Ar}), 140.35 (C_{Ar}), 142.74 (CH = C_{Ar}), 165.34 (C = O). Anal. Calcd. for $C_{22}H_{17}BrClNO$ (MW 425.02): C, 61.92; H, 4.02%. Found: C, 61.86; H, 4.09%. HRMS (Q-TOF) m/z Calcd for $[M+H]^+$ 426.0182, found. 426.0254.

The chemical structures of the synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS (see the supporting file for details).

2.2. Pharmacological/biological assays

AChE and BuChE inhibition assay

Tertiary benzamide/ nicotinamide/ cinnamamide derivatives were evaluated against AChE (E.C. 3.1.1.7, Type VI-S, Electrophorus electricus) and BuChE (E.C. 3.1.1.8, equine serum) spectrophotometrically by the method of Ellman with slight modifications using commercially available Tacrine as the reference compound [56]. Stock solutions were dissolved in dimethylsulfoxide and then diluted in a 50 mM Tris buffer (pH 8.0) to provide a final concentration range. In a 96-well polystyrene photometric microplates, the assay medium in each well consisted of 50 µL of a Tris buffer, 125 µL of 3 mM 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), 25 µL of 0.2 U/mL enzyme (AChE or BuChE), and a 15 mM substrate acetylthiocholine iodide (ATCI) or butyrylthiocholine iodide (BTCI). The assay mixture containing the enzyme, buffer, DTNB, and 25 µL of the inhibitor compound was preincubated for 15 min at 37 °C before the substrate was added to begin the reaction. All test compounds were prepared at different concentrations: 4–65 ng/mL. The absorbance of the reaction mixture was then measured three times at 412 nm every 45 s using a microplate reader (Bio-Tek

ELx800, Winooski, VT). IC50 values were obtained from activity (%) versus compounds plots.

Molecular docking studies

Docking simulation was carried out by using the Autodock4.2 program [57]. The crystal structures of AChE (PDB: 1EVE) and BuChE (PDB: 1P0I) enzymes in PDB format were used for the preparation of proteins. Molecules were drawn by using Chemdraw 19.0, and then passed to Chemdraw 3D 19.0. The molecules were minimized in the MM2 area and saved in PDB format.

Using Autodock Tools (ADT ver 1.5.6): All other hydrogens, water molecules, and non-standardized residues were removed, except polar hydrogens in the structure of proteins. Kollman united atom charges and solvation parameters were assigned to the proteins and the Gasteiger charge was assigned to the ligand. The modified structures obtained were converted to PDBQT format in ADT for AutoDock calculations. The grid size for specifying the search space was set at $60 \times 60 \times 60$ cenrred on the macromolecule with a default grid point spacing of 0.375 Å. The Lamarckian Genetic Algorithm was implemented with a population size of 10 dockings. Analysis of clustering conformations was performed on the docked results using an RMSD tolerance of 2.0 Å. The conformation with the lowest binding energy was evaluated by using Pyton Molecule Viewer (PMV ver.1.5.6) and Protein–Ligand Interaction Profiler (PLIP).

Prediction of physicochemical and ADME properties

The free SwissADME web tool was used for in silico prediction of the pharmacokinetic properties and ADME parameters of the synthesized compounds. The chemical structures of the compounds were drawn and converted to SMILES (simplified molecular-input line-entry system) by using the SwissADME. Finally, the program was run to calculate the physicochemical and pharmacokinetic parameters of the compounds. (http://www.swissadme.ch/index.php) [58].

Results

Chemistry

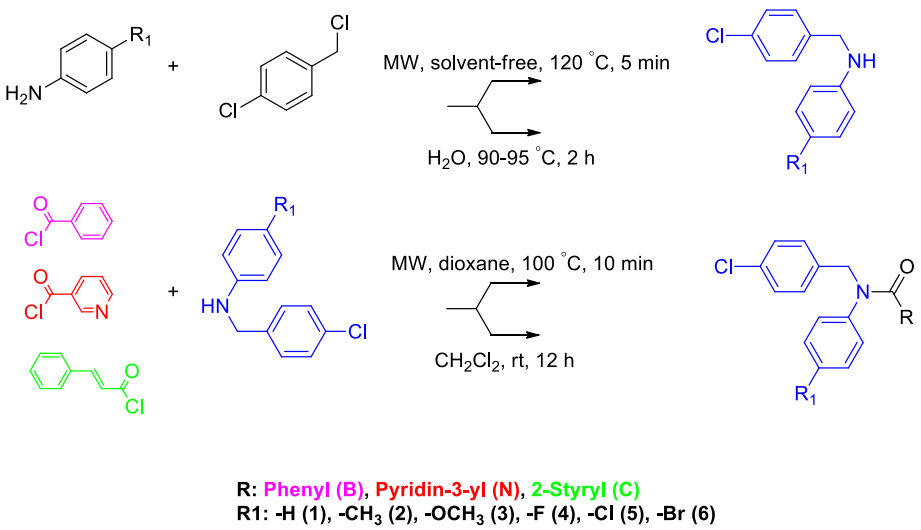
The compounds were synthesized and purified for the first time (except **B1** and **B5**) by conventional and microwave irradiation methods according to Scheme 1. First, secondary amines were synthesized by using a suitable aniline [aniline (**1**), 4-methylaniline (**2**), 4-methoxyaniline (**3**), 4-floroaniline (**4**), 4-chloroaniline (**5**), and 4-bromoaniline (**6**)]. Then, amides were synthesized successfully in the second step by using a suitable acid chloride (Scheme 1). The acid chlorides used in the series are as follows: benzoyl chloride for B series, nicotinamide chloride for N series, and cinnamamide chloride for the C series.

AChE and BuChE inhibitory activity

In vitro cholinesterase inhibitory activities of the compounds were reported for the first time in this study and the inhibition results were presented in Table 1.

Molecular docking studies

Among the benzamide, nicotinamide, and cinnamamide series, the compounds with the highest AChE inhibition potential (**B4, N4, and C4**) were docked at the binding site of AChE to explain the binding pattern of the compounds. The X-ray crystallographic structures of AChE



Scheme 1 Synthesis of the target compounds

Table 1 Inhibitory activity (IC50) of the compounds against ChEs

Compounds	R1	R–C = O	AChE (nM)	r^2	BuChE (nM)	r^2
B1	–H	Benzoyl	83.03	0.9853	66.68	0.9787
B2	–CH3	Benzoyl	79.98	0.9675	59.28	0.9857
B3	$–OCH_3$	Benzoyl	45.63	0.9979	38.37	0.9864
B4	–F	Benzoyl	15.42	0.9776	37.88	0.9732
B5	–Cl	Benzoyl	43.52	0.9881	49.16	0.9970
B6	–Br	Benzoyl	34.95	0.9957	54.73	0.9782
N1	–H	Nicotinoyl	51.09	0.9738	59.89	0.9897
N2	$–CH_3$	Nicotinoyl	64.00	0.9810	55.68	0.9724
N3	$–OCH_3$	Nicotinoyl	26.35	0.9911	42.02	0.9871
N4	–F	Nicotinoyl	12.14	0.9676	43.32	0.9946
N5	–Cl	Nicotinoyl	32.86	0.9676	45.55	0.9558
N6	–Br	Nicotinoyl	28.23	0.9890	48.75	0.9759
C1	–H	Cinnamoyl	48.94	0.9858	69.86	0.9806
C2	$–CH_3$	Cinnamoyl	57.62	0.9984	62.77	0.9322
C3	$–OCH_3$	Cinnamoyl	24.12	0.9516	32.74	0.9854
C4	–F	Cinnamoyl	10.67	0.9629	37.78	0.9847
C5	–Cl	Cinnamoyl	21.76	0.9947	49.50	0.9674
C6	–Br	Cinnamoyl	26.90	0.9946	54.03	0.9949
Tacrine*			20.85	0.9839	15.66	0.9874

*Tacrine was used as a standard inhibitor towards both AChE and BuChE enzymes. r^2: is a statistical measure of how close the data are to the fitted regression line. It is also known as the coefficient of determination, or the coefficient of multiple determinations for multiple regressions[59]

Table 2 Binding free energies of **B4, N4,** and **C4** in the AChE binding site

Compound	Binding Free Energy (kcal/mol)
B4	− 9.09
N4	− 9.30
C4	− 9.58

(1EVE) were obtained from the Protein Data Bank (PDB, https://www.rcsb.org/). The binding free energies of the most active compounds **B4, N4,** and **C4** were presented in Table 2.

Prediction of physicochemical and ADME properties

Molecular weights, the numbers of hydrogen bond donors and acceptors, the topological surface areas (TPSA; a sum of polar atoms' surfaces), lipophilicities, and water solubilities of all compounds were calculated and presented in the supporting file. ADME profiles of the most active compounds in each series were calculated and presented in Table 3.

Table 3 In silico physicochemical properties of the compounds **B4**, **N4**, and **C4**

Compound	MW[a]	HBA[b]	HBD[c]	TPSA[d]	$CLogP_{o/w}$[e]	logS[f]	Violations[g]
B4	339.79	2	0	20.31	4.92	− 5.42	1
N4	340.78	3	0	33.20	4.10	− 4.75	0
C4	365.83	2	0	20.31	5.29	− 5.75	1
Tacrine	198.26	1	1	38.91	2.59	− 3.27	0

[a]Molecular weight (< 500 Da),

[b]Number of hydrogen bond acceptors (< 10),

[c]Number of hydrogen bond donors (< 5),

[d]Topological polar surface area (20–130 $Å^2$),

[e]Octanol/water partition coefficient (recommended range: − 2.0 to 6.5),

[f]Aqueous solubility prediction (not higher than 6),

[g]Number of Lipinski's rule of 5 violations

Discussion

Chemistry

Secondary amines were synthesized in the range of 80–90% yields by both conventional and microwave irradiation methods. The reaction took place in a solvent-free environment in a shorter period under the microwave irradiation method while it took place in the aqueous medium by the conventional method. In both methods, the completion of the reaction was checked by TLC and all of the compounds were purified by column chromatography on silica gel with hexane: ethyl acetate (2:1) as the mobile phase.

Amides were also synthesized in similar yields (72–85%) by both conventional and microwave irradiation methods (Scheme 1). The fact that the reaction takes place in lower solvent conditions (1 mL) and the procedure can be carried out in 10 min makes microwave assisted synthesis preferable over the conventional method. Moreover, the methodologies proceeds with high atom economy which is in harmony with the green chemistry laws. The reaction processes includes low energy consumption process; it either occurs at room temperature in CH_2Cl_2 or with a moderate temperature by microwave irradiation in dioxane. The products can be isolated with high yields and selectivity in both cases (microwave assisted and conventional). As a result, the microwave irradiation method provided great advantages in terms of reaction periods, especially for the synthesis of amide derivatives.

AChE and BuChE inhibitory activity

Tacrine was used as the reference drug and its IC_{50} values were 20.85 nM and 15.66 nM against AChE and BuChE, respectively. According to Table 1, IC50 values of the compounds were in the range from 10.67 nM to 83.03 nM against AChE, while they were in the range from 32.74 nM to 66.68 nM against BuChE.

According to Table 1, compound **C4** had a higher IC50 value (about 2 times) than the reference drug towards AChE. The inhibition results in Table 1 pointed out that the synthesized compounds had stronger inhibition effects towards AChE than BuChE enzyme. The most active compound in the series was 4-fluoro substituted compound **C4** (cinnamamide derivative) having IC_{50} value of 10.67 nM in terms of AChE inhibitory activity. On the other hand, the results in Table 1 showed that halogen substitution at the 4th position of the phenyl ring (series 4, 5, 6) was a useful modification in terms of AChE inhibitory activity. When the inhibition results in Table 1 were evaluated, it was seen that the substitution of a methoxy group rather than a methyl group at the 4th position of the phenyl ring led to an increase in AChE inhibitory activity (about 2 times).

The inhibition results presented in Table 1 showed that all compounds synthesized had higher IC50 values than Tacrine against BuChE. According to BuChE inhibition results presented in Table 1, the most active compound was 4-methoxy substituted and cinnamamide derivative compound **C3** having IC_{50} value of 32,74 nM against BuChE.

Molecular docking studies

According to the results in Table 2, the binding free energy of the **C4** (-9.58 kcal/mol) was higher than the free binding energy of the compounds **B4** (-9.09 kcal/mol) and **N4** (-9.30 kcal/mol). Also, the results showed that the binding free energy scores of the compounds B4, N4, and C4 presented in Table 2 were in an agreement with in vitro AChE inhibition results presented in Table 1.

According to the **B4**-1EVE complex presented in Fig. 1, π-π charge transfer interactions were observed between the indole ring of TRP84A and the 4-fluoro substituted phenyl ring of the compound **B4** (3.63 Å and 4.02 Å, respectively). On the other hand, many hydrophobic interactions were determined between **B4** and the active site of the enzyme, 1EVE (Fig. 1).

According to the **N4**-1EVE complex presented in Fig. 2, hydrogen bondings were observed between N atom in the pyridine ring of the compound **N4** and the amino acids GLY118A (NH; 3.13 Å), GLY119A (NH; 3.01 Å), HIS440A (ArNH; 3.33 Å), SER200A (OH; 1.89 Å). In addition, the carbonyl group of the compound **N4** realized a hydrogen

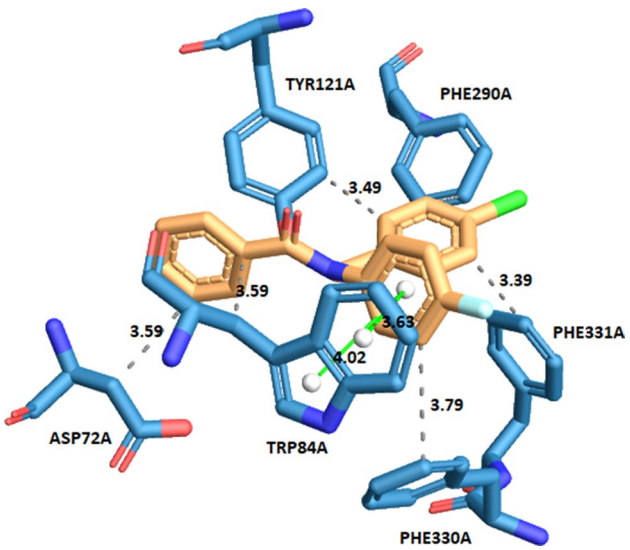


Fig. 1 Schematic presentation of interactions between **B4** and AChE (1EVE). Green colour represents *π-π* charge transfer interactions and grey represents pink represents hydrophobic interactions

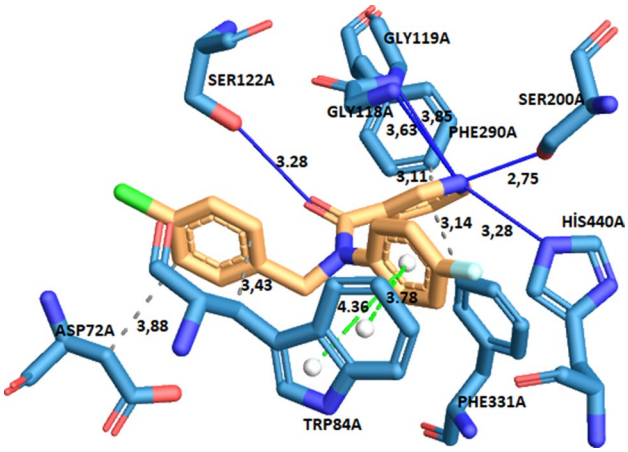


Fig. 2 Schematic presentation of interactions between the compound **N4** and AChE (1EVE). Blue colour represents hydrogen bondings, green colour represents π-π charge transfer interactions and grey represents hydrophobic interactions

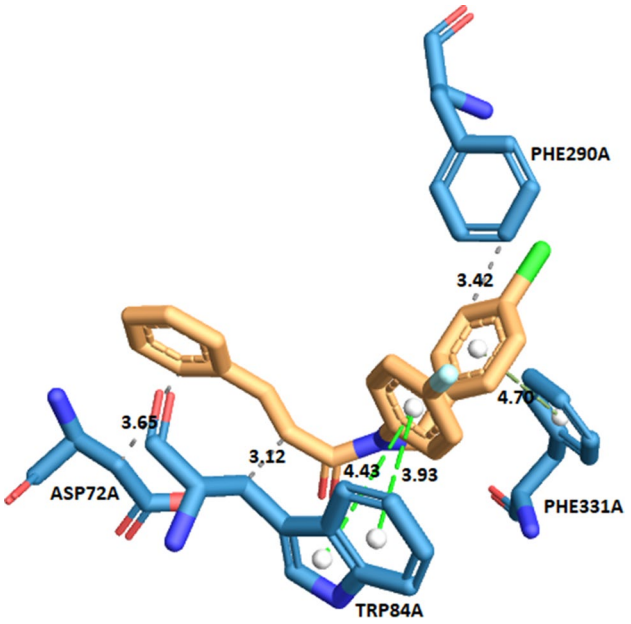


Fig. 3 Schematic presentation of interactions between the compound C4 and AChE (1EVE). Green colour represents π-π charge transfer interactions and grey represents hydrophobic interactions

bonding with the side chain of the amino acid SER122 (OH; 2.86 Å). π-π charge transfer interactions were determined between the indole ring of TRP84A and the 4-fluoro phenyl ring of the compound **N4** (4.02 Å and 3.63 Å, respectively). Besides, many hydrophobic interactions were identified between **N4** and the active site of the enzyme, 1EVE (Fig. 2).

According to the **C4**—1EVE complex presented in Fig. 3, π-π charge transfer interactions were observed between the indole structure of TRP84A and the 4-fluoro substituted phenyl ring of the compound **C4** (4.43 Å and 3.93 Å, respectively). Also, the benzene ring of the 4-chlorobenzyl of the molecule realized a π-π charge-transfer interaction with the benzene ring of PHE331A (4.70 Å) (Fig. 3).

The molecular modelling studies showed that the benzene rings in the amide part of the molecules realized the charge-transfer interaction with the anionic side of AChE (TRP84A, PHE331A). Besides, the nicotinoyl structure replaced by the benzoyl structure of the compounds led to the formation of hydrogen bonds with both the catalytic (SER200A, HİS440A) and oxyanion side (GLY118A, GLY119A) of the enzyme.

Prediction of physicochemical and ADME properties

The physicochemical properties of a molecule are very important in designing drug-candidate because they determine the pharmacokinetic properties of the molecule. 'Drug-likeness' is a term explained earlier in the literature that describes the possibility for a molecule to be used as an oral drug in terms of bioavailability [58]. In medicinal chemistry, Lipinski's 5 rules are used to score the drug-likeness properties of a compound [60, 61].

According to the results presented in the supporting file, ClogP values of the compounds were in the range of 4.59–5.21(<5) for benzamide (B) series, 3.76–4.38 (<5) for nicotinamide (N) series, and 4.94–5.59 for cinnamamide (C) series. The molecular weights of the compounds were in the range of 321.80–400.70 (<500) for benzamide (B) series, 322.79–401.68 (<500) for nicotinamide (N) series, and 347.84–426.76 (<500) for cinnamamide (C) series. HBA values of the compounds ranged 1–3 (≤ 10) and HBD values of the compounds were 0 (<5) for each series. When these results were compared with the reference drug Tacrine, it was seen that the ADME profiles of the N series had the closest values to the physicochemical properties of Tacrine. Besides, the most active compounds in each series towards AChE **B4, N4,** and **C4** had desired logP values (Table 3) in terms of penetrating the blood–brain barrier. This means that the compounds had CNS effects in terms of being used as AChE inhibitors for the treatment of AD. According to Table 3, both compounds **B4** and **C4** had 1 violation in terms of Lipinski's rule of five because of having a MlogP value higher than 4.15.

Conclusion

The newly designed (except **B1** and **B5**) total of 18 compounds, N-(4-chlorobenzyl)-N-(4-substituted phenyl)benzamide/nicotinamide/cinnamamide, were synthesized and purified successfully for the first time with their potential

inhibitory effects on AChE and BuChE enzymes. According to the inhibition results, the IC50 values of the compounds synthesized were in the range of 10.66–83.03 nM towards AChE, while they were in the range of 32.74–66.68 nM towards BuChE. The inhibition results showed that the newly synthesized compounds had higher inhibitory effects towards AChE than BuChE. According to the AChE inhibition results, among the synthesized benzamide/nicotinamide/cinnamamide derivatives, 4-floroaniline derivatives (**B4, N4, C4**) showed the highest AChE inhibitory activities in each series and had the highest selectivity to AChE. The most active compounds **B4** (IC50: 15.42 nM), **N4** (IC50: 12.14 nM), and **C4** (IC50: 10.67 nM) in each series had higher in vitro AChE inhibitory activity than the reference drug TAC (IC50: 20.85 nM). The most active compound towards AChE in the series was the cinnamamide derivative compound **C4** having a lower IC50 value (about two times) than TAC. Besides, the inhibition results of the compounds towards AChE suggested that replacing the cinnamamide structure with benzamide and nicotinamide structure led to an increase in AChE inhibitory effect. On the other hand, the most active compounds **B4**, **N4**, and **C4** in each series towards AChE were docked at the binding sites of the enzyme to explain the inhibitory activities of each series. ADME prediction studies of the compounds showed that the newly designed compounds were not only potent AChE inhibitors but also had desired physicochemical and ADME profiles for further studies as drug candidates.

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Declaration

Conflict of interest The authors declare that there is no conflict of interest.

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