Synthesis and Biological Activity of New Donepezil-Hydrazinonicotinamide Hybrids

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Abstract

Currently available treatment used in Alzheimer’s disease is based on acetylcholinesterase inhibitors, e.g. donepezil, tacrine, galantamine, and rivastigmine. In the present study some derivatives of donepezil were synthesized, and their potential anticholinesterase properties were investigated using the colorimetric Ellman’s method. These compounds were synthesized by condensation between indanone derivatives and the hydrazine nicotinated moiety (Hynic). For received derivatives, the selectivity and the IC₅₀ values for acetylcholinesterase and butyrylcholinesterase were calculated. All the tested compounds exhibited lower affinity for AChE than donepezil and higher affinity for BChE than donepezil. Compound 33 showed the most selectivity for AChE among the obtained indanone derivatives.

Introduction

Alzheimer’s disease (AD) is a slow progressive, degenerative disorder of the CNS. It is the most common form of dementia accounting for about 50–60% of all cases of dementia among persons over 65 years of age. Currently, an estimated 4.5 million older people suffer with Alzheimer’s disease (AD), and researchers predict that by 2050 the number could nearly triple, to 13.5 million [1]. This disease is important not only because of the number of affected patients but also because it leads to significant physical and emotional burden on families and caregivers. Alzheimer’s disease is characterized by the loss of memory and learning ability, together with a reduced ability to perform basic activities of daily living. AD patients exhibit marked neuropsychiatric symptoms such as apathy, irritability, anxiety, depression, hallucinations and verbal and physical agitation [2].

Studies performed during the last 20 years revealed that disordered cholinergic transmission is behind cognitive impairment present in Alzheimer’s disease patients. The disorders in transmission result from the reduced number of cholinergic neurons in brain regions associated with higher cognitive functions, i.e. in the neocortex and hippocampus, as well as from the decreased level of choline acetyltransferase, leading to impaired synthesis and uptake of acetylcholine (ACh) neurotransmitter [3,4]. As ACh is degraded by cholinesterase, it stands to reason that cholinergic transmission can be improved by inhibiting the activity of this enzyme. 2 kinds of choline esterases catalyzing hydrolysis of choline esters exist in the central nervous system: acetylcholinesterase (AChE), the so-called true esterase, and butyrylcholinesterase (BChE), referred to as pseudocholinesterase or non-specific cholinesterase. AChE is bound to the membrane of cholinergic neurons, whereas BChE is present both in the neurons and in the glial cells. Both enzymes show 65% homology, and the main differences pertain to their substrate specificity.

Acetylcholinesterase selectively and rapidly hydrolyzes acetylcholine in cholinergic synapses. In turn, butyrylcholinesterase can also hydrolyze butyrylcholine and some medications and drugs, aside from acetylcholine. Moreover, it regulates cholinergic transmission in states of acetylcholinesterase deficiency. In healthy individuals, AChE accounts for 80% of esterase activity within the central nervous system, whereas the remaining 20% is provided by BChE. In Alzheimer’s disease, the activity of AChE can be reduced to 67% of normal level in certain brain regions; simultaneously, an
increase in BChE level is observed, reaching up to 165% of its normal level [5,6].

Cholinesterase inhibitors (ChEi) constitute an important group of compounds used in the symptomatic treatment of Alzheimer’s disease. They support cholinergic transmission by inhibiting acetylcholinesterase cleavage. Most commonly administered agents from this group include donepezil and galantamine, both being acetylcholinesterase inhibitors (AChEi), and rivastigmine, which inhibits both acetylcholinesterase and butyrylcholinesterase (AChEi, BChEi). Studies revealed that the administration of such double inhibitors is particularly favorable at later stages of Alzheimer’s disease [7–10].

In the study, synthesis and biological evaluation of a series of new donepezil derivatives with hydrazine nicotinate moiety as potential cholinesterase inhibitors are described. Inhibitory activity of acetylcholinesterase and butyrylcholinesterase of the obtained compounds was studied using the method of Ellman, to determine the rate of hydrolysis of acetylthiocholine (ATCh) in the presence the inhibitor [11].

Material and Methods

Chemistry

Reaction was monitored by TLC using DC-Alufolien Kieselgel 60F254 plates (Merck), with detection by UV lamp (254 nm). Melting points were measured on an Electrothermal apparatus in open capillaries and are uncorrected. Column chromatography was performed using silica gel 60 (230–400 mesh, Merck). IR spectra were recorded in KBr using a Mattson Infinity Series FT-IR spectrophotometer. 1H NMR spectra were recorded with a Varian Mercury 300 MHz spectrometer, using tetramethylsilane as internal standard. Elemental analyses were recorded using Perkin Elmer series II, CHNSO, Analyzer 2400. Mass spectra were performed by the Centre of Molecular and Macromolecular Studies in Lodz.

6-hydrizinopyridine-3-carboxylic acid (1)
6-chloronicotinic acid (50.8 mmol) was added to 8% hydrazine hydrate (930.0 mmol) and placed in a 100 °C oil bath for 4 h. The homogenous reaction mixture was cooled to room temperature, and concentrated to dryness to give a white solid. The solid was recrystallized from ethyl acetate, giving a yellow solid. Recrystallization from ethyl acetate gave product 2 as a white solid.

Yield 75%. Mp. 285–287 °C. IR (KBr) cm−1: 3162, 1651, 1549, 1482, 1412, 1355, 1287, 1228, 1150, 1093, 1051, 989, 813, 671. MS (FAB) (m/z): 246 (M + 1). Anal. calc. for C15H20ClNO2 (%): C 52.17, H 5.93, N 4.87.

N-Boc-3-bromopropylamine (7)

To a solution of 1 M NaOH (20.1 mmol) in tert-butyl alcohol (6 mL) was added the corresponding n-bromoalkylamine hydrobromide (9.1 mmol) and di-tert-butyl dicarbonate (10.1 mmol). The reaction mixture was stirred for 12 h in room temperature, and then was washed with 0.1 M HCl and 5% NaHCO3, and brine. The organic layer was dried over Na2SO4 and concentrated in vacuo to give N-Boc-3-bromoalkylamine as yellow oil.

Yield 84%. Mp. 170–172°C. IR (KBr) cm−1: 739, 1692, 2951, 3285. 1H NMR (DMSO) δ: 6.9 (1H, s, NH), 3.6 (2H, t, BrCH2), 2.9 (2H, m, NCH2), 1.4 (9H, s, Boc).
General procedure for synthesis of compounds (10–13)

A solution of N-Boc-3-bromoalkylamine 7, 8 (3.5 mmol) in CH₂CN (30 mL) was added to a mixture of 5, 6 (3.0 mmol) and K₂CO₃ (9.0 mmol) in CH₂CN (30 mL). The mixture was heated under reflux for 12h. The inorganic material was filtered off and the solvent was evaporated in vacuo. The crude residue was extracted with CH₂Cl₂, washed with H₂O and brine, dried over Na₂SO₄, and concentrated in vacuo to give oil.

5-(2-Boc-aminoethoxy)-2-piperidin-1-ylmethylindan-1-one (10)

Yield 65%. IR (KBr) cm⁻¹: 3035, 2953, 1642, 1558, 1509, 1414, 1229, 1123, 1072. ¹H NMR (DMSO) δ: 7.7 (1H, m, Ar), 7.2–7.4 (5H, m, Ar), 6.8–6.9 (2H, m, Ar), 6.7 (1H, t, NH), 4.1 (2H, t, OCH₂), 3.6 (2H, d, CH₂CH), 3.1–3.2 (4H, m, 2-NCH₂), 3.0 (2H, m, NHCH₂), 2.7–2.8 (1H, m, COCH), 2.7 (2H, t, ArCH₂), 1.5–1.7 (5H, m, 2-CH₂, 1-CH), 1.4–1.5 (2H, m, CCH₂), 1.1 (9H, s, Boc). Anal. calc. for C₂₄H₃₁ClN₂O₂ ( %): C 69.46, H 7.53, N 6.75; Found ( %): C 69.11, H 7.23, N 6.61.

General procedure for preparation of compounds (16–21)

A solution of 10–15 (0.5 mmol) in anhydrous THF (5 mL) was cooled to −20°C and stirred for 30 min. Etherate HCl was added dropwise to the reaction mixture under pH = 1. The precipitate was collected by suction filtration, washed with ether and dried in desiccator to give a white solid.

5-(2-aminoethoxy)-2-piperidin-1-ylmethylindan-1-one hydrochloride (16)

Yield 78%. Mp. 145–148°C. IR (KBr) cm⁻¹: 3075, 1701, 3370. ¹H NMR (DMSO) δ: 7.7 (1H, s, Ar), 6.8–6.9 (2H, m, Ar), 4.1 (2H, t, OCH₂), 3.6 (2H, d, CH₂CH), 3.0 (2H, m, NHCH₂), 1.5–1.6 (6H, m, 2-CH₂, 1-CH), 1.3–1.4 (6H, m, CCH₂), 1.1 (9H, s, Boc). Anal. calc. for C₁₈H₂₇ClN₂O₂ ( %): C 63.80, H 8.03, N 8.27; Found ( %): C 63.28, H 7.52, N 8.41.

5-(3-Boc-aminopropoxy)-2-piperidin-1-ylmethylindan-1-one hydrochloride (16)

Yield 70%. Mp. 168–171°C. IR (KBr) cm⁻¹: 738, 1695, 2694, 2944, 3370. ¹H NMR (DMSO) δ: 7.7 (1H, s, Ar), 7.2–7.4 (5H, m, Ar), 6.8–6.9 (2H, m, Ar), 6.7 (1H, t, NH), 4.1 (2H, t, OCH₂), 3.6 (2H, d, CH₂CH), 3.4 (2H, d, NCH₂), 3.1–3.2 (4H, m, 2-NCH₂), 3.0 (2H, m, NHCH₂), 2.7–2.8 (1H, m, COCH), 1.7–1.8 (6H, m, 3-CH₂), 1.4–1.5 (2H, m, CCH₂), 1.1 (9H, s, Boc). Anal. calc. for C₁₈H₂₇ClN₂O₂ ( %): C 63.80, H 8.03, N 8.27; Found ( %): C 63.28, H 7.52, N 8.41.
C$_{27}$H$_{37}$ClN$_2$O$_2$ (%): C 69.99, H 7.75, N 6.53; Found (%): C 69.58, H 7.43, N 6.32.

5-(5-aminopyl oxy)-2-piperidin-1- ylmethyldi an-1-one hydrochloride (20)

Yield 69%. Mp. 125–128°C. IR (KBr) cm$^{-1}$: 1649, 1700, 2835, 3086. $^1$H NMR (DMSO) δ: 7.7 (1H, s, Ar), 6.8–6.9 (2H, m, Ar), 3.9 (2H, t, OCH$_2$), 3.6 (2H, d, CH$_2$CH), 3.4 (2H, d, NCH$_2$), 3.3–3.2 (4H, m, 2 · NCH$_2$), 3.1 (2H, m, NHCH$_2$), 2.8–2.9 (1H, m, COCH), 2.6–2.7 (1H, m, COCH$_2$), 1.7–1.8 (6H, m, 3 · CH$_2$), 1.5–1.6 (6H, m, 3 · CH$_2$). Anal. calc. for C$_{27}$H$_{37}$ClN$_2$O$_2$: C 70.95, H 8.16, N 6.13; Found ( %): C 70.61, H 7.97, N 6.32.

5-(5-aminopyl oxy)-2-(4-benzylpiperidin-1-ylmethyl)- indan-1-one hydrochloride (21)

Yield 74%. Mp. 160–163°C. IR (KBr) cm$^{-1}$: 1736, 1700, 2948, 3375. $^1$H NMR (DMSO) δ: 7.6 (1H, s, Ar), 7.2–7.4 (5H, m, Ar), 6.8–6.9 (2H, m, Ar), 3.9 (2H, t, OCH$_2$), 3.6 (2H, d, CH$_2$CH), 3.4 (2H, d, NCH$_2$), 3.3–3.3 (4H, m, 2 · NCH$_2$), 3.2 (1H, m, NHCH$_2$), 2.8–2.9 (1H, m, COCH), 2.7 (2H, m, COCH$_2$), 2.2 (2H, s, NH$_2$), 1.3–1.7 (5H, m, 2 · CH$_2$-1-CH), 1.2–1.3 (2H, m, CH$_2$CH). Anal. calc. for C$_{27}$H$_{37}$ClN$_2$O$_2$: C 70.95, H 8.16, N 6.13; Found (%): C 70.61, H 7.96, N 5.87.

General procedure for preparation of compounds (22–27)

1.1'-Carbonyldimidazole (1.0 mmol) was added to solution 2 in anhydrous THF (10 mL). The mixture was stirred for 4 h in room temperature. Next, the corresponding amine 16–21 (1.0 mmol) and TEA (1.0 mmol) were added. The resultant mixture was stirred for 20 h. The solvent was evaporated under reduced pressure, water was added, and the resultant mixture was extracted twice with CH$_2$Cl$_2$. The combined organic extracts were washed with brine and then dried with anhydrous Na$_2$SO$_4$. The solvent was evaporated under reduced pressure and the residue was purified by crystallization from methanol.

6-Boc-hydrazino-N-[2-(1-oxo-2-piperidin-1-ylmethyldi an-5-yl-oxy)-ethyl]-nicotinamide (22)

Yield 45%. Mp. 175–178°C. IR (KBr) cm$^{-1}$: 1734, 1595, 1692, 1707, 2990, 3297. $^1$H NMR (DMSO) δ: 8.8 (1H, s, NH), 8.4 (1H, s, Ar), 8.1 (1H, s, CONH), 7.9 (1H, d, Ar), 7.6 (1H, s, Ar), 7.2–7.4 (5H, m, Ar), 6.8–6.9 (2H, m, Ar), 6.5 (1H, d, Ar), 4.1 (2H, t, OCH$_2$), 3.6 (2H, d, CH$_2$CH), 3.4 (2H, d, NCH$_2$), 3.3 (3H, d, CNH), 3.1–3.2 (4H, m, 2 · NCH$_2$), 3.0 (2H, m, NHCH$_2$), 2.7–2.8 (1H, m, COCH), 2.6 (2H, m, COCH$_2$), 1.7–1.8 (5H, m, 2 · CH$_2$-1-CH), 1.2–1.3 (2H, m, CH$_2$CH). Anal. calc. for C$_{23}$H$_{39}$N$_5$O$_5$: C 65.82, H 7.66, N 12.38; Found (%): C 65.46, H 6.91, N 10.77.

6-Boc-hydrazino-N-[5-(1-oxo-2-piperidin-1-y lmethyldi an-5-yl-oxy)-pentyl]-nicotinamide (26)

Yield 45%. Mp. 181–183°C. IR (KBr) cm$^{-1}$: 1639, 1697, 1706, 2991, 3352. $^1$H NMR (DMSO) δ: 8.7 (1H, s, NH), 8.5 (1H, s, Ar), 8.2 (1H, s, Ar), 7.9 (1H, d, Ar), 7.6 (1H, s, Ar), 7.2–7.4 (5H, m, Ar), 6.8–6.9 (2H, m, Ar), 6.5 (1H, d, Ar), 6.3 (2H, t, OCH$_2$), 3.6 (2H, d, CH$_2$CH), 3.4 (2H, d, NCH$_2$), 3.3 (1H, d, CNH), 3.1–3.2 (4H, m, 2 · NCH$_2$), 3.0 (2H, m, NHCH$_2$), 2.8–2.9 (1H, m, COCH), 1.6–1.7 (6H, m, 3 · CH$_2$), 1.1–1.3 (6H, m, CH$_2$CH$_2$), 1.0 (9H, s, Boc). Anal. calc. for C$_{31}$H$_{45}$N$_5$O$_5$: C 65.82, H 7.66, N 12.38; Found (%): C 65.46, H 7.50, N 12.21.

N-[2-(4-benzylpiperidin-1-ylmethyl)-1-oxoindan-5-yl-oxy]-pentyl-6-Boc-hydrazinonicotinamide (27)

Yield 42%. Mp. 201–204°C. IR (KBr) cm$^{-1}$: 732, 1601, 1690, 1711, 2985, 3297. $^1$H NMR (DMSO) δ: 8.8 (1H, s, NH), 8.4 (1H, s, Ar), 8.1 (1H, s, CONH), 7.9 (1H, d, Ar), 7.6 (1H, s, Ar), 7.2–7.4 (5H, m, Ar), 6.8–6.9 (2H, m, Ar), 6.4 (1H, d, Ar), 3.9 (2H, t, OCH$_2$), 3.7 (2H, d, CH$_2$CH), 3.5 (2H, d, NCH$_2$), 3.2–3.4 (5H, m, 2 · NCH$_2$), 3.1 (2H, m, NHCH$_2$), 2.8–2.9 (1H, m, COCH), 1.6–1.7 (6H, m, 3 · CH$_2$), 1.2–1.3 (6H, m, CH$_2$CH$_2$), 1.1 (9H, s, Boc). Anal. calc. for C$_{22}$H$_{35}$N$_5$O$_5$: C 69.59, H 7.53, N 10.68; Found (%): C 69.14, H 7.38, N 10.31.

General procedure for preparation of compounds (28–33)

0.5 mmol 22–27 was dissolved in anhydrous THF (5 mL) and ether saturated HCl was dropwise added. Mixture was stirred in room temperature and a precipitate was formed. The precipitate was isolated and the solid was washed with ether.

6-Boc-hydrazino-N-[2-(1-oxo-2-piperidin-1-ylmethyl)-1-oxoindan-5-yl-oxy)-ethyl]-nicotinamide hydrochloride (28)

Yield 65%. Mp. 175–178°C. IR (KBr) cm$^{-1}$: 1639, 1696, 2787, 2974, 3154. $^1$H NMR (DMSO) δ: 8.5 (1H, s, Ar), 8.2 (1H, s, CNH), 7.9 (1H, d, Ar), 7.6 (1H, s, Ar), 6.8–6.9 (2H, m, Ar), 6.5 (1H, d, Ar), 4.1 (2H, t, OCH$_2$), 3.6 (2H, d, CH$_2$CH), 3.4 (2H, d, NCH$_2$), 3.3 (1H, d, CNH), 3.1–3.2 (4H, m, 2 · NCH$_2$), 3.0 (2H, m, NHCH$_2$), 2.6–2.7 (1H, m, COCH), 2.2 (2H, m, NH$_2$), 1.5–1.6 (6H, m, 2 · CH$_2$-1-CH).

N-[2-(4-benzylpiperidin-1-ylmethyl)-1-oxoindan-5-yloxy]-ethyl]-6-hydrazinonicotinamide hydrochloride (29)

Yield 62%. Mp. 185–188°C. IR (KBr) cm$^{-1}$: 740, 1633, 1696, 2693, 2989, 3252. $^1$H NMR (DMSO) δ: 8.4 (1H, s, Ar), 8.3 (1H, s, CONH), 7.8 (1H, d, Ar), 7.7 (1H, s, Ar), 7.2–7.4 (5H, m, Ar), 6.8–6.9 (2H, m, Ar), 6.5 (1H, Ar), 4.1 (2H, t, OCH$_2$), 3.6 (2H, d, CH$_2$), 3.4 (2H, d, NCH$_2$), 3.3 (1H, s, CNH), 3.1–3.2 (4H, m, 2 · NCH$_2$), 3.0 (2H, m, NH$_2$), 2.8 (2H, t, ArCH$_2$), 2.5–2.6 (1H, m, COCH), 2.2 (2H, d, NH$_2$), 1.2–1.4 (5H, m, 2 · CH$_2$, 1 · CH). MS (FAB) (m/z): 516 (M+1). Anal. calc. for C$_9$H$_{15}$ClN$_5$O$_3$ (%): C 65.28, H 6.46, N 12.59.

Inhibition Studies on AChE and BChE

The activity of acetylcholinesterase (AChE) and butyrylcholinesterase inhibitors was measured spectrophotometrally according to the colorimetric method of Ellman (1961) with some modification. The AChE activity was determined in a mixture containing the assay solution consisting of a phosphate buffer (0.1M, pH 8.0) with the addition of a solution of 5,5′-dithiobisnitrobenzoic acid (DTNB, 0.05mL, 0.5mM), AChE (5U/mL) and the appropriate inhibitor (tested compounds). The final assay volume was 3mL.

Inhibitor curves of the different derivatives were obtained using 7 concentrations of acetylcholine iodide as the substrate of the enzymatic reaction lasting 1 min. Enzyme activity was determined by measuring the absorbance at 412 nm after 1 min at 37°C with Perkin Elmer Lambda 25 UV/VIS Spectrophotometer. Sample without inhibitor was consistently available to provide 100% of AChE activity. The reaction rates and the percent of inhibition due to the presence of tested compounds were compared. Each reaction was independently repeated at least 3 times. Determination of butyrylcholinesterase (BChE) inhibitory activity was carried out in a similar manner using 5units/L of BChE instead of AChE in the final volume of 3mL. The IC$_{50}$ defined as the concentration of each compound that reduces the enzymatic activity (AChE or BChE) by 50% with respect to that without inhibitors, was calculated by non-linear and linear regression.

DTNB, enzymes (C2629 and C4290) and acetylcholine iodide were purchased from Sigma-Aldrich.

Results and Discussion

Chemistry

In the present investigation compound 2 was synthesized following the method described by Abrams ($\odot$ Fig. 1) [12]. 6-chloronicotinic acid and hydrazine hydrate were refluxed at 100°C. In the next step, hydrazine N-atom was protected by di-tert butyl dicarbonate ((t-BuOCO)$_2$O) to give 6-Boc-hydrazinopyridine-3-carboxylic acid instead of AChE in the final volume of 3mL. The IC$_{50}$, defined as the concentration of each compound that reduces the enzymatic activity (AChE or BChE) by 50% with respect to that without inhibitors, was calculated by non-linear and linear regression. The IC$_{50}$ was used to perform O-alkylation with N-Boc-2-bromoethylamine ($\odot$ Fig. 3a) [16–18]. N-Atom of n-bromoalkylamine and hydrazine were refluxed at 100°C. Treatment of 5-hydroxyindan-1-one and secondary amines (piperidine 3, 4-benzylpiperidine 4) with parafomaldehyde gave 5, 6 ($\odot$ Fig. 2) [13, 14]. In the next step, derivatives of 5-hydroxyindan-1-one 5, 6 coupled with alky linker through etheric bond. Based on our study, we determined 2 methods for synthesizing compounds with a variable length of the carbon chain at C-5. N-Boc-aminoalcohol derivatives 10, 11 were used to perform O-alkylation of 5, 6 with N-Boc-2-bromoethylamine 7 using potassium carbonate in refluxing acetoniitrile [15]. Similar reaction conditions employing N-Boc-3-bromopropylamine 8, led to the desired 12, 13 ($\odot$ Fig. 3a). Treatment of 5, 6 under Mitsunobu conditions, using 5-aminopentanol with N-atom protected by Boc 9 provided 14, 15 ($\odot$ Fig. 3b) [16–18], N-atom of n-bromoalcoylamine and N-atom of 5-aminopentanol were protected following the methods described previously [15, 19]. All the obtained N-Boc-aminoalcohol derivatives 10–15 were deprotected in the presence of ether saturated HCl, affording 16–21 ($\odot$ Fig. 3c). Coupling reac-

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tion of the intermediates 16–21 with 6-Boc-hydrazinopyridine-carboxylic acid 2 in the presence carbonyldimidazole (CDI) led to the formation of the desired compound 22–27 (Fig. 3d) [20–22]. The final compounds 28–33 were obtained from 22–27, which were deprotected in the presence of the ether saturated HCl (Fig. 3e).

Biochemistry
Anticholinesterase activities of the obtained compounds 28–33 were determined by modification of Ellman’s spectrophotometric method. Parameters of the enzymatic reaction $K_m$ and $V_{max}$ were obtained by linear regression of the reaction rate as a function of substrate concentration (Table 1). To determine the type of inhibition, the Michaelis-Menten equation was plotted using Lineweaver-Burk linear transformation ($1/v$ vs. $1/[S]$). $K_i$ constants were then calculated using non linear regression [23–26].

The inhibitory activities against both AChE and BChE of the compounds 28–33 together with the reference donepezil are reported in Table 1, expressed as IC$_{50}$ values [27]. All the tested compounds showed a higher inhibitory activity on AChE than BChE, and showed lower inhibition of AChE compared with donepezil. Compound 33 showed the greatest affinity for AChE, while compound 28 was found to be the least effective against this enzyme.

We also assessed the impact of the activity of the constructed derivatives obtained against AChE. After analyzing biological activity of the compounds with the same length of carbon chain, it can be concluded that replacing piperidine with benzylpiperidine substituent in compounds 28, 30, and 32 (and obtaining compounds 29, 31, and 33, respectively) was reflected by a slight increase in activity against AChE. For example, the activity of ethyl derivative of benzylpiperidine 29 increased 9-fold as compared to the ethyl derivative of piperidine 28. Variation of the alkyl linker length between the 2 groups, indanone and Hynic, for piperidine derivatives 28, 30, 32 revealed the pentylene-linked substance to be more active than their propylene and ethylene linked counterparts. In the case of derivatives of N-benzylpiperidine 29, 31, 33, the influence of alkylene-linker length was negligible and compounds 31 and 33 showed nearly equal potency in anti-AChE assay.

All the tested compounds showed higher inhibition of BChE than the reference (donepezil). The selectivity toward AChE (IC$_{50}$ ratio of BChE/AChE) and BChE (IC$_{50}$ ratio of AChE/BChE) of the obtained compounds and reference inhibitor (donepezil) were also determined. All the tested compounds showed a higher selectivity for AChE than for BChE, while the selectivity with respect to AChE was less than in the case of the standard (donepezil), selectivity to BChE was greater than for donepezil. Compound 33 was found to be the most potent with regards to AChE activity and the most selective for AChE among the obtained indanone derivatives. The selectivity of this derivative was nearly 50-fold higher compared to the least selective derivative 28. Concurrently, compound 28 was characterized by the highest selectivity against BChE.

Drug-modeling studies
Docking studies of donepezil-hydrazinonicotinamide hybrids on the active site of electric eel AChE inhibited by donepezil (PDB: 1EA5) revealed their mode of structural and positional requirements for potential activity. These studies elucidated the interaction between electric eel AChE and inhibitors 29–33. The AChE-Inhibitor complex was generated using Cache software (Fujitsu). Docking analysis performed with these inhibitors...
show that compounds 29–33 bind with the total score of −184.794, −196.681, −165.113, −195.338, −181.939, respectively. (Fig. 4)

**Conclusion**

In summary, series of indanone derivatives with hydrazine nicotinate moiety were synthesized and their anticholinesterase activities were evaluated. All tested compounds exhibited a higher affinity to AChE than for BChE. Similarly, their selectivity for AChE was greater than for BChE. When compared to donepezil, all synthesized molecules were less active with regards to inhibiting AChE and slightly more active in inhibiting BChE. All tested derivatives were less selective for AChE and BChE, their selectivity is higher than the reference (donepezil). The results indicate that compound 33 (N-benzylpiperidine derivative) was the most effective inhibitor of AChE, additionally, it...
had the highest selectivity for the enzyme. Compound 30 (piperidine derivative) was most active against BChE, however, compound 28 showed the highest selectivity for BChE.

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Conflict of Interest

The authors have declared no conflict of interest.

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