Acta Poloniae Pharmaceutica – Drug Research, Vol. 69 No. 3 pp. 449–455, 2012 ISSN 0001-6837
Polish Pharmaceutical Society

SYNTHESIS OF NOVEL N-BENZYL SUBSTITUTED PIPERIDINE AMIDES OF 1H-INDOLE-5-CARBOXYLIC ACID AS POTENTIAL INHIBITORS OF CHOLINESTERASES

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Abstract: A series of novel *N*-benzyl substituted amides of 1*H*-indole-5-carboxylic acid were synthesized and evaluated for their ability to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). The target compounds (**6b–6e**) displayed moderate potency to inhibit BuChE. One of the compounds tested, i.e., 1-benzylpiperidine amide of 1*H*-indole-5-carboxylic acid (**6a**) was a weak, non-selective inhibitor for both enzymes. The highest inhibitory activity towards BuChE (30.06% [10 μM]) was determined for compound (**6c**) which is 1-(3-chloro)benzylpiperidine amide of 1*H*-indole-5-carboxylic acid.

Keywords: Alzheimer's disease, acetylcholinesterase inhibitors, butyrylcholinesterase inhibitors, *N*-benzylpiperidines, 1*H*-indole-5-carboxylic acid derivatives

Alzheimer's disease (AD) is said to be the leading cause of dementia in elderly individuals. The neuropathologic hallmarks of this illness are the presence of extracellular deposits of β-amyloid peptide as fibril aggregates that form neurotic plaques and intracellular neurofibrillary tangles. Neurochemically AD is characterized by a consistent deficit in cholinergic neurotransmission, particularly affecting cholinergic neurons in specific regions of the brain (1, 2). Due to several theoretical possibilities of AD therapy, the earliest (clinically relevant) approach is using acetylcholinesterase inhibitors (AChEIs). This finding resulted in the introduction of 1,2,3,4-tetrahydro-9-aminoacridine (tacrine) as the first AChEI, specifically approved for the treatment of AD (3). Currently, three cholinesterase inhibitors such as donepezil (4), galantamine (5) and rivastigmine (6) are widely used for the symptomatic treatment of patients with mild-to-moderate AD. These drugs represent different chemical classes of compounds and exhibit different selectivity for both cholinesterases, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Rivastigmine is an inhibitor of both enzymes, whereas donepezil and galantamine are inhibitors of AChE alone. Tacrine, because of its hepatotoxicity, is not used in clinical practice; it is used as reference inhibitor of both enzymes.

Currently, some investigations indicated that BuChE could also play a significant role in the regulation of acetylcholine in the human brain. Therefore, compounds which inhibit BuChE may also provide added benefits in the therapy of AD. It is known that BuChE is mainly associated with glial cells and it is found in much lower concentrations than AChE in the healthy brain. However, over the course of AD, AChE activity progressively decreases, while BuChE activity increases and BuChE may act as a compensatory mechanism for acetylcholine metabolism (7, 8). Consequently, with AD progress, AChE regulation may become increasingly dependent on BuChE, and dual inhibitors may provide more sustained efficacy than AChE selective agent. As part of our studies (9) aimed at the development of cholinesterase inhibitors a series of *N*-benzyl substituted amides of 1*H*-indole-5-carboxylic acid derivatives (**6a–f**) was synthesized. The designed structures contain pharmacophoric *N*-benzylpiperidine moiety connected by amide group with heteroaromatic indole fragment. The benzylpiperidine fragment of molecules designed is based on donepezil structure and is a very well known fragment in numerous AChE inhibitors (Fig. 1) (9–11).

It was assumed that the molecules designed might interact with both the catalytic anionic site (CAS) and the peripheral anionic site (PAS) of the

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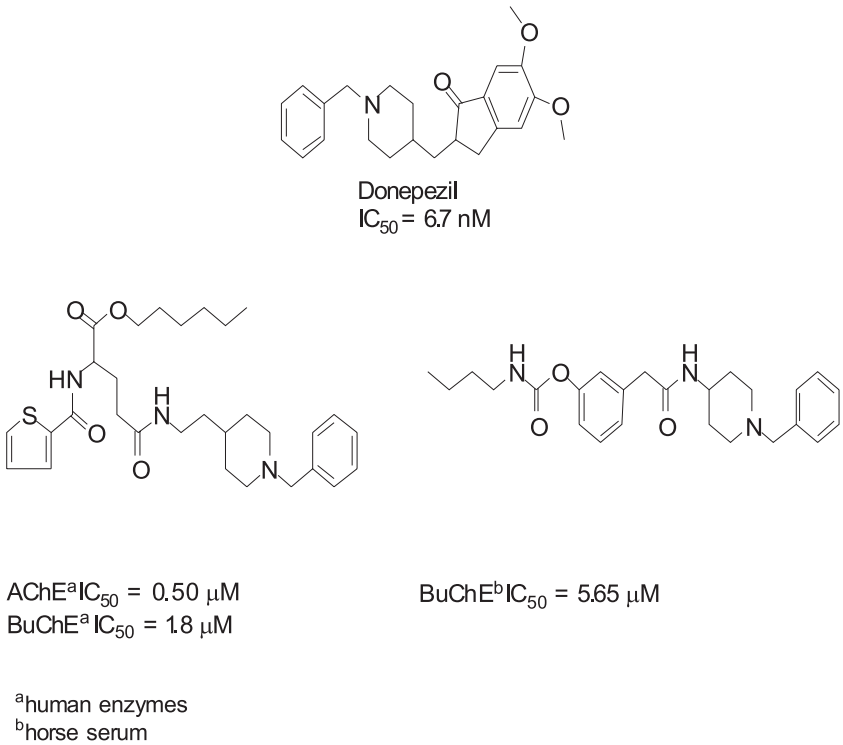


Figure 1. Donepezil and AChE inhibitors (4, 9–11)

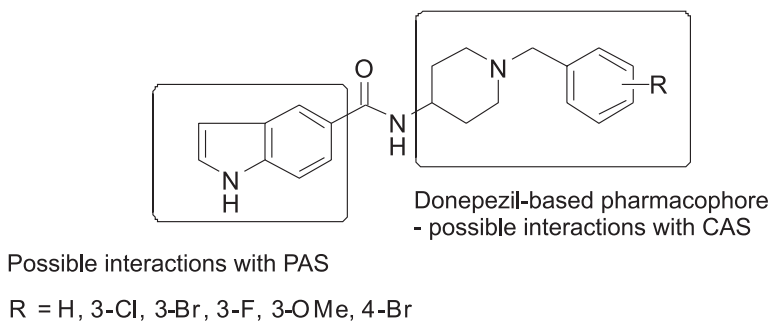


Figure 2. Schematic structure of compounds design

enzyme. In the present study, the synthesis of new *N*-benzyl substituted piperidine amides of 1*H*-indole-5-carboxylic acid and their *in vitro* biological evaluation towards AChE and BuChE inhibitions is described (Fig. 2).

EXPERIMENTAL

Chemistry

As a starting material for the synthesis the final amides, piperidin-4-ol (**1**) was chosen. In the first step, compound (**1**) reacted under the phase transfer catalysis conditions with appropriate substituted benzylbromide derivatives (**2a–f**), giving relevant *N*-benzylpiperidin-4-ol derivatives (**3a–f**) with good to excellent (72–99%) yields. These reactions were carried out in the mixture of acetonitrile, K_2CO_3 and tetrabutylammonium iodide. In the next step, the obtained alcohols (**3a–f**) were transformed into relevant substituted 2-(1-benzylpiperidin-4-yl)isoindoline-1,3-dione derivatives (**4a–f**) in the Mitsunobu reaction (12), which was carried out in dry THF

using phtalimide, triphenylphosphine (Ph_3P) and diisopropylazodicarboxylate (DIAD) under argon atmosphere. The yields of this process were rather low between 17 and 31%. Hydrazinolysis of compounds (**4a–f**) gave primary amines (**5a–f**), which have been used for further synthesis without purification. Finally, the commercially available 1*H*-indole-5-carboxylic acid was activated with carbonyldiimidazole (CDI) and reacted with the appropriate amine (**5a–f**) in dry THF (13) giving the final compounds (**6a–f**) with low to good yield (**6c**) (25–85%). The structures of the new compounds were confirmed by elemental analysis and spectral data (^{1}H NMR, MS), their purity by thin layer chromatography.

Reagents and solvents were purchased from common commercial suppliers, and were used without further purification. All experiments, in which air-sensitive materials were used, were carried out in oven-dried glassware under a dry argon atmosphere. Standard vacuum techniques were used for handling air-sensitive materials. Tetrahydrofuran (THF) was dried, kept under argon and freshly distilled over sodium-benzophenone before use. Melting points were determined in open capillaries on an Electrothermal 9300 apparatus and are uncorrected. Merck silica gel 60 (70–230 mesh ASTM) was used for column chromatography. As solvents for chromatography mixtures: S_1 dichloromethane/methanol (9:1), S_2 dichloromethane/methanol (95:5), S_3 acetone, were used. Analytical thin layer chromatography was performed on Merck TLC plates, silica gel 60 F_{254}. TLC visualization was achieved with a UV lamp or ninhydrin solution (0.3% ninhydrin in *n*-BuOH with 3% AcOH). Nuclear magnetic resonance spectra (^{1}H NMR) were recorded with Varian Mercury VX 300 MHz (Hansen Way, USA) spectrometer using $CDCl_3$ or DMSO-d_6 at ambient temperature using the solvent signal as an internal standard. The chemical shifts (δ) are reported in parts per million (ppm). In the case of solid products elemental analyses were performed on Vario EL III Elemental analyzer (Elementar Analysensystem, Hanau, Germany). Mass spectras were recorded on Waters Acquity TQD spectrometer (Waters, USA). Mass spectra were obtained by electronic impact (EI) at 70 eV.

General procedure for the synthesis of compounds 3a–f

Piperidin-4-ol (**1**) (2.03 g, 20 mmol) and relevant benzylbromide derivatives (**2a–f**) (20 mmol) were dissolved in 20 mL of acetonitrile. Then, anhydrous K_2CO_3 (2.76 g, 20 mmol) and TBAI (0.08 g, 0.2 mmol) were added. The reaction mixture was refluxed for 24 h. The inorganic salt was filtered and washed with methanol. The filtrate was evaporated and the oil obtained was purified by column chromatography using a mixture of dichloromethane/methanol (9:1).

1-Benzylpiperidin-4-ol (3a)

Yellow oil. Yield: 72%; TLC: R_f = 0.36 (S_1); ^{1}H NMR ($CDCl_3$, δ, ppm): 1.22 (s, CHO*H*, 1H), 1.51–1.69 (m, piper, 2H), 1.82–1.95 (m, piper, 2H), 2.09–2.22 (m, piper, 2H), 2.69–2.82 (m, piper, 2H), 3.51 (s, NCH_2, 2H), 3.62–3.77 (m, C*H*OH, 1H), 7.19–7.36 (m, arom, 5H).

1-(3-Bromo)benzylpiperidin-4-ol (3b)

Yellow oil. Yield: 99%; TLC: R_f = 0.50 (S_1); ^{1}H NMR ($CDCl_3$, δ, ppm): 1.49–1.68 (m, CHO*H*, piper, 3H), 1.80–1.94 (m, piper, 2H), 2.07–2.21 (m, piper, 2H), 2.66–2.79 (m, piper, 2H), 3.47 (s, NCH_2, 2H), 3.64–3.75 (m, C*H*OH, 1H), 7.12–7.50 (m, arom, 4H).

1-(3-Chloro)benzylpiperidin-4-ol (3c)

Yellow oil. Yield: 90%; TLC: R_f = 0.41 (S_1); ^{1}H NMR ($CDCl_3$, δ, ppm): 1.50–1.70 (m, CHO*H*, piper, 3H), 1.82–1.94 (m, piper, 2H), 2.08–2.22 (m, piper, 2H), 2.67–2.79 (m, piper, 2H), 3.47 (s, NCH_2, 2H), 3.63–3.76 (m, C*H*OH, 1H), 7.14–7.35 (m, arom, 4H).

1-(3-Fluoro)benzylpiperidin-4-ol (3d)

Yellow oil. Yield: 86%; TLC: R_f = 0.46 (S_1); ^{1}H NMR ($CDCl_3$, δ, ppm): 1.51–1.68 (m, piper, 2H), 1.82–1.96 (m, CHO*H*, piper, 3H), 2.10–2.23 (m, piper, 2H), 2.68–2.80 (m, piper, 2H), 3.49 (s, NCH_2, 2H), 3.62–3.77 (m, C*H*OH, 1H), 6.88–7.31 (m, arom, 4H).

1-(3-Methoxy)benzylpiperidin-4-ol (3e)

Yellow oil. Yield: 98%; TLC: R_f = 0.20 (S_1); ^{1}H NMR ($CDCl_3$, δ, ppm): 1.51–1.67 (m, CHO*H,* piper, 3H), 1.82–1.94 (m, piper, 2H), 2.09–2.24 (m, piper, 2H), 2.69–2.83 (m, piper, 2H), 3.48 (s, NCH_2 arom, 2H), 3.64–3.75 (m, C*H*OH, 1H), 3.80 (s, OCH_3, 3H), 6.75–7.28 (m, arom, 4H).

1-(4-Bromo)benzylpiperidin-4-ol (3f)

Yellow oil. Yield: 98%; TLC: R_f = 0.40 (S_1); ^{1}H NMR ($CDCl_3$, δ, ppm): 1.49–1.73 (m, CHO*H*, piper, 3H), 1.80–1.94 (m, piper, 2H), 2.07–2.21 (m, piper, 2H), 2.66–2.77 (m, piper, 2H), 3.47 (s, NCH_2, 2H), 3.62–3.77 (m, C*H*OH, 1H), 7.14–7.48 (m, arom, 4H).

General procedure for the synthesis of compounds 4a–f

To the stirred solution of Ph_3P (6.4 g, 24 mmol) in dry THF (150 mL), maintained at 0°C under an atmosphere of dry argon, DIAD (4.4 g, 20 mmol) was added dropwise. The resulting solution was stirred at the same temperature for 20 min whereupon it showed a creamy white color. Then, relevant 1-benzylpiperidin-4-ol (**3a–f**) (20 mmol) dissolved in THF (26 mL) was added dropwise. Finally, phthalimide (3.28 g, 20 mmol) was added in one portion. The resulting mixture was stirred at 0°C for another 20 min and then for 72 h at room temperature. The solvent was removed in vacuum. Diethyl ether was added to the residue to precipitate triphenylphosphine oxide, which was filtered off after 24 h. The filtrate was evaporated, and toluene was added to the residue to precipitate diisopropyl hydrazinedicarboxylate. The obtained precipitate was filtered off after 48 h. The filtrate was evaporated, and the product was purified by crystallization from methanol.

2-(1-Benzylpiperidin-4-yl)isoindoline-1,3-dione (4a)

White crystals. Yield: 23%; m.p. 157–158°C, lit. 156–157°C (14); Analysis: calcd. for $C_{20}H_{20}N_2O_2$: C 74.98, H 6.29, N 8.74%; found: C 74.90, H 5.30, N 8.76%; TLC: R_f = 0.78 (S_2); MS (m/z): 321 $[M+H]^+$; ^{1}H NMR ($CDCl_3$, δ, ppm): 1.55–1.71 (m, piper, 2H), 2.04–2.16 (m, piper, 2H), 2.48–2.65 (m, piper, 2H), 2.95–3.06 (m, piper, 2H), 3.56 (s, NC*H*$_2$, 2H), 4.06–4.12 (m, C*H*OH, 1H), 7.22–7.40 (m, arom, 3H), 7.66–7.91 (m, arom, 6H).

2-[1-(3-Bromo)benzylpiperidin-4-yl]isoindoline-1,3-dione (4b)

White crystals. Yield: 22%; m.p. 108–113°C; Analysis: calcd. for $C_{20}H_{19}N_2O_2Br$: C 60.16, H 4.80, N 7.02%; found: C 60.10, H 4.82, N 7.06%; TLC: R_f: = 0.88 (S_2); MS (m/z): 399 $[M+H]^+$; ^{1}H NMR ($CDCl_3$, δ, ppm): 1.57–1.73 (m, piper, 2H), 2.04–2.17 (m, piper, 2H), 2.48–2.65 (m, piper, 2H), 2.92–3.03 (m, piper, 2H), 3.51 (s, NC*H*$_2$, 2H), 4.06–4.20 (m, C*H*OH, 1H), 7.14–7.42 (m, arom, 4H), 7.65–7.86 (m, arom, 4H).

2-[1-(3-Chloro)benzylpiperidin-4-yl]isoindoline-1,3-dione (4c)

White crystals. Yield: 31%; m.p. 96–99°C; Analysis: calcd. for $C_{20}H_{19}N_2O_2Cl$: C 67.70, H 5.40, N 7.89%; found: C 67.74, H 5.43, N 7.86%; TLC: R_f = 0.95 (S_2); MS (m/z): 355 $[M+H]^+$; ^{1}H NMR ($CDCl_3$, δ, ppm): 1.60–1.77 (m, piper, 2H), 2.04–2.18 (m, piper, 2H), 2.48–2.66 (m, piper, 2H), 2.93–3.04 (m, piper, 2H), 3.52 (s, NC*H*$_2$, 2H), 4.06–4.20 (m, C*H*OH, 1H), 7.20–7.40 (m, arom, 4H), 7.63–7.88 (m, arom, 4H).

2-[1-(3-Fluoro)benzylpiperidin-4-yl]isoindoline-1,3-dione (4d)

White crystals. Yield: 17%; m.p. 99°C; TLC: R_f = 0.90 (S_2); Analysis: calcd. for $C_{20}H_{19}N_2O_2F$: C 70.99, H 5.66, N 8.25%; found: C 70.90, H 5.62, N 8.26%; MS (m/z): 339 $[M+H]^+$; ^{1}H NMR ($CDCl_3$, δ, ppm): 1.59–1.76 (m, piper, 2H), 2.04–2.18 (m, piper, 2H), 2.48–2.65 (m, piper, 2H), 2.93–3.04 (m, piper, 2H), 3.54 (s, NC*H*$_2$, 2H), 4.06–4.21 (m, C*H*OH, 1H), 6.89–7.33 (m, arom, 2H), 7.65–7.87 (m, arom, 6H).

2-[1-(3-Methoxy)benzylpiperidin-4-yl]isoindoline-1,3-dione (4e)

White crystals. Yield: 22%; m.p. 131°C. Analysis: calcd. for $C_{21}H_{22}N_2O_3$: C 71.98, H 6.33, N 7.99%; found: C 71.91, H 6.23, N 7.92%; TLC: R_f = 0.67 (S_2); MS (m/z): 351 $[M+H]^+$; ^{1}H NMR ($CDCl_3$, δ, ppm): 1.58–1.75 (m, piper, 2H), 2.04–2.18 (m, piper, 2H), 2.48–2.66 (m, piper, 2H), 2.95–3.07 (m, piper, 2H), 3.53 (s, NC*H*$_2$, 2H), 3.83 (s, OC*H*$_3$, 3H), 4.05–4.20 (m, C*H*OH, 1H), 6.76–7.29 (m, arom, 4H), 7.65–7.88 (m, arom, 4H).

2-[1-(4-Bromo)benzylpiperidin-4-yl]isoindoline-1,3-dione (4f)

White crystals. Yield: 30%; m.p. 131°C; Analysis: calcd. for $C_{20}H_{19}BrN_2O_2$: C 60.16, H 4.80, N 7.02%; found: C 60.18, H 4.70, N 7.01%; TLC: R_f = 0.82 (S_2); MS (m/z): 399 $[M+H]^+$; ^{1}H NMR ($CDCl_3$, δ, ppm): 1.55–1.71 (m, piper, 2H), 2.02–2.14 (m, piper, 2H), 2.47–2.64 (m, piper, 2H), 2.91–3.02 (m, piper, 2H), 3.49 (s, NC*H*$_2$, arom, 2H), 4.06–4.19 (m, C*H*OH, 1H), 7.21–7.48 (m, aromat, 2H), 7.66-7.88 (m, aromat, 6H).

General procedure for the synthesis of compounds 5a-f

To a stirred solution of various substituted 2-(1-benzylpiperidin-4-yl)isoindoline-1,3-diones (**4a-f**) (3 mmol) in methanol (60 mL) hydrazine hydrate (3 g, 60 mmol) was added dropwise. The resulting mixture was stirred at room temperature for 24 h. Then, the obtained white precipitate was filtered off and washed with methanol. The filtrate was evaporated, and the remaining oil was dissolved in water (60 mL) and extracted with dichloromethane (3 × 60 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vac-

uum. The obtained oil was used in further synthesis without purification.

General procedure for the synthesis of compounds 6a–f

CDI (1 eq.) was added to a solution of 1*H*-indole-5-carboxylic acid (1 eq. for compounds **5a–c** or 1.5 eq for compounds **5d–f**) in dry THF under an atmosphere of dry argon. Thirty minutes later, various substituted 4-amine-1-benzylpiperidines (**5a–f**) (1 eq.) dissolved in THF were added, and the reaction mixture was stirred at room temperature for 24 h. Then, the solvent was removed in vacuum and the oil obtained was purified by column chromatography using an acetone as a solvent.

1H-indole-5-carboxylic acid (1-benzylpiperidin-4-yl)-amide (6a)

Reagents: 4-amine-1-benzylpiperidine (0.38 g, 2 mmol), 1*H*-indole-5-carboxylic acid (0.32 g, 2 mmol), CDI (0.32 g, 2 mmol), THF (6 mL). Yellow semi-solid. Yield: 83%. Analysis: calcd. for $C_{21}H_{23}N_3O$: C 75.65, H 6.95, N 12.60%; found: C 75.24, H 6.92, N 12.14%; TLC: R_f = 0.51 (S_3); MS (m/z): 334 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, δ, ppm): 1.49–1.85 (m, piper, 4H), 1.93–2.07 (m, piper, 2H), 2.72–2.90 (m, piper, 2H), 3.45 (s, NCH_2, 2H), 3.68–3.87 (m, C*H*NH, 1H), 6.50 (s, N*H*CO, 1H), 7.15–7.67 (m, arom, 8H), 7.99–8.15 (m, arom, 2H), 11.28 (s, N*H* ind, 1H).

1H-indole-5-carboxylic acid [1-(3-bromo)benzylpiperidin-4-yl]-amide (6b)

Reagents: 4-amine-1-(3-bromo)benzylpiperidine (0.54 g, 2.04 mmol), 1*H*-indole-5-carboxylic acid (0.33 g, 2.04 mmol), CDI (0.33 g, 2.04 mmol), THF (6 mL). Yellow oil. Yield: 59%; TLC: R_f = 0.74 (S_3); MS (m/z): 412 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, δ, ppm): 1.50–1.67 (m, piper, 2H), 1.72–1.83 (m, piper, 2H), 1.94–2.06 (m, piper, 2H), 2.74–2.86 (m, piper, 2H), 3.46 (s, NCH_2, 2H), 3.70–3.86 (m, C*H*NH, 1H), 6.50 (s, N*H*CO, 1H), 7.23–7.63 (m, arom, 7H), 8.01–8.12 (m, arom, 2H), 11.28 (s, N*H* ind, 1H).

1H-indole-5-carboxylic acid [1-(3-chloro)benzylpiperidin-4-yl]-amide (6c)

Reagents: 4-amine-1-(3-chloro)benzylpiperidine (0.5 g, 2.26 mmol), 1*H*-indole-5-carboxylic acid (0.36 g, 2.26 mmol), CDI (0.36 g, 2.26 mmol), THF (6 mL). Yellow oil. Yield: 25%; TLC: R_f = 0.82 (S_3); MS (m/z): 368 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, δ, ppm): 1.50–1.69 (m, piper, 2H), 1.71–1.85 (m, piper, 2H), 1.94–2.13 (m, piper, 2H), 2.72–2.86 (m, piper, 2H), 3.48 (s, NCH_2, 2H), 3.70–3.87 (m, C*H*NH, 1H), 6.50 (s, N*H*CO, 1H), 7.21–7.57 (m, arom, 7H), 8.02–8.14 (m, arom, 2H), 11.28 (s, N*H* ind, 1H).

1H-indole-5-carboxylic acid [1-(3-fluoro)benzylpiperidin-4-yl]-amide (6d)

Reagents: 4-amine-1-(3-fluoro)benzylpiperidine (0.65 g, 2.36 mmol), 1*H*-indole-5-carboxylic acid (0.57 g, 3.5 mmol), CDI (0.57 g, 3.5 mmol), THF (6 mL). Yellow oil. Yield: 85%; TLC: R_f 0,80 (S_3); MS (m/z): 352 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, δ, ppm): 1.48–1.70 (m, piper, 2H), 1.72–1.85 (m, piper, 2H), 1.92–2.12 (m, piper, 2H), 2.74–2.88 (m, piper, 2H), 3.48 (s, NCH_2, 2H), 3.66–3.88 (m, C*H*NH, 1H), 6.50 (s, N*H*CO, 1H), 6.95–7.76 (m, arom, 7H), 8.01–8.17 (m, arom, 2H), 11.28 (s, N*H* ind, 1H).

1H-indole-5-carboxylic acid [1-(3-metoxy)benzylpiperidin-4-yl]-amide (6e)

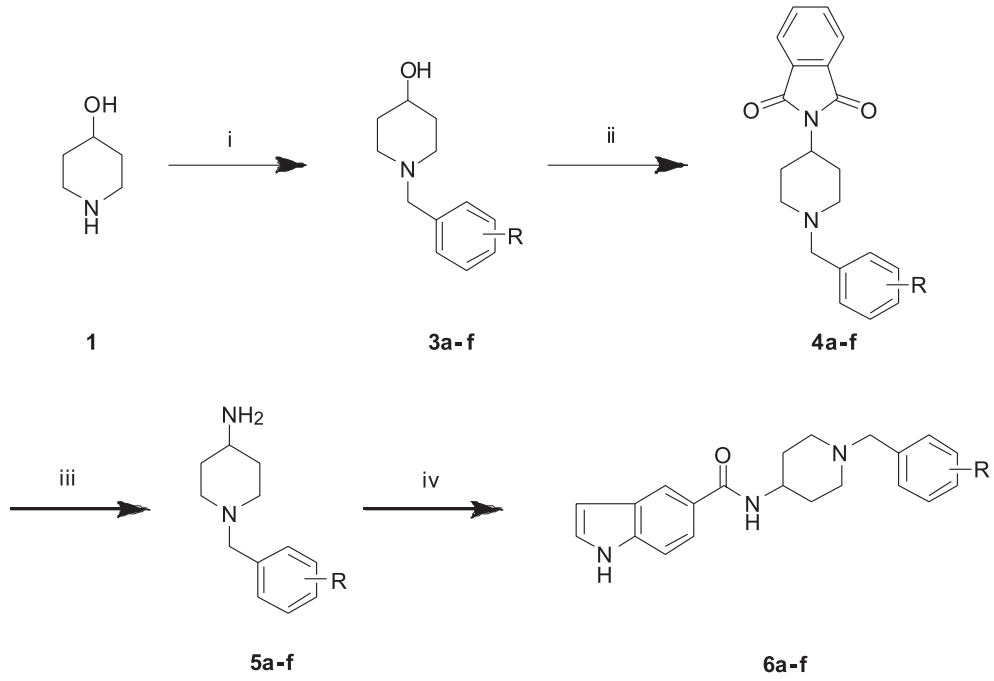
Reagents: 4-amine-1-(3-metoxy)benzylpiperidine (0.4 g, 1.85 mmol), 1*H*-indole-5-carboxylic acid (0.46 g, 2.8 mmol), CDI (0.46 g, 2.8 mmol), THF (6 ml). Yellow oil. Yield: 85%; TLC: R_f 0.80 (S_3); MS (m/z): 364 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, δ, ppm): 1.52–1.70 (m, piper, 2H), 1.72–1.84 (m, piper, 2H), 1.92–2.08 (m, piper, 2H), 2.74–2.90 (m, piper, 2H), 3.43 (s, NCH_2, 2H), 3.72 (s, OCH_3, 3H), 3.70–3.88 (m, C*H*NH, 1H), 6.51 (s, N*H*CO, 1H), 7.03–7.48 (m, arom, 7H), 8.03–8.15 (m, arom, 2H), 11.29 (s, N*H* ind, 1H).

1H-indole-5-carboxylic acid [1-(4-bromo)benzylpiperidin-4-yl]-amide (6f)

Reagents: 4-amine-1-(4-bromo)benzylpiperidine (0.45 g, 1.7 mmol), 1*H*-indole-5-carboxylic acid (0.41 g, 2.6 mmol), CDI (0.41 g, 2.6 mmol), THF (6 mL). Yellow oil. Yield: 67%; TLC: R_f = 0.74 (S_3); MS (m/z): 412 $[M+H]^+$; ^{1}H NMR (DMSO-d_6, δ, ppm): 1.49–1.67 (m, piper, 2H), 1.71–1.82 (m, piper, 2H), 1.94–2.13 (m, piper, 2H), 2.73–2.84 (m, piper, 2H), 3.43 (s, NCH_2, 2H), 3.69–3.87 (m, C*H*NH, 1H), 6.65 (s, N*H*CO, 1H), 7.12–7.41 (m, arom, 7H), 8.20–8.26 (m, arom, 2H), 11.28 (s, N*H* ind, 1H).

AChE/BuChE inhibitory activity

AChE and BuChE inhibitory activities were evaluated by spectrophotometrical Ellman's method (15) using AChE from electric eel and BuChE from horse serum (2.5 units/1 mL). DNTB, acetylthiocholine, butyrylthiocholine and the enzymes were purchased from Sigma Aldrich. The reaction was



R	H	3-Br	3-Cl	3-F	3-OMe	4-Br
Comp	**a**	**b**	**c**	**d**	**e**	**f**

Scheme 1. Synthesis of compounds **3a–f** – **6a–f.** Reagents and conditions: (i) benzyl bromide **(2a–f),** TBAI, K_2CO_3, acetonitrile, reflux 24 h; (ii) phthalimide, Ph_3P, DIAD, THF, 0°C – r.t. 72 h; (iii) $NH_2NH_2 \times H_2O$, methanol, r.t. 24 h; (iv) 1*H*-indole-5-carboxylic acid, CDI, THF, r.t. 24 h

Table 1. Cholinoesterases inhibitory activity of compounds tested (**6a**–**f**) measured at concentration 10 μM.

Compound %I	AChE ± SEM %I	BuChE ± SEM
6a	20.20 ± 0.72	13.45 ± 0.62
6b	na	27.94 ± 1.42
6c	na	30.06 ± 1.72
6d	na	8.60 ± 1.93
6e	na	5.39 ± 1.53
6f	na	na
Tacrine[a]	AChE pIC_{50} 7.76 ± 0.02 nM	BuChE pIC_{50} 8.31 ± 0.01 nM
Donepezil[b]	6.7 nM	-

na – not active; [a] ref. (9), [b] ref. (4)

taken place in a final volume of 3.32 mL of 100 mM phosphate buffer, pH 8.0, containing 0.25 unit of AChE or BuChE, 0.3 mmol 5,5'-dithio-bis-(2-nitrobenzoic) acid (DTNB, Ellman's reagent) and 0.45 mmol acetylthiocholine or butyrylthiocholine as substrates. The compounds tested were dissolved in a mixture water/DMSO (9:1) giving 10 mM stock solution. Each compound was tested at concentra-

tion 10 μM, which was obtained by dissolving appropriate amount of stock solution in water. Each measurement has been done in triplicate. The tested compounds were preincubated with the enzyme for 5 min at 20°C before starting the reaction by adding a substrate. Enzyme activity was determined by measuring the absorbance at 412 nm during 5 min with the Perkin Elmer Lambda 12 spectrometer. As a reference, sample without inhibitor was used (100% enzyme activity). The reaction rates were compared, and the percent of inhibition due to the presence of the test compounds was calculated.

RESULTS AND DISCUSSION

New *N*-benzylpiperidine amides derivatives (**6a–f**) were prepared according to the synthetic routes shown in Scheme 1.

The final amides were evaluated for their inhibitory potencies against AChE (electric eel) and BuChE (horse serum) using spectrophotometric method elaborated by Ellman (15). Tacrine was used as reference compound. In this test, the sulfhydryl group of acetylthiocholine or butyrylthiocholine reacts with 5,5'-dithio-bis-(2-nitrobenzoic) acid (DTBN, Ellman's reagent) giving a yellow-colored product. Absorbance determined at 412 nm is the measure of the activity of compounds tested. Because of the insolubility of compounds in concentrations higher than 10 μM of potential inhibitors, it was not possible to determine IC_{50}. Therefore, the activities of compounds designed are expressed as a percentage of enzyme inhibition at 10 μM concentration. The results obtained are presented in Table 1.

It was found that the only one of the compounds tested, i.e., 1-benzylpiperidine amide of 1*H*-indole-5-carboxylic acid (**6a**) was weak, non-selective inhibitor for both enzymes. Introduction of the substituent into *meta* position of the phenyl ring of the *N*-benzylpiperidine fragment resulted in selective BuChE inhibitors. The most active BuChE inhibitor (30.06% [10 μM]) was amide (**6c**) having chlorine atom in the *N*-benzylpiperidine moiety.

CONCLUSION

In summary, the synthesis of substituted *N*-benzylamides of 1*H*-indole-5-carboxylic acid (**6a–f**) is described. Some of compounds obtained at concentration 10 μM were found to possess moderate inhibitory activity towards BuChE. The results of biochemical studies suggest that their BuChE inhibitory activity might depend on the character of the substituent of the *N*-benzylpiperidine fragment of the molecule.

Acknowledgment

This work was supported by funds from grant of Ministry of Science and High Education no. N N405 163339.

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Received: 23. 11. 2011