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ORIGINAL PAPER

Synthesis, characterization and investigation of AChE and BuChE inhibitory activity of 1-alkyl-4-[(5,6-dimethoxy-1-indanone-2-yl) methylene]pyridinium halide derivatives

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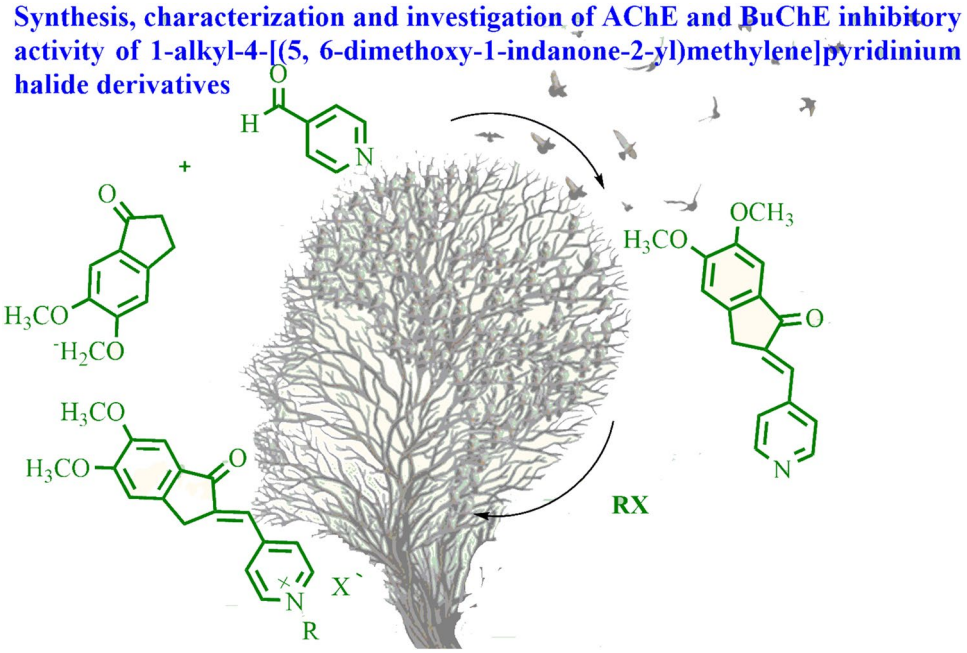
Abstract

The design, synthesis and characterization of a series of 1-alkyl-4-[(5,6-dimethoxy-1-indanone-2-yl)methylene]pyridinium halide derivatives as acetylcholinesterase and butyrylcholinesterase inhibitors for the treatment of Alzheimer's disease were reported. The strategy for this synthesis was based on aldol condensation reaction between 4-pyridinecarboxaldehyde with 5,6-dimethoxy-1-indanone in the presence of NaOH in EtOH-H_2O solution as the first step and N-alkylation reaction of the produced 5,6-dimethoxy-2-[(pyridin-4-yl)methylene]-1-indanone with various alkyl halides (R–X) in the second step. This reaction was carried out under reflux temperature in polar aprotic solvents such as acetone or acetonitrile. ^{1}H and ^{13}C NMR and FTIR spectroscopy along with CHN analysis were used to confirm the structure of our synthesized compounds. Biological activities including acetylcholinesterase and butyrylcholinesterase (BuChE) inhibitions for synthesized compounds were tested using the Ellman method. The results were compared with donepezil and galantamine, and among the synthesized compounds, the highest inhibitory activity with BuChE $IC_{50} = 0.55$ μM was observed.

Graphic abstract



Keywords 1-Indanone · Acetylcholinesterase (AChE) · Butyrylcholinesterase (BuChE) · Enzyme inhibitor

Extended author information available on the last page of the article

Introduction

Alzheimer's disease (AD) is the most common form of dementia, which seems to be directly related to age [1]. The etiology of AD has not been fully understood yet; but there are several reasons such as β-amyloid (Aβ) deposits, τ-protein aggregation, oxidative stress and low levels of acetylcholine (Ach) have been considered as the main causes of this disease [2, 3]. Acetylcholine (ACh), the main neurotransmitter of the cholinergic pathway, plays an important role in cognition impairment (e.g., Alzheimer), and reducing the activity of this substance is one of the main processes involved in AD development. Serine hydrolase enzymes include that acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are responsible for the hydrolysis of acetylcholine (ACh) [4]. AChE inhibition has been known as a strategy to the AD treatment through the ACh-level enhancement for years.

According to several reports, various derivatives of 1-indanone moiety have important role in biological activities such as antiproliferative activity, acetylcholinesterase inhibition in donepezil (Aricept) for the treatment of Alzheimer's disease, and etc. [5–8]. Tacrine, donepezil, rivastigmine and galantamine include the acetylcholine esterase inhibitors (AChE) for the treatment of Alzheimer's disease [8–12]. Donepezil hydrochloride is the second drug approved by the US FDA for the treatment of AD [13, 14]. At first glance, donepezil is divided into four parts that are shown in Fig. 1; part 1: 1-indanone moiety, part 2: linkage moiety, part 3: piperidine moiety and part 4: benzyl moiety. Choosing the best moiety to replace with other analogous to improve the inhibition activity is one of the most challenging debates among the scientists [15].

According to different results that were mentioned in researches on compounds having high acetylcholinesterase inhibitions, 5,6-dimethoxy-1-indanone was chosen as part 1, carbon with double-bond form as linker, pyridine as part 3 and pyridinium cation form of various alkyls as part 4 for increasing solubility in water of acetylcholinesterase and butyrylcholinesterase inhibitors.

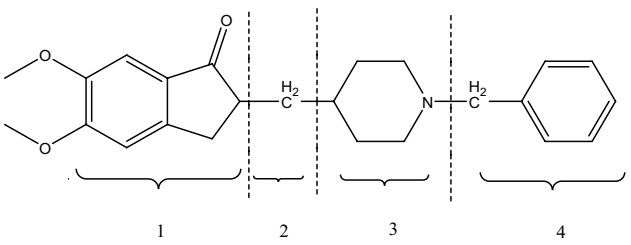


Fig. 1 Donepezil molecular structure

Organic heterocyclic salts such as pyridinium, quinolinium and N-methylimidazolium salts are an important class of new compounds, which were used as nonlinear optics, ionic liquids, key intermediate in organic reactions and biological applications. It is obvious that the compounds in salt form were better dissolved in aqueous media; therefore, most drugs are in the form of salt [16–21]. In this context, a series of 1-alkyl-4-[(5,6-dimethoxy-1-indanone-2-yl)methylene]pyridinium halide derivatives were prepared from 5,6-dimethoxy-2-[(pyridin-4-yl)methylene]-1-indanone and various alkyl halides. Biological activities including acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitions for synthesized compounds were assayed. The results were compared with donepezil and galantamine.

Experimental

Materials and instruments

All materials used in synthesis were purchased from Merck and Sigma-Aldrich. All reactions were carried out under argon atmosphere. Column chromatography was performed using SiO_2 (60 Å, 230–400 mesh, particle size 0.040–0.063 mm) at 25 °C. Melting points were determined on a MEL-TEMP model 1202D and are uncorrected. FTIR spectra were recorded on a Bruker Tensor 27 spectrometer as KBr disks. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Spectrospin Avance 400 and 100 MHz spectrometer, respectively, used solvents was $CDCl_3$ and DMSO-d_6. ^{13}C NMR spectra were determined on the same instrument at 100 MHz. All chemical shifts were reported as δ (ppm), and coupling constants (J) were given in Hz. The elemental analyses were carried out with an Elementor Vario EL. III instrument.

Synthesis

Synthesis of 5,6-dimethoxy-2-[(pyridin-4-yl) methylene]-1-indanone (3)

To a solution of 1 mmol of 4-pyridinecarboxaldehyde and 1 mmol of 5,6-dimethoxy-1-indanone in 10 ml EtOH, aqueous solution of NaOH (10%) was added dropwise. The reaction mixture was stirred overnight at room temperature. The obtained solid was filtered and recrystallized from EtOH to give **3** as an off-white solid [22]; yield 67%; mp: 118–120 °C; FTIR (KBr) ν 3008, 2937, 1691, 1600, 1465, 1315, 1268, 1029 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): 3.82 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 4.03 (2H, s, CH_2), 7.17 (1H, s, Ar), 7.22 (1H, s, Ar), 7.42 (1H, s, =CH), 7.67 (2H, d, $^3J_{H-H}$ = 5.36, pyridine-H), 8.64 (2H, d, $^3J_{H-H}$ = 5.46, pyridine-H).

General procedure for the synthesis of compounds (5a–o)

5,6-Dimethoxy-2-[(pyridin-4-yl)methylene)-1-indanone (0.35 mmol) was dissolved in 4 cc acetone (for synthesis of **5a**) or acetonitrile (for synthesis of **5b–o**) under reflux temperature, and then 1.05 mmol of appropriate alkyl halides was added. The reaction mixture was stirred for 48 h under reflux condition. The precipitate was filtered and washed with appropriate solvent. The obtained solid was dried under reduced pressure to afford related compounds.

1-Methyl-4-[(5,6-dimethoxy-1-indanone-2-yl)methylene] pyridinium iodide (5a) From 0.14 g of methyliodide, 0.11 g (0.8 mmol) of cloudy white solid was obtained in 80% yield; mp: 222–224 °C; FTIR (KBr) ν 3008, 2934, 1685, 1640, 1462, 1317, 1273, 1026 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 3.84 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 4.17 (2H, s, CH_2), 4.28 (3H, s, CH_3), 7.12 (1H, s, Ar–H), 7.28 (1H, s, Ar–H), 7.55 (1H, s, =CH), 8.18 (2H, d, $^3J_{H-H}$ = 5.90, pyridine-H), 9.15 (2H, d, $^3J_{H-H}$ = 5.70, pyridine-H); ^{13}CNMR (100 MHz, DMSO): 32.24, 55.46, 56.23, 61.24, 123.07, 128.44, 129.31, 138.02, 138.55, 139.12, 145.62, 146.24, 150.01, 155.62, 156.16, 192.04; Anal. Calc. for $C_{18}H_{18}INO_3$: C, 51.08; H, 4.29; N, 3.31%. Found: C, 51.11; H, 4.27; N, 3.32%.

1-Benzyl-4-[(5,6-dimethoxy-1-indanone-2-yl)methylene] pyridinium chloride (5b) From 0.13 g of benzylchloride, 0.12 g (0.31 mmol) of white solid was obtained in 90% yield; mp: 226–228 °C; FTIR (KBr) ν 3008, 2833, 1683, 1600, 1462, 1317, 1272,1071 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 3.92 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 4.18 (2H, s, CH_2), 5.76 (2H, s, CH_2), 7.18 (1H, s, Ar–H),7.27 (1H, s, Ar–H), 6.47–7.58 (3H, m, Ar–H, =CH), 7.61–7.72 (3H, m, Ar–H), 8.15 (2H, d, $^3J_{H-H}$ = 6.20, pyridine-H), 9.13 (2H, d, $^3J_{H-H}$ = 6.20, pyridine-H); ^{13}C NMR (100 MHz, DMSO): 191.20, 157.18, 151.71, 150.12, 147.02, 146.30, 145.11,134.18, 130.02, 129.71, 129.70, 129.21, 128.52, 125.25, 108.31, 105.20, 63.12, 56.71, 55.92, 31.19; Anal. Calc. for $C_{24}H_{22}ClNO_3$: C, 70.67; H, 5.44; N, 3.43%. Found: C, 70.63; H, 5.45; N, 3.42%.

1-(4-Bromobenzyl)-4-[(5,6-dimethoxy-1-indanone-2-yl) methylene]pyridinium bromide (5c) From 0.26 g of 4-bromobenzylbromide, 0.12 g (0.23 mmol) of white solid was obtained in 66% yield; mp: 228–229 °C; FTIR (KBr) ν 3048, 2834, 1682, 1600, 1464, 1320, 1272, 1020, 803, 533 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 3.84 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 4.13 (2H, s, CH_2), 5.70 (2H, s, CH_2), 7.18 (1H, s, Ar–H), 7.29 (1H, s, Ar–H), 7.56–7.61 (3H, m, Ar–H, =CH), 7.66–7.72 (2H, d, $^3J_{H-H}$ = 8.37, Ar–H), 8.39 (2H, d, $^3J_{H-H}$ = 6.50, pyridine-H), 9.21 (2H, d, $^3J_{H-H}$ = 6.50, pyridine-H); ^{13}C NMR (100 MHz, DMSO): 190.99, 156.81, 152.03, 150.21, 146.99, 146.31, 145.20, 134.32, 132.60, 131.61, 130.01, 128.61, 124.96, 123.42, 108.41, 105.30, 62.41, 57.17, 56.31, 32.01; Anal. Calc. for $C_{24}H_{21}Br_2NO_3$: C, 54.26; H, 3.98; N, 2.64%. Found: C, 54.29; H, 3.97; N, 2.63%.

1-(3-Bromobenzyl)-4-[(5,6-dimethoxy-1-indanone-2-yl) methylene]pyridinium bromide (5d) From 0.26 g of 3-bromobenzylbromide, 0.11 g (0.22 mmol) of white solid was obtained in 63% yield; mp: 232–234 °C; FTIR (KBr) ν 3004, 2939, 1690, 1600, 1467, 1312, 1268, 1038, 699, 785, 845 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 3.69 (3H, s, OCH_3), 3.91 (2H, s, OCH_3), 4.16 (2H, s, CH_2), 5.78 (2H, s, CH_2),7.19 (1H, s, Ar–H), 7.26 (1H, s, Ar–H), 7.44 (1H, m, Ar–H), 7.58 (1H, s, =CH), 7.68–7.59 (2H, m, Ar–H), 7.89 (1H, s, Ar–H), 8.42 (2H, d, $^3J_{H-H}$ = 6.16, pyridine-H), 9.16 (2H, d, $^3J_{H-H}$ = 6.14, pyridine-H); ^{13}C NMR (100 MHz, DMSO): 191.18, 156.81, 151.78, 150.20, 146.90, 46.31, 144.98, 137.21, 132.80, 132.22, 132.01, 129.80, 128.35, 128.50, 124.93, 122.71, 108.40, 105.32, 61.99, 56.77, 56.30, 32.08; Anal. Calc. for $C_{24}H_{21}Br_2NO_3$: C, 54.26; H, 3.98; N, 2.64%. Found: C, 54.28; H, 3.97; N, 2.65%.

1-(2-Bromobenzyl)-4-[(5,6-dimethoxy-1-indanone-2-yl) methylene]pyridinium bromide (5e) From 0.26 g of 2-bromobenzylbromide, 0.12 g (0.23 mmol) of off-white solid was obtained in 66% yield; mp: 212–214 °C; FTIR (KBr) ν 2932, 1689, 1590, 1464, 1309, 1027, 847, 752, 539 cm^{-1}; ^{1}HNMR (400 MHz, DMSO): δ 3.76 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 4.18 (2H, s, CH_2), 5.86 (2H, s, CH_2), 7.20 (1H, s, Ar), 7.28 (1H, s, Ar), 7.37 (1H, dd, $^3J_{H-H}$ = 7.6 Hz, Ar), 7.44 (1H, m, Ar), 7.51 (1H, s, =CH), 7.60 (1H, s, Ar), 7.79 (1H, dd, $^3J_{H-H}$ = 7.9, Hz, Ar), 8.48 (2H, d, $^3J_{H-H}$ = 6.55, pyridine-H), 9.15 (2H, d, $^3J_{H-H}$ = 6.55, pyridine-H); ^{13}C NMR (100 MHz, DMSO): 191.33, 156.11, 152.30, 150.17, 147.22, 146.30, 145.71, 133.90, 133.68,131.92, 131.60, 129.79, 129.22, 128.50, 125.31, 123.76, 108.40, 105.28, 63.30, 56.70, 56.28, 31.90; Anal. Calc. For $C_{24}H_{21}Br_2NO_3$: C, 54.26; H, 3.98; N, 2.64%. Found: C, 54.23; H, 3.97; N, 2.63%.

1-(2-Chlorobenzyl)-4-[(5,6-dimethoxy-1-indanone-2-yl) methylene]pyridinium chloride (5f) From 0.17 g of 1-chloro-2-(chloromethyl)benzene, 0.09 g (0.21 mmol) of white solid was obtained in 60% yield; mp: 222–224 °C; FTIR (KBr) ν 3004, 2944, 1691, 1600, 1640, 1464, 1317, 1272, 1024, 757, 855, 525 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 3.76 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 4.17 (2H, s, CH_2), 5.92 (2H, s, CH_2), 7.36 (1H, s, Ar), 7.45 (1H, s, Ar), 7.52 (1H, dd, $^3J_{H-H}$ = 7.5 Hz, Ar), 7.58 (1H, m, Ar), 7.49 (1H, s, =CH), 7.63 (1H, s, Ar), 7.80 (1H, dd, $^3J_{H-H}$ = 7.5, Hz, Ar), 8.79 (2H, d, $^3J_{H-H}$ = 6.50, pyridine-H), 9.14 (2H, d, $^3J_{H-H}$ = 6.50, pyridine-H); ^{13}C NMR (100 MHz, DMSO): 191.36, 156.11, 152.30, 150.17, 147.22, 146.30, 145.71,

133.90, 133.67,131.92, 138.60, 129.81, 129.23, 128.50, 125.33, 123.76, 108.40, 105.27, 63.32, 55.93, 56.25, 31.89; Anal. Calc. for $C_{24}H_{21}Cl_2NO_3$: C, 65.17; H, 4.79; N, 3.17%. Found: C, 65.21; H, 4.80; N, 3.16%.

1-Hexyl-4-[(5,6-dimethoxy-1-indanone-2-yl)methylene] pyridinium bromide (5g) From 0.17 g of 1-bromohexane, 0.08 g (0.19 mmol) of white solid was obtained in 54% yield; mp: 228–231 °C; FTIR (KBr) ν 2931, 1686, 1642, 1591, 1466, 1312, 1271, 1121, 845 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 0.80 (3H, s, CH_3), 1.05–1.15 (6H, m, CH_2), 1.91–1.94 (2H, m, CH_2), 3.73 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 4.11 (2H, m, CH_2), 4.93 (2H, t, CH_2), 7.40 (1H, s, Ar–H), 7.43 (1H, s, =CH), 7.56 (1H, s, Ar–H), 8.75 (2H, d, $^3J_{H-H}$=6.71, pyridine-H), 9.09 (2H, d, $^3J_{H-H}$=6.71, pyridine-H); ^{13}C NMR (100 MHz, DMSO): 14.25, 22.73, 27.21, 31.52, 32.28, 52.06, 55.47, 56.26, 60.96, 122.93, 128.42, 129.33, 137.99, 138.56, 139.14, 145.61, 146.22, 150.13, 155.54, 156.14, 191.84; Anal. Calc. for $C_{23}H_{28}BrNO_3$: C, 61.89; H, 6.32; N, 3.14%. Found: C, 61.87; H, 6.31; N, 3.15%.

1-(2-Propenyl)-4-[(5,6-dimethoxy-1-indaanone-2-yl)methylene]pyridinium chloride (5h) From 0.08 g of 3-chloroprop-1-ene, 0.07 g (0.26 mmol) of white solid was obtained in 74% yield; mp: 225–230 °C; FTIR (KBr) ν 2937, 1688, 1505, 1316, 1269, 1028, 830 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 3.75 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 4.15 (2H, s, CH_2), 5.28 (2H, m, CH_2), 5.24–5.31 (2H, m, $=CH_2$), 6.12 (1H, m, =CH), 7.35 (1H, s, Ar–H), 7.42 (1H, s, =CH), 7.60 (1H, s, Ar–H), 8.70 (2H, d, $^3J_{H-H}$=5.25, pyridine-H), 9.12 (2H, d, $^3J_{H-H}$=5.48, pyridine-H); ^{13}C NMR (100 MHz, DMSO): 52.06, 55.47, 56.32, 58.28, 117.2, 122.94, 128.41, 129.32, 132.90, 138.01, 138.56, 139.14, 145.56, 146.25, 151.05, 155.56, 156.12, 192.03; Anal. Calc. for $C_{20}H_{20}ClNO_3$: C, 67.13; H, 5.63; N, 3.91%. Found: C, 67.15; H, 5.62; N, 3.90%.

1-[2-(Pyrrolidin-1-yl)ethyl]-4-[(5,6-dimethoxy-1-indanone-2-yl)methylene]pyridinium bromide (5i) From 0.18 g of 1-(2-bromoethyl)pyrrolidine, 0.08 g (0.17 mmol) of white solid was obtained in 49% yield; mp: 230–236 °C; FTIR (KBr) ν 2929, 2861, 1687, 1463, 1316, 1268, 1031, 870 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 1.83–1.90 (4H, m, CH_2), 2.46–2.59 (6H, m, CH_2–N), 3.74 (3H, s, CH_3), 3.96 (3H, s, OCH_3), 4.16 (2H, m, CH_2), 5.03 (2H, t, CH_2), 7.28 (1H, s, Ar–H), 7.45 (1H, s, =CH), 7.63 (1H, s, Ar–H), 8.92 (2H, d, $^3J_{H-H}$=6.00, pyridine-H), 9.08 (2H, d, $^3J_{H-H}$=5.93, pyridine-H); ^{13}C NMR (100 MHz, DMSO): 23.67, 32.41, 48.94, 55.24, 56.16, 56.24, 57.05, 115.6, 120.71, 133.58, 138.5, 139.33, 139.82, 142.50, 144.51, 147.32, 150.16, 155.52, 191.64; Anal. Calc. for $C_{23}H_{27}BrN_2O_3$: C, 60.14; H, 5.92; N, 6.10%. Found: C, 60.15; H, 5.93; N, 6.09%.

1-(2-Hydroxyethyl)-4-[(5,6-dimethoxy-1-indanone-2-yl) methylene]pyridinium chloride (5j) From 0.08 g of 2-chloroethanol, 0.07 g (0.19 mmol) of white solid was obtained in 54% yield; mp: 229–232 °C; FTIR (KBr) ν 3400, 2935, 1692, 1591, 1463, 1316, 1032, 853 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 3.63–3.66 (2H, m, CH_2-OH), 3.75 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 4.17 (2H, s, CH_2), 4.69 (2H, t, CH_2), 7.34 (1H, s, Ar–H), 7.38 (1H, s, =CH), 7.63 (1H, s, Ar–H), 8.46 (2H, d, $^3J_{H-H}$=6.55, pyridine-H), 9.14 (2H, d, $^3J_{H-H}$=6.56, pyridine-H), –OH not detected; ^{13}C NMR (100 MHz, DMSO): 32.45, 55.19, 55.26, 56.24, 60.52, 116.06, 120.72, 133.61, 138.51, 139.32, 139.79, 142.51, 145.01, 147.33, 150.17, 155.44, 191.82; Anal. Calc. for $C_{19}H_{20}ClNO_4$: C, 63.07; H, 5.57; N, 3.87%. Found: C, 63.09; H, 5.58; N, 3.86%.

1-(Acetic acid)-4-[(5,6-dimethoxy-1-indanone-2-yl)methylene]pyridinium chloride (5k) From 0.10 g of 2-chloroacetic acid, 0.09 g (0.25 mmol) of white solid was obtained in 71% yield; mp: 231–236 °C; FTIR (KBr) ν 3426, 2943, 1688, 1503, 1314, 1272, 1029, 848 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 3.76 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 4.15 (4H, s, CH_2), 4.98 (1H, m, CH_2), 7.35 (1H, d, Ar–H), 7.42 (1H, s, =CH), 7.66 (1H, s, Ar–H), 8.12 (2H, d, $^3J_{H-H}$=5.70, pyridine-H), 9.18 (2H, d, $^3J_{H-H}$=5.68, pyridine-H), –OH not detected; ^{13}C NMR (100 MHz, DMSO): 33.06, 56.18, 56.33, 58.86, 115.6, 120.71, 133.58, 138.50, 138.96, 139.31, 142.50, 144.51, 147.32, 150.16, 155.52, 178.99, 192.15; Anal. Calc. for $C_{19}H_{18}ClNO_5$: C, 60.73; H, 4.38; N, 3.73%. Found: C, 60.75; H, 4.37; N, 3.74%.

1-(Propionic acid)-4-[(5,6-dimethoxy-1-indanone-2-yl)methylene]pyridinium chloride (5l) From 0.11 g of 3-chloropropanoic acid, 0.11 g (0.28 mmol) of white solid was obtained in 80% yield; mp: 241–243 °C; FTIR (KBr) ν 3532, 3052, 2836, 1689, 1730, 1641, 1568, 1466, 1317, 1271, 1025, 846 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 3.02 (2H, m, CH_2 –COOH), 3.75 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 4.16 (2H, s, CH_2), 4.72 (2H, m, –CH_2–N), 7.33 (1H, s, Ar–H), 7.41 (1H, s, =CH), 7.65 (1H, s, Ar–H), 8.13 (2H, d, $^3J_{H-H}$=6.10, pyridine-H), 9.11 (2H, d, $^3J_{H-H}$=6.00, pyridine-H), –OH not detected; ^{13}C NMR (100 MHz, DMSO): 32.95, 34.92, 48.32, 56.14, 56.25, 115.6, 120.71, 133.56, 138.52, 138.84, 139.29, 142.51, 144.53, 147.32, 151.01, 155.52, 176.91, 192.18; Anal. Calc. for $C_{20}H_{20}ClNO_5$: C, 61.62; H, 5.17; N, 3.59%. Found: C, 61.64; H, 5.18; N, 3.60%.

1-(Butyric acid)-4-[(5,6-dimethoxy-1-indanone-2-yl)methylene]pyridinium chloride (5m) From 0.13 g of 4-chlorobutanoic acid, 0.11 g (0.30 mmol) of white solid was obtained in 86% yield; mp: 232–236 °C; FTIR (KBr) ν 3392, 2937, 1691, 1593, 1499, 1315, 1263, 1026, 823 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 2.98 (2H, t, CH_2 –COOH), 2.36 (2H,

m, CH_2), 3.75 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 4.17 (2H, s, CH_2), 4.68 (2H, m, –CH_2–N), 7.34 (1H, s, Ar–H), 7.40 (1H, s, =CH), 7.68 (1H, s, Ar–H), 8.12 (2H, d, $^3J_{H-H}$ = 6.10, pyridine-H), 9.11 (2H, d, $^3J_{H-H}$ = 6.00, pyridine-H), –OH not detected; ^{13}C NMR (100 MHz, DMSO): 24.86, 32.54, 34.92, 48.32, 53.21, 56.14, 56.25, 115.6, 120.71, 133.55, 137.94, 138.81, 138.97, 142.51, 144.53, 147.32, 151.05, 155.52, 178.43, 192.18; Anal. Calc. for $C_{21}H_{22}ClNO_5$: C, 62.46; H, 5.49; N, 3.47%. Found: C, 62.44; H, 5.50; N, 3.46%.

1-(4-Oxopentyl)-4-[(5,6-dimethoxy-1-indanone-2-yl)methylene]pyridinium chloride (5n) From 0.12 g of 5-chloropentan-2-one, 0.07 g (0.19 mmol) of white solid was obtained in 54% yield; mp: 228–234 °C; FTIR (KBr) ν 2834, 1689, 1593, 1316, 1265, 1026, 824 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 2.10 (3H, s, CH_3), 2.15 (2H, t, CH_2), 2.40 (2H, t, CH_2-CO), 3.75 (3H, s, OCH_3), 3.94 (3H, s,OCH_3), 4.18 (2H, s, CH_2), 4.68 (2H, s, CH_2–N), 7.35 (1H, s, Ar–H), 7.43 (1H, s, =CH), 7.68 (1H, s, Ar–H), 8.16 (2H, d, $^3J_{H-H}$ = 5.38, pyridine-H), 9.13 (2H, d, $^3J_{H-H}$ = 5.36, pyridine-H); ^{13}C NMR (100 MHz, DMSO): 22.93, 29.81, 32.26, 42.71, 53.31, 55.61, 56.25, 122.93, 128.42, 129.33, 138.01, 138.54, 139.17, 145.62, 146.22, 150.13, 155.54, 156.14, 192.13, 207.71; Anal. Calc. for $C_{22}H_{24}ClNO_4$: C, 65.75; H, 6.02; N, 3.49%. Found: C, 65.77; H, 6.01; N, 3.48%.

1-[(5-Hydroxy-4H-pyran-4-one-2-yl)methyl]-4-[(5,6-dimethoxy-1-indanone-2-yl)methylene] pyridinium chloride (5o) From 0.17 g of 5-(chloromethyl)-2-hydroxy-4*H*-pyran-4-one, 0.11 g (0.26 mmol) of light brown solid was obtained in 74% yield; mp: 254–256 °C; FTIR (KBr) ν 3395, 3051, 2835, 1688, 1644, 1596, 1467, 1317, 1272, 1052, 855 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 3.75 (3H, s, OCH_3), 3.95 (3H, s,OCH_3), 4.16 (2H, s, CH_2), 4.40 (2H, s, CH_2–N), 6.68 (1H, s, =CH_2 pyran), 6.82 (1H, s =CH_2 pyran), 7.34 (1H, s, Ar–H), 7.44 (1H, s, =CH), 7.67 (1H, s, Ar–H), 8.15(2H, d, $^3J_{H-H}$ = 5.46, pyridine-H), 9.13 (2H, d, $^3J_{H-H}$ = 5.42, pyridine-H), –OH not detected; ^{13}C NMR (100 MHz, DMSO): 32.44, 55.46, 56.18, 64.41, 113.31, 122.91, 128.01, 128.42, 129.33, 137.99, 138.56, 139.12, 145.63, 146.28, 150.13, 155.54, 156.14, 163.54, 172.71, 181.32, 191.91; Anal. Calc. for $C_{23}H_{20}ClNO_6$: C, 62.52; H, 4.56; N, 3.17%. Found: C, 62.54; H, 4.55; N, 3.18%.

Biochemical studies: cholinesterase inhibitory activities

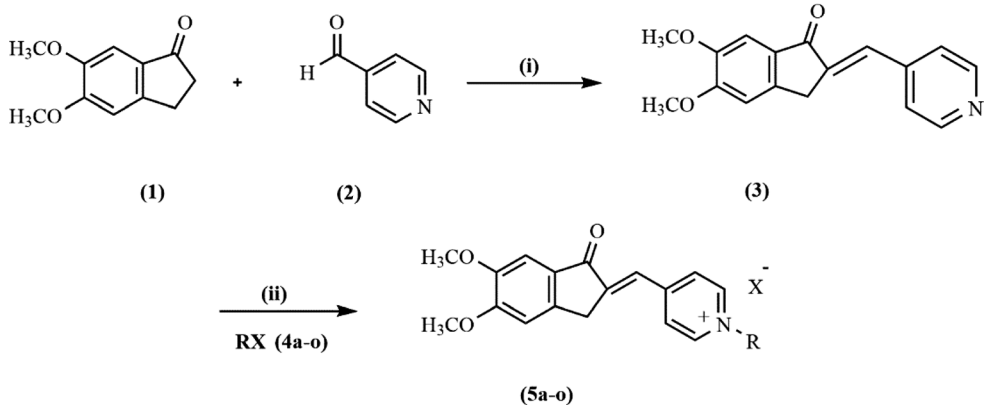
Cholinesterase activity of the new synthesized compounds was measured using the Ellman method [23], and acetylthiocholine or butyrylthiocoline was used as substrate for AChE or BuChE, respectively. The 50% inhibitory concentration was measured and expressed as IC_{50}. Human erythrocytes AChE (hAChE) and human plasmatic BuChE (hBuChE) were produced from fresh blood [24]. 5,5′-Dithiobis (2-nitrobenzoic acid) (Ellman's reagent, DTNB), phosphate buffer (PB), acetylthiocholine (ATC) and butylthiocholine (BTC) were purchased from Sigma-Aldrich, Praque. Quartz cuvettes were used for measuring purposes. All of the synthesized compounds were solved in DMSO. The Na_2HPO_4 buffers (0.1 M) with pH = 7 and 8 were used to prepare 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB, 3.5 mM) and acetylthiocholine (ATC, 7 mM) or butylthiocholine (BTC, 7 mM) solutions, respectively. All tests were carried out in 0.1 M of Na_2HPO_4 buffer, pH = 8. The assay medium (1 mL) consists of 550 µL of phosphate buffer (pH 8), 150 µL of DTNB, and 150 µL of substrate, 150 µL of inhibitor (10^{-4} to 10^{-10} M) in test cuvettes and 700 µL of phosphate buffer (pH 8), 150 µL of DTNB, and 150 µL of inhibitor solutin (10^{-4} to 10^{-10} M) in control cuvettes were incubation for 5 min in 37 °C. The reaction was initiated by an immediate addition of 50 µL of enzyme. The activity was determined by measuring the increase in absorbance at 412 nm at 5-min interval using a spectrophotometer Helios Zeta (Thermospectronic, Cambridge, UK). Each experiment was carried out twice. BuChE study was conducted in a similar situation that is described above. Nonlinear and linear regressions were used to estimate the drug concentration inducing 50% inhibition of the AChE or BuChE activity.

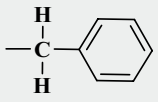
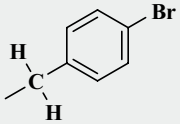
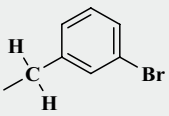
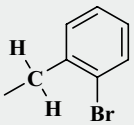
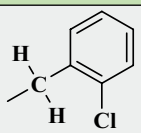
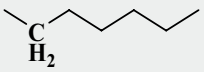
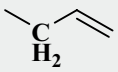
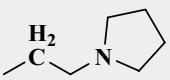
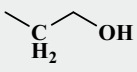
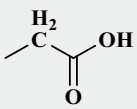
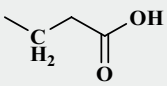
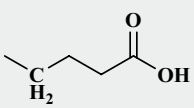
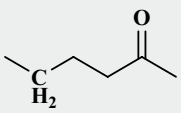
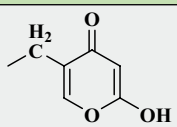
Results and discussion

Chemistry

4-Pyridinecarboxaldehyde (1) is one of the well-known aromatic aldehydes used in organic synthesis. Synthesis of 5,6-dimethoxy-2-[(pyridin-4-yl)methylene)-1-indanone (**3**) was reported in the literature as an aldol condensation reaction between 5,6-dimethoxy-1-indanone (**2**) and 4-pyridinecarboxaldehyde (**1**) under alkaline or acidic conditions [22]. This compound was prepared in the presence of NaOH 10% in ethanol. In continuation, the 1-alkyl-4-[(5,6-dimethoxy-1-indanone-2-yl)methylene]pyridinium halide derivatives (**4a-p**) were synthesized via N-alkylation reaction of 5,6-dimethoxy-2-[(pyridin-4-yl)methylene]-1-indanone (**3**) with various alkyl halides (R–X). This reaction was carried out under reflux temperature in acetone or acetonitrile as solvent (Scheme 1).

According to the results previously reported, polar aprotic solvents such as acetone, acetonitrile and DMSO are the suitable solvents for this type of reactions [6]. According to the results obtained from experiments in the case of iodide alkyl halides, the reaction was completed in mild temperature. Acetone was chosen as solvent, because the iodide is good leaving groups. In the case of chloride and bromide alkyl halides, higher temperature was



Compound	5a	5b	5c	5d	5e
-R	-CH3				
X	I	Cl	Br	Br	Br
Compound	5f	5g	5h	5i	5j
-R					
X	Cl	Br	Cl	Br	Cl
Compound	5k	5l	5m	5n	5o
-R					
X	Cl	Cl	Cl	Cl	Cl

Scheme 1 Synthesis of pyridinium halide derivatives (i) EtaOH, NaOH 10% (ii) acetone or acetonitrile, reflux

required for completion of reaction and acetonitrile was used as solvent for these compounds.

With the precursor **3** in hand, the focus was on the optimization of reaction condition for compound **5b** as representative of the final products. As can be seen in Table 1, the N-alkylation reaction was surveyed in the variety of indanone:alkylhalide ratio, time and temperature. Among different times, the optimal results were obtained in 48 h and for reflux temperature (80 °C for CH_3CN). By increasing the indanone:alkylhalide ratio until 1:3, the yield was increased up to 90%, but there was no change in yield afterward.

Table 1 Obtained yield in % for compound **5b** in different indanone:alkylhalide ratio, time and temperature

	Temperature (°C)																	
Indanone:alkylhalide	1:1			1:1.5			1:2			1:2.5			1:3			1:3.5		
Time	r.t	40	80	r.t	40	80	r.t	40	80	r.t	40	80	r.t	40	80	r.t	40	80
24 h	0	10	12	0	10	14	7	13	18	10	15	25	10	20	60	10	20	60
48 h	10	13	20	10	20	27	10	18	48	12	20	56	25	38	90	25	38	90
72 h	10	13	20	10	20	27	10	18	48	12	20	56	25	38	90	25	38	90

Table 2 In vitro hAChE and hBuChE inhibitory activity and selectivity index of indanone derivatives

Compound	Yield (%)	AChE IC_{50} (μM)	BuChE IC_{50} (μM)	AChE IC_{50}/ BuChEIC_{50}
Donepezil	–	0.04	14.4	0.003
Galantamine	–	3	31.6	0.095
3	67	16.2%	9.94%	–
5a	81	108	2.06%	–
5b	90	46	1.92	23.958
5c	66	17.3	7.12	2.430
5d	64	48.6	2.19	22.192
5e	48	10.8	5.48	1.971
5f	60	22.1	0.55	40.182
5g	57	4.6	26.03	0.177
5h	59	24.25%[a]	4.13%[a]	–
5i	50	4.85%[a]	12.97%[a]	–
5j	56	11.13%[a]	9.71%[a]	–
5k	71	22.95%[a]	0.5%[a]	–
5l	81	34	221.94	0.153
5m	84	21.35%[a]	1.54%[a]	–
5n	56	18.16%[a]	0	–
5o	77	22.60%	13.12%	–

[a]Percent inhibition at a concentration of 100 μM

All molecular structures of the synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR and FTIR spectroscopy and elemental analysis.

Inhibition of human AChE and BuChE

The extent of inhibition was expressed as the chemical concentration at which 50% of enzyme activity was inhibited (IC_{50}). The IC_{50} values of compounds **3** and **5a–o** were determined against human erythrocyte AChE and human plasmatic butyrylcholinesterase using the method of Ellman et al. [23]. The IC_{50} values and selectivity index (SI) of synthesized pyridinium halide derivatives and the control compounds, donepezil and galantamine are summarized in Table 2. These synthesized derivatives demonstrated inhibitory activity against both hAChE and hBuChE with IC_{50} values ranging from micro-molar to submicromolar concentrations. The IC_{50} values of the synthesized compounds suggest strong BuChE inhibition in comparison with AChE for these derivatives. Among these compounds, the highest inhibition of hAChE was recorded for **5g** (IC_{50}: 4.6 μM, weaker than donepezil and galantamine). The most potent inhibitor of hBuChE is **5f** which showed IC_{50}: 0.55 μM, while this value is approximately 26.18 times higher than donepezil and 57.45 times higher than galantamine; but in the case of hAChE, this compound showed IC_{50}: 22.1 μM that is weaker than donepezil and galantamine. It is notable that the compounds with chlorine and bromine atoms substitution on phenyl ring show better inhibition compared with the others. Summarized results in Table 1 indicated that bromine atom in ortho position shows a better inhibition for hAChE (compound **5e**); meanwhile, a better inhibition of hBuChE was obtained when the bromine set in meta position (compound **5d**).

Conclusion

In summary, a series of 1-alkyl-4-[(5,6-dimethoxy-1-indanone-2-yl)methylene]pyridinium halide were designed and synthesized with acceptable yield using N-alkylation reaction of 5,6-dimethoxy-2-[(pyridin-4-yl)methylene)1-indanone. Anti-AChE and anti-BuChE effects of all synthesized compounds were tested, 1-hexyl-4-[(5,6-dimethoxy-1-indanone-2-yl)methylene]pyridinium bromide (**5g**) displayed excellent inhibition of hAChE, and the most potent inhibitor of hBuChE was 1-(2-chlorobenzyl)-4-[(5,6-dimethoxy-1-indanone-2-yl)methylene]pyridinium chloride (**5f**).

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