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Synthesis and Cholinesterase Inhibitory Activity of Selected Indole-Based Compounds

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Abstract

Synthesis and anticholinesterase activity of 18 previously unpublished indole- and tryptophan-derived compounds are disclosed. These compounds containing an indole structural unit exhibit selective submicromolar inhibition of human butyrylcholinesterase (hBChE). The structures of the newly synthesized compounds were confirmed by ^{1}H and ^{13}C NMR, IR spectroscopy, and high-resolution mass spectrometry.

Keywords: Indole derivatives, Tryptophan derivatives, Amidations, Cholinesterase (ChE) inhibitors, 1,1'-Carbonyldiimidazole (CDI), Rearrangements, Catalytic hydrogenation

1. Introduction

Indole is a privileged scaffold found in countless natural products that have diverse biological activities and functions.[1–6] In addition to the well-known skatole (3-methylindole), serotonin, *L*-tryptophan, tryptamine, the plant growth hormone 3-indoleacetic acid, and others, indole-containing alkaloids represent one of the most important alkaloid subgroups.[7,8] Indoles exhibit diverse biological activities ranging from antitumor to antibacterial activity.[9–12] Commercially available drugs with indole moiety include ajmaline[13] (antiarrhythmic agent), physostigmine[14] (for the treatment of glaucoma and anticholinergic poisoning), and vincristine[15] (antitumor agent), among others.[1,16,17]

Dementia is a serious neurological condition that severely affects patient's quality of life. It is estimated that Alzheimer's disease (AD), the most common form of dementia, affects more than 50 million people worldwide, and this number is expected to triple by 2050, mainly due to the aging of the population.[18] Selective human butyrylcholinesterase (hBChE) inhibitors improved cognitive functions, memory, and learning ability in a scopolamine mice model of cognitive deficit without causing peripheral cholinergic side effects typical of acetylcholinesterase (AChE) inhibitors.[19,20] These data suggest that hBChE may be considered a promising therapeutic target to improve cognitive functions in late-stages of AD.[21] Recently, we have disclosed a hit-to-lead development of a new series of tryptophan-derived selective hBChE inhibitors with nanomolar inhibitory potencies, which were developed from (+)-isocampholenic acid-derived tryptophan amide hit **A**[22] by a medicinal chemistry-based approach (Figure 1).[23,24] Lead compounds **B** and **C** inhibited hBChE in the low nanomolar range with high selectivity over AChE, and possessed advantageous physicochemical properties for high blood-brain barrier permeability. Furthermore, compound **B** showed beneficial effects on fear-motivated long-term memory and spatial long-term memory retrieval in a scopolamine AD mouse model, with no adverse peripheral cholinergic side effects.[23–25]

While the structure-activity relationships (SAR) has been explored, several of the indole products were not included in our earlier reports. Therefore, we report here the synthesis and cholinesterase inhibitory activity of those unpublished indole-based compounds.

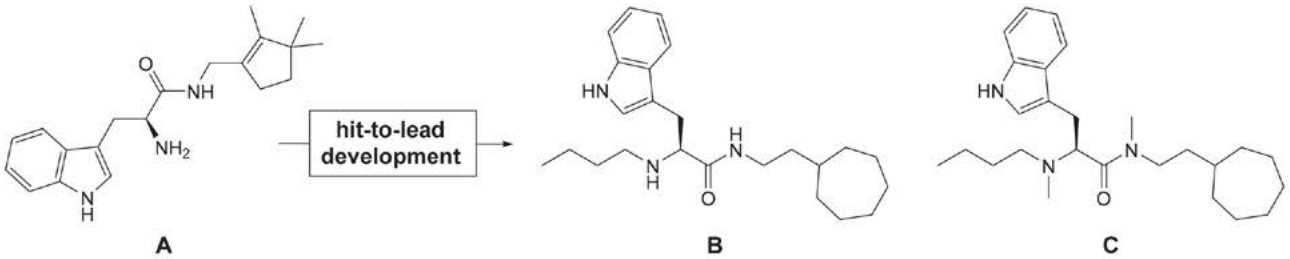


Figure 1. Hit compound **A** and selected lead compounds **B** and **C**.

2. Experimental

2. 1. Materials and Measurements

Solvents for extractions and chromatography were of technical grade and were distilled prior to use. Extracts were dried over technical grade anhydrous Na_2SO_4. Melting points were determined on a Kofler micro hot stage and using the SRS OptiMelt MPA100 – Automated Melting Point System (Stanford Research Systems, Sunnyvale, CA, USA). NMR spectra were recorded on a Bruker UltraShield 500 plus (Bruker, Billerica, MA, USA) at 500 MHz for the ^1H nucleus and 126 MHz for the ^{13}C nucleus, using $CDCl_3$ with TMS as the internal standard, as solvents. Mass spectra were recorded using an Agilent 6224 Accurate Mass TOF LC/MS (Agilent Technologies, Santa Clara, CA, USA), IR spectra using a Perkin-Elmer Spectrum BX FTIR spectrophotometer (Perkin-Elmer, Waltham, MA, USA). Column chromatography was performed on silica gel (silica gel 60, particle size: 0.035–0.070 mm (Sigma-Aldrich, St. Louis, MO, USA)). All commercial chemicals used were purchased from Sigma-Aldrich (St. Louis, MO, USA). Catalytic hydrogenation was performed in a Parr Pressure Reaction Hydrogenation Apparatus (Moline, IL, USA). Microanalyses were performed by combustion analysis on a Perkin-Elmer Series II CHNS/O Analyser (Perkin-Elmer, Waltham, MA, USA).

General procedure 1 (GP1) – amide formation. To a solution/suspension of acid (1 equivalent) in anhydrous MeCN under argon at room temperature was added CDI (1.20 equivalents). The resulting reaction mixture was stirred at room temperature for 1 h, then amine (1.13 equivalents) was added. After stirring the reaction mixture at room temperature for 16 h, the volatile components were evaporated *in vacuo* and the residue was purified by column chromatography on silica gel 60. The fractions containing the pure product (amide) were combined and the volatiles were evaporated *in vacuo*.

General procedure 2 (GP2) – Boc-deprotection and double bond isomerization. To a solution of the starting compound in anhydrous CH_2Cl_2 at 0 °C was added trifluoroacetic acid (TFA). The resulting reaction mixture was stirred at 0 °C for 30 minutes and then stirred at room temperature for 2 h. Volatile components were evaporated *in vacuo*. The residual trifluoroacetic acid was removed by azeotropic evaporation with anhydrous toluene.

General procedure 3 (GP3) – acetamidation. To a solution of the trifluoroacetate salt in anhydrous CH_2Cl_2 under argon at room temperature was added *N*,*N*-diisopropylethylamine (DIPEA) followed by CH_3COCl. After stirring the reaction mixture at room temperature for 12 h, the volatiles were evaporated *in vacuo*. The residue was dissolved in EtOAc (50 mL) and washed with $NaHSO_4$ (1 M in H_2O, 2×5 mL), $NaHCO_3$ (aq. sat, 2 × 5 mL), and NaCl (aq. sat, 2 × 5 mL). The organic phase was dried under anhydrous Na_2SO_4, filtered, and the volatiles were evaporated *in vacuo*. The residue was purified by column chromatography on silica gel 60. The fractions containing the pure product were combined and the volatiles were evaporated *in vacuo*.

General procedure 4 (GP4) – amine N-Boc protection. To a solution of the trifluoroacetate salt in anhydrous CH_2Cl_2 under argon at room temperature were added Et_3N and Boc_2O. After stirring the reaction mixture at room temperature for 16 h, the volatile components were evaporated *in vacuo*. The residue was purified by column chromatography on silica gel 60. The fractions containing the pure product were combined and the volatiles were evaporated *in vacuo*.

General procedure 5 (GP5) – alkene hydrogenation. To a solution of alkene in MeOH, Pd–C (10% Pd on C; 20% by mass reagent was used) was added under argon. The resulting reaction mixture was thoroughly purged with hydrogen and shaken on a Parr apparatus under H_2 (4 bar) at room temperature. The reaction mixture was filtered through a short pad of Celite® on a ceramic frit and Celite® was washed with MeOH. The volatiles were evaporated *in vacuo*. If necessary, the product was additionally purified by column chromatography on silica gel 60. The fractions containing the pure product were combined and the volatiles were evaporated *in vacuo*.

***N*-(Cycloheptylmethyl)-3-(1*H*-indol-3-yl)propanamide (8)**

Following *GP1*. Prepared from 3-(1*H*-indol-3-yl) propanoic acid (**1**) (283 mg, 1.50 mmol), MeCN (3 mL), CDI (280 mg, 1.73 mmol), cycloheptylmethanamine (**5**)

(250 μL, 1.74 mmol); column chromatography (EtOAc/petroleum ether = 1:1). Yield: 348 mg (1.17 mmol, 78%) of white solid; mp 80.9–85.0 °C. Anal. Calcd for $C_{19}H_{26}N_2O$: C, 76.47; H, 8.78; N, 9.39. Found: C, 76.38; H, 8.85; N, 9.31. ESI-HRMS Calcd for $C_{19}H_{27}N_2O$: *m/z* 299.2118 (MH^+). Found: *m/z* 299.2119 (MH^+). IR ν_{max} 3258, 3087, 2914, 2848, 1612, 1564, 1492, 1455, 1429, 1350, 1274, 1217, 1181, 1102, 1065, 1008, 979, 790, 767, 733, 698 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 1.00–1.11 (m, 2H), 1.28–1.64 (m, 11H), 2.44 (dd, *J* = 6.9, 8.4 Hz, 2H), 2.84–2.96 (m, 4H), 6.93–6.99 (m, 1H), 7.02–7.09 (m, 2H), 7.32 (dt, *J* = 0.9, 8.1 Hz, 1H), 7.52 (dd, *J* = 1.0, 7.9 Hz, 1H), 7.79 (t, *J* = 5.8 Hz, 1H), 10.73 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 21.15, 25.87, 27.99, 31.63, 36.36, 45.11, 111.28, 113.89, 118.09, 118.37, 120.85, 122.08, 127.07, 136.25, 171.87 (one signal missing).

***tert*-Butyl (*R*)-(1-((2-Cyclohexylethyl)amino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)carbamate (9)**

Following *GP1*. Prepared from (*tert*-butoxycarbonyl)-*D*-tryptophan (**2**) (182 mg, 0.598 mmol), MeCN (2 mL), CDI (112 mg, 0.691 mmol), 2-cyclohexylethan-1-amine (**6**) (100 μL, 0.677 mmol); column chromatography (EtOAc). Yield: 221 mg (0.534 mmol, 89%) of white solid; mp 103.9–106.2 °C. Anal. Calcd for $C_{24}H_{35}N_3O_3$: C, 69.70; H, 8.53; N, 10.16. Found: C, 69.77; H, 8.60; N, 10.08. ESI-HRMS Calcd for $C_{24}H_{36}N_3O_3$: *m/z* 414.2751 (MH^+). Found: *m/z* 414.2762 (MH^+). IR ν_{max} 3413, 3324, 2920, 2849, 1682, 1650, 1521, 1455, 1390, 1366, 1247, 1166, 1090, 1065, 1046, 1011, 857, 795, 736 cm^{-1}. $[\alpha]_D^{r.t.}$ = –14.4 (*c* = 1.1 mg/mL, CH_2Cl_2). ^1H NMR (500 MHz, $CDCl_3$): δ 0.71–0.85 (m, 2H), 0.99–1.21 (m, 6H), 1.43 (s, 9H), 1.51–1.68 (m, 5H), 3.04–3.20 (m, 3H), 3.31 (dd, *J* = 5.0, 14.3 Hz, 1H), 4.38 (s, 1H), 5.19 (s, 1H), 5.55 (s, 1H), 7.05 (d, *J* = 2.3 Hz, 1H), 7.11–7.16 (m, 1H), 7.18–7.23 (m, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.67 (d, *J* = 7.9 Hz, 1H), 8.15 (s, 1H, NH). ^{13}C NMR (126 MHz, $CDCl_3$): δ 26.26, 26.60, 28.47, 28.78, 33.12, 33.18, 35.23, 36.80, 37.36, 55.43, 80.08, 111.17, 111.30, 119.14, 119.95, 122.47, 123.18, 127.56, 136.37, 155.60, 171.47.

***tert*-Butyl (*S*)-(1-((2-Cyclohexylethyl)amino)-3-(1-methyl-1*H*-indol-3-yl)-1-oxopropan-2-yl)carbamate (10)**

Following *GP1*. Prepared from N^{α}-(*tert*-butoxycarbonyl)-1-methyl-*L*-tryptophan (**3**) (159 mg, 0.499 mmol), MeCN (2 mL), CDI (99.6 mg, 0.614 mmol), 2-cyclohexylethan-1-amine (**6**) (83.3 μL, 0.564 mmol); column chromatography (EtOAc/petroleum ether = 1:1). Yield: 198 mg (0.463 mmol, 93%) of white solid; mp 118.2–123.8 °C. Anal. Calcd for $C_{25}H_{37}N_3O_3$: C, 70.23; H, 8.72; N, 9.83. Found: C, 70.15; H, 8.89; N, 9.76. ESI-HRMS Calcd for $C_{25}H_{38}N_3O_3$: *m/z* 428.2908 (MH^+). Found: *m/z* 428.2910 (MH^+). IR ν_{max} 3341, 3320, 2921, 2852, 1679, 1655, 1543, 1514, 1486, 1463, 1452, 1420, 1391, 1369, 1321, 1291, 1238, 1206, 1165, 1123, 1093, 1062, 1045, 1024, 1001, 963, 924, 887, 867, 783, 767, 734 737 cm^{-1}. $[\alpha]_D^{r.t.}$ = –1.03 (*c* = 2.8 mg/mL, CH_2Cl_2). ^1H NMR (500 MHz, $CDCl_3$): δ 0.73–0.85 (m, 2H), 0.98–1.21 (m, 6H), 1.43 (s, 9H), 1.51–1.70 (m, 5H), 3.03–3.23 (m, 3H), 3.30 (dd, *J* = 5.2, 14.5 Hz, 1H), 3.74 (s, 3H), 4.37 (s, 1H), 5.18 (s, 1H), 5.59 (s, 1H), 6.90 (s, 1H), 7.10–7.14 (m, 1H), 7.21–7.25 (m, 1H), 7.29 (dt, *J* = 0.9, 8.3 Hz, 1H), 7.64 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 26.24, 26.57, 28.45, 28.61, 32.81, 33.12, 33.18, 35.27, 36.86, 37.33, 55.41, 80.04, 109.36, 119.20, 119.38, 121.96, 127.99, 137.07, 155.61, 171.49 (one signal missing).

***tert*-Butyl ((S)-1-((2-((*R*)-2,2-Dimethyl-3-methylenecyclopentyl)ethyl)amino)-3-(1-methyl-1*H*-indol-3-yl)-1-oxopropan-2-yl)carbamate (11)**

Following *GP1*. Prepared from N^{α}-(*tert*-butoxycarbonyl)-1-methyl-*L*-tryptophan (**3**) (318 mg, 0.999 mmol), MeCN (3 mL), CDI (188 mg, 1.16 mmol), (*R*)-2-(2,2-dimethyl-3-methylenecyclopentyl)ethan-1-amine (**7**)[22] (182 μL, 1.13 mmol); column chromatography (EtOAc/petroleum ether = 1:2). Yield: 183 mg (0.403 mmol, 40%) of yellow oil. ESI-HRMS Calcd for $C_{27}H_{40}N_3O_3$: *m/z* 454.3064 (MH^+). Found: *m/z* = 454.3068 (MH^+). IR ν_{max} 3306, 3068, 2958, 2932, 2867, 1651, 1524, 1473, 1436, 1390, 1365, 1325, 1240, 1166, 1046, 1013, 878, 780, 737 cm^{-1}. $[\alpha]_D^{r.t.}$ = +5.40 (*c* = 1.8 mg/mL, CH_2Cl_2). ^1H NMR (500 MHz, $CDCl_3$): δ 0.72 (s, 3H), 0.95 (s, 3H), 1.00–1.08 (m, 1H), 1.10–1.20 (m, 1H), 1.21–1.31 (m, 1H), 1.31–1.39 (m, 1H), 1.43 (s, 9H), 1.64–1.79 (m, 1H), 2.16–2.28 (m, 1H), 2.35–2.45 (m, 1H), 3.03–3.22 (m, 3H), 3.30 (dd, *J* = 5.2, 14.5 Hz, 1H), 3.73 (s, 3H), 4.38 (s, 1H), 4.72 (t, *J* = 2.5 Hz, 1H), 4.74 (t, *J* = 2.2 Hz, 1H), 5.18 (s, 1H), 5.67 (s, 1H), 6.91 (s, 1H), 7.09–7.14 (m, 1H), 7.20–7.25 (m, 1H), 7.28 (d, *J* = 8.2 Hz, 1H), 7.64 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 23.43, 26.51, 28.04, 28.45, 28.57, 29.71, 30.68, 32.82, 38.94, 44.00, 47.92, 55.43, 80.13, 103.20, 109.38, 119.19, 119.40, 122.01, 127.96, 128.00, 137.08, 155.64, 162.07, 171.54 (one signal missing).

***tert*-Butyl ((*R*)-1-((2-((*R*)-2,2-Dimethyl-3-methylenecyclopentyl)ethyl)amino)-3-(1-methyl-1*H*-indol-3-yl)-1-oxopropan-2-yl)carbamate (12)**

Following *GP1*. Prepared from N^{α}-(*tert*-butoxycarbonyl)-1-methyl-*D*-tryptophan (**4**) (200 mg, 0.628 mmol), MeCN (3 mL), CDI (123 mg, 0.759 mmol), (*R*)-2-(2,2-dimethyl-3-methylenecyclopentyl)ethan-1-amine (**7**)[22] (114 μL, 0.707 mmol); column chromatography (EtOAc/petroleum ether = 1:1). Yield: 140 mg (0.309 mmol, 49%) of orange oil. ESI-HRMS Calcd for $C_{27}H_{40}N_3O_3$: *m/z* 454.3064 (MH^+). Found: *m/z* 454.3048 (MH^+). IR ν_{max} 3307, 2958, 1651, 1523, 1474, 1365, 1325, 1240, 1166, 1046, 1013, 877, 781, 737 cm^{-1}. $[\alpha]_D^{r.t.}$ = +4.21 (*c* = 1.4 mg/mL, CH_2Cl_2). ^1H NMR (500 MHz, $CDCl_3$): δ 0.73 (s, 3H), 0.96 (s, 3H), 0.99–1.10 (m, 1H), 1.13–1.22 (m, 1H), 1.28–1.37 (m, 2H), 1.43 (s, 9H), 1.70–1.78 (m, 1H), 2.19–2.29 (m, 1H), 2.37–2.45 (m, 1H), 3.01–3.25 (m, 3H), 3.31 (dd, *J* = 5.2, 14.3 Hz, 1H), 3.74 (s, 3H), 4.37 (s, 1H), 4.73 (t, *J* = 2.5 Hz, 1H), 4.75

(t, *J* = 2.3 Hz, 1H), 5.17 (s, 1H), 5.66 (s, 1H), 6.92 (s, 1H), 7.10–7.14 (m, 1H), 7.21–7.25 (m, 1H), 7.29 (dt, *J* = 0.9, 8.3 Hz, 1H), 7.64 (d, *J* = 7.9 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 23.47, 26.54, 28.06, 28.51, 28.45, 29.71, 30.70, 32.84, 39.03, 44.01, 47.97, 55.49, 80.14, 103.21, 109.37, 119.20, 119.41, 122.00, 127.98, 128.04, 137.08, 155.61, 162.09, 171.53 (one signal missing).

(*R*)-1-((2-Cyclohexylethyl)amino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-aminium 2,2,2-Trifluoroacetate (13)

Following *GP2*. Prepared from Boc-amine **9** (199 mg, 0.481 mmol), CH_2Cl_2 (2 mL), TFA (1.8 mL); the product **13** was thoroughly dried in high vacuum. Yield: 187 mg (0.437 mmol, 91%) of orange oil. ESI-HRMS Calcd for $C_{19}H_{28}N_3O$: *m/z* 314.2227 (MH^+). Found: *m/z* = 314.2242 (MH^+). IR ν_{max} 3293, 2923, 2851, 1448, 1661, 1341, 1180, 1135, 1011, 838, 799, 741, 722 cm^{-1}. $[\alpha]_D^{r.t.}$ = –36.4 (*c* = 1.8 mg/mL, CH_2Cl_2). ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.75–0.87 (m, 2H), 1.04–1.25 (m, 6H), 1.52–1.69 (m, 5H), 2.97–3.22 (m, 4H), 3.84–3.97 (m, 1H), 6.98–7.03 (m, 1H), 7.07–7.11 (m, 1H), 7.19 (d, *J* = 2.5 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.62 (d, *J* = 7.8 Hz, 1H), 8.15 (s, 3H), 8.38 (t, *J* = 5.6 Hz, 1H), 11.05 (d, *J* = 2.5 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 25.67, 26.09, 27.43, 32.52, 32.56, 34.31, 36.13, 36.45, 52.90, 107.00, 111.47, 118.39, 121.11, 124.73, 127.07, 136.27, 158.13 (q, *J* = 31.9 Hz), 168.08 (one signal missing).

(*S*)-3-(1-Methyl-1*H*-indol-3-yl)-1-oxo-1-((2-(2,3,3-trimethylcyclopent-1-en-1-yl)ethyl)amino)propan-2-aminium 2,2,2-Trifluoroacetate (14)

Following *GP2*. Prepared from Boc-amine **11** (98.5 mg, 0.217 mmol), CH_2Cl_2 (2 mL), TFA (1 mL); the product **14** was thoroughly dried in high vacuum. Yield: 92.9 mg (0.199 mmol, 92%) of dark brown oil. ESI-HRMS Calcd for $C_{22}H_{32}N_3O$: *m/z* 354.2540 (MH^+). Found: *m/z* 354.2541 (MH^+). IR ν_{max} 3056, 2951, 2934, 2862, 1779, 1662, 1549, 1474, 1435, 1378, 1359, 1330, 1199, 1175, 1134, 1014, 965, 837, 798, 739, 722 cm^{-1}. $[\alpha]_D^{r.t.}$ = +6.52 (*c* = 2.3 mg/mL, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (s, 3H), 0.89 (s, 3H), 1.34–1.36 (m, 3H), 1.43–1.56 (m, 2H), 1.88–2.06 (m, 4H), 2.95–3.11 (m, 2H), 3.24 (d, *J* = 7.2 Hz, 2H), 3.70 (s, 3H), 4.16 (t, *J* = 7.3 Hz, 1H), 6.71 (t, *J* = 5.5 Hz, 1H), 7.00–7.07 (m, 2H), 7.11–7.20 (m, 1H), 7.22–7.30 (m, 1H), 7.51 (d, *J* = 7.9 Hz, 1H), 7.94 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 9.26, 26.44, 26.47, 27.72, 27.99, 32.06, 32.78, 38.36, 38.76, 46.92, 54.42, 106.28, 109.75, 118.54, 119.65, 122.26, 127.41, 129.05, 129.30, 138.02, 142.05, 161.69 (q, *J* = 36.9 Hz), 168.64 (one signal missing).

(*R*)-3-(1-Methyl-1*H*-indol-3-yl)-1-oxo-1-((2-(2,3,3-trimethylcyclopent-1-en-1-yl)ethyl)amino)propan-2-aminium 2,2,2-Trifluoroacetate (15)

Following *GP2*. Prepared from Boc-amine **12** (99.7 mg, 0.220 mmol), CH_2Cl_2 (2 mL), TFA (1 mL); the product **15** was thoroughly dried in high vacuum. Yield: 89.2 mg (0.191 mmol, 87%) of dark orange oil. ESI-HRMS Calcd for $C_{22}H_{32}N_3O$: *m/z* 354.2540 (MH^+). Found: *m/z* 354.2535 (MH^+). IR ν_{max} 3061, 2951, 2862, 1779, 1663, 1550, 1473, 1435, 1378, 1359, 1330, 1251, 1172, 1135, 1013, 960, 836, 798, 739 cm^{-1}. $[\alpha]_D^{r.t.}$ = –9.0 (*c* = 1.9 mg/mL, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (s, 3H), 0.89 (s, 3H), 1.36 (t, *J* = 2.2 Hz, 3H), 1.44–1.57 (m, 2H), 1.89–2.07 (m, 4H), 2.96–3.13 (m, 2H), 3.24 (d, *J* = 7.3 Hz, 2H), 3.71 (s, 3H), 4.18 (t, *J* = 7.3 Hz, 1H), 6.64 (t, *J* = 5.5 Hz, 1H), 7.02 (s, 1H), 7.03–7.07 (m, 1H), 7.13–7.19 (m, 1H), 7.23–7.28 (m, 1H), 7.50 (d, *J* = 7.9 Hz, 1H), 7.85 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 9.27, 26.44, 26.47, 27.76, 27.97, 32.05, 32.81, 38.39, 38.76, 46.94, 54.43, 106.13, 109.80, 118.47, 119.71, 122.33, 127.34, 129.05, 129.24, 138.02, 142.18, 161.60 (q, *J* = 37.6 Hz), 168.61 (one signal missing).

(*R*)-2-Acetamido-*N*-(2-cyclohexylethyl)-3-(1*H*-indol-3-yl)propanamide (19)

Following *GP3*. Prepared from trifluoroacetate salt **13** (160 mg, 0.374 mmol), CH_2Cl_2 (2 mL), DIPEA (196 μL, 1.13 mmol), CH_3COCl (32.1 μL, 0.450 mmol); column chromatography (EtOAc/petroleum ether = 1:1). Yield: 78 mg (0.219 mmol, 59%) of white solid; mp 195.7–198.6 °C. ESI-HRMS Calcd for $C_{21}H_{30}N_3O_2$: *m/z* 356.2333 (MH^+). Found: *m/z* 356.2347 (MH^+). IR ν_{max} 3407, 3287, 2914, 2849, 1636, 1561, 1539, 1455, 1370, 1287, 1243, 1091, 1023, 1012, 813, 779, 741 cm^{-1}. $[\alpha]_D^{r.t.}$ = –15.3 (*c* = 1.2 mg/mL, CH_2Cl_2). ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.74–0.88 (m, 2H), 1.04–1.25 (m, 6H), 1.54–1.67 (m, 5H), 1.78 (s, 3H), 2.87 (dd, *J* = 8.5, 14.5 Hz, 1H), 2.96–3.11 (m, 3H), 4.42–4.50 (m, 1H), 6.94–6.98 (m, 1H), 7.02–7.07 (m, 1H), 7.10 (d, *J* = 2.3 Hz, 1H), 7.31 (dt, *J* = 0.9, 8.1 Hz, 1H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.84 (t, *J* = 5.6 Hz, 1H), 7.99 (d, *J* = 8.4 Hz, 1H), 10.77 (s, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6): δ 22.57, 25.72, 26.09, 28.07, 32.60, 34.50, 36.24, 36.41, 53.47, 110.26, 111.19, 118.09, 118.42, 120.76, 123.36, 127.32, 136.00, 168.88, 171.25.

(*R*)-2-Acetamido-3-(1*H*-indol-3-yl)-*N*-(2-(2,3,3-trimethylcyclopent-1-en-1-yl)ethyl)propanamide (20)

Following *GP3*. Prepared from trifluoroacetate salt **16**[23] (267 mg, 0.589 mmol), CH_2Cl_2 (3.5 mL), DIPEA (307 μL, 1.76 mmol), CH_3COCl (50.3 μL, 0.705 mmol); column chromatography (EtOAc/petroleum ether = 1:1). Yield: 148.6 mg (0.389 mmol, 66%) of yellowish solid; mp 78.2–83.8 °C. ESI-HRMS Calcd for $C_{23}H_{32}N_3O_2$: *m/z* 382.2489 (MH^+). Found: *m/z* 382.2489 (MH^+). IR ν_{max} 3282, 3079, 2951, 2861, 1636, 1533, 1457, 1435, 1372, 1358, 1287, 1234, 1204, 1138, 1043, 1011, 908, 838, 801, 732 cm^{-1}. $[\alpha]_D^{r.t.}$ = –4.9 (*c* = 2.3 mg/mL, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (s, 3H), 0.87 (s, 3H), 1.33 (t, *J* = 2.1 Hz, 3H), 1.44–1.54 (m, 2H), 1.91 (s, 3H), 1.94–2.02 (m, 4H), 3.05–3.17 (m, 3H), 3.23 (dd, *J* = 5.9, 14.5, Hz, 1H), 4.68 (q, *J* = 7.3 Hz, 1H), 6.02 (t, *J* = 5.6 Hz, 1H), 6.79 (d, *J* = 7.8 Hz, 1H), 6.98 (d, *J* = 2.4 Hz, 1H), 7.05–7.10 (m, 1H), 7.12–7.19 (m, 1H), 7.31 (d, *J* = 8.1 Hz, 1H), 7.61 (d, *J* = 7.8 Hz, 1H),

8.61 (s, 1H, NH). ^{13}C NMR (126 MHz, $CDCl_3$): δ 9.30, 23.14, 26.42, 26.46, 28.27, 28.62, 32.10, 37.94, 38.73, 46.86, 54.35, 110.60, 111.41, 118.70, 119.68, 122.19, 123.22, 127.51, 129.58, 136.28, 141.83, 170.52, 171.47.

***tert*-Butyl (*R*)-(3-(1*H*-Indol-3-yl)-1-oxo-1-((2-(2,3,3-trimethylcyclopent-1-en-1-yl)ethyl)amino)propan-2-yl)carbamate (21)**

Following *GP4*. Prepared from trifluoroacetate salt **17**[23] (382 mg, 0.842 mmol), CH_2Cl_2 (5 mL), Et_3N (500 µL, 3.59 mmol), Boc_2O (377 mg, 1.73 mmol); column chromatography (EtOAc/petroleum ether = 1:2). Yield: 245 mg (0.557 mmol, 66%) of colorless oil. ESI-HRMS Calcd for $C_{26}H_{38}N_3O_3$: *m/z* 440.2908 (MH^+). Found: *m/z* 440.2903 (MH^+). IR ν_{max} 3307, 2931, 2861, 1698, 1654, 1493, 1457, 1436, 1391, 1365, 1246, 1163, 1102, 1065, 1046, 1011, 909, 857, 780, 735 cm^{-1}. $[\alpha]_D^{r.t.}$ = –11.1 (*c* = 2.2 mg/mL, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.84 (s, 3H), 0.88 (s, 3H), 1.32 (s, 3H), 1.42 (s, 9H), 1.46–1.52 (m, 2H), 1.92–2.02 (m, 4H), 3.05–3.20 (m, 3H), 3.34 (d, *J* = 14.7 Hz, 1H), 4.39 (s, 1H), 5.06 (s, 1H), 5.63 (s, 1H), 7.04 (d, *J* = 2.5 Hz, 1H), 7.10–7.15 (m, 1H), 7.17–7.22 (m, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.63 (d, *J* = 7.9 Hz, 1H), 8.24 (s, 1H, NH). ^{13}C NMR (126 MHz, $CDCl_3$): δ 9.32, 26.42, 26.55, 28.23, 28.43, 28.64, 32.07, 37.68, 38.78, 46.93, 55.37, 80.14, 110.89, 111.30, 119.08, 119.91, 122.45, 123.21, 127.63, 129.72, 136.36, 142.12, 155.52, 171.47.

***tert*-Butyl (*R*)-(3-(1*H*-Indol-3-yl)-1-oxo-1-(((2,3,3-trimethylcyclopent-1-en-1-yl)methyl)amino)propan-2-yl) carbamate (22)**

Following *GP4*. Prepared from trifluoroacetate salt **18**[22] (216 mg, 0.491 mmol), CH_2Cl_2 (5 mL), Et_3N (386 µL, 2.77 mmol), Boc_2O (350 mg, 1.60 mmol); column chromatography (EtOAc/petroleum ether = 1:2). Yield: 109 mg (0.256 mmol, 52%) of colorless oil. ESI-HRMS Calcd for $C_{25}H_{36}N_3O_3$: *m/z* 426.2751 (MH^+). Found: *m/z* 426.2751 (MH^+). IR ν_{max} 3303, 2929, 2860, 1691, 1655, 1492, 1457, 1437, 1390, 1365, 1246, 1163, 1099, 1056, 1011, 859, 739 cm^{-1}. $[\alpha]_D^{r.t.}$ = +1.92 (*c* = 1.9 mg/mL, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (s, 3H), 0.90 (s, 3H), 1.34–1.46 (m, 12H), 1.47–1.57 (m, 2H), 1.84–2.02 (m, 2H), 3.09–3.22 (m, 1H), 3.23–3.36 (m, 1H), 3.73 (s, 2H), 4.33–4.49 (m, 1H), 5.05–5.28 (m, 1H), 5.54–5.75 (m, 1H), 7.03 (d, *J* = 2.5 Hz, 1H), 7.09–7.15 (m, 1H), 7.17–7.21 (m, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.65 (d, *J* = 7.9 Hz, 1H), 8.41 (s, 1H, NH). ^{13}C NMR (126 MHz, $CDCl_3$): δ 9.45, 26.31, 28.41, 28.63, 31.07, 37.93, 38.61, 47.08, 55.34, 80.11, 110.81, 111.35, 118.98, 119.79, 122.31, 123.26, 127.49, 128.86, 136.39, 143.51, 155.59, 171.63 (one signal missing).

***tert*-Butyl (*S*)-(3-(1-Methyl-1*H*-indol-3-yl)-1-oxo-1-((2-(2,3,3-trimethylcyclopent-1-en-1-yl)ethyl)amino)propan-2-yl)carbamate (23)**

Following *GP4*. Prepared from trifluoroacetate salt **14** (62.9 mg, 0.134 mmol), CH_2Cl_2 (3 mL), Et_3N (170 µL, 1.22 mmol), Boc_2O (203 mg, 0.930 mmol); column chromatography (EtOAc/petroleum ether = 1:4). Yield: 45.2 mg (0.0996 mmol, 74%) of yellow oil. ESI-HRMS Calcd for $C_{27}H_{40}N_3O_3$: *m/z* 454.3064 (MH^+). Found: *m/z* = 454.3068 (MH^+). IR ν_{max} 3305, 2950, 2931, 2861, 1652, 1523, 1474, 1365, 1325, 1241, 1166, 1121, 1045, 1013, 859, 779, 737 cm^{-1}. $[\alpha]_D^{r.t.}$ = +6.95 (*c* = 1.7 mg/mL, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.82 (s, 3H), 0.87 (s, 3H), 1.31 (s, 3H); 1.42 (s, 9H), 1.44–1.51 (m, 2H), 1.90–1.97 (m, 2H), 2.00 (t, *J* = 6.9 Hz, 2H), 3.04–3.22 (m, 3H), 3.33 (d, *J* = 11.9 Hz, 1H), 3.74 (s, 3H), 4.37 (s, 1H), 5.04 (s, 1H), 5.63 (s, 1H), 6.91 (s, 1H), 7.09–7.13 (m, 1H), 7.20–7.25 (m, 1H), 7.28 (dt, *J* = 1.0, 8.3 Hz, 1H), 7.61 (d, *J* = 7.9 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 9.28, 26.36, 26.51, 28.16, 28.42, 31.98, 32.79, 37.62, 38.76, 46.91, 55.48, 80.13, 109.24, 109.34, 119.17, 119.39, 122.00, 127.91, 128.14, 129.74, 137.08, 142.11, 155.51, 171.48.

***tert*-Butyl (*R*)-(3-(1-Methyl-1*H*-indol-3-yl)-1-oxo-1-((2-(2,3,3-trimethylcyclopent-1-en-1-yl)ethyl)amino) propan-2-yl)carbamate (24)**

Following *GP4*. Prepared from trifluoroacetate salt **15** (61.8 mg, 0.132 mmol), CH_2Cl_2 (3 mL), Et_3N (97.6 µL, 0.700 mmol), Boc_2O (110 mg, 0.504 mmol); column chromatography (EtOAc/petroleum ether = 1:3). Yield: 45.7 mg (0.101 mmol, 76%) of orange oil. ESI-HRMS Calcd for $C_{27}H_{40}N_3O_3$: *m/z* 454.3064 (MH^+). Found: *m/z* 454.3067 (MH^+). IR ν_{max} 3305, 3052, 2950, 2931, 2861, 1652, 1522, 1474, 1365, 1325, 1242, 1165, 1046, 1013, 923, 860, 778, 734 cm^{-1}. $[\alpha]_D^{r.t.}$ = +28.7 (*c* = 1.7 mg/mL, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.82 (s, 3H), 0.87 (s, 3H), 1.32 (t, *J* = 2.1 Hz, 3H), 1.42 (s, 9H), 1.45–1.52 (m, 2H), 1.88–1.97 (m, 2H), 2.00 (t, *J* = 7.0 Hz, 2H), 3.04–3.22 (m, 3H), 3.34 (d, *J* = 14.8 Hz, 1H), 3.74 (s, 3H), 4.36 (s, 1H), 5.03 (s, 1H), 5.62 (s, 1H), 6.91 (s, 1H), 7.10–7.14 (m, 1H), 7.20–7.25 (m, 1H), 7.28 (dt, *J* = 1.0, 8.3 Hz, 1H), 7.61 (d, *J* = 7.9 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 9.29, 26.36, 26.52, 28.17, 28.43, 31.99, 32.80, 37.62, 38.77, 46.92, 55.47, 80.11, 109.25, 109.34, 119.18, 119.40, 122.01, 127.91, 128.15, 129.74, 137.09, 142.12, 155.53, 171.49.

2-(1*H*-Indol-3-yl)-*N*-(2-((1*R*,3*R*)-2,2,3-trimethylcyclopentyl)ethyl)acetamide (*cis*-27) and 2-(1*H*-Indol-3-yl)-*N*-(2-((1*R*,3*S*)-2,2,3-trimethylcyclopentyl)ethyl)acetamide (*trans*-27)

Following *GP5* Prepared from alkene **25**[23] (126 mg, 0.406 mmol), MeOH (20 mL), Pd–C (29 mg); column chromatography (EtOAc/petroleum ether = 1:2). The product was isolated and characterized as a diastereomer mixture of ***cis*-27**:***trans*-27** = 86:14. Yield: 83.9 mg (0.268 mmol, 66%) of orange oil. ESI-HRMS Calcd for $C_{20}H_{29}N_2O$: *m/z* 313.2274 (MH^+). Found: *m/z* 313.2277 (MH^+). IR ν_{max} 3407, 3273, 2949, 2867, 1639, 1526, 1456, 1435, 1365, 1339, 1252, 1227, 1186, 1126, 1099, 1009, 925, 878, 778, 738 cm^{-1}. $[\alpha]_D^{r.t.}$ = +9.78 (*c* = 1.7 mg/mL, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) for ***cis*-27**: δ 0.41 (s,

3H), 0.71 (s, 3H), 0.78 (d, *J* = 6.8 Hz, 3H), 0.99–1.21 (m, 4H), 1.33–1.47 (m, 2H), 1.62–1.68 (m, 2H), 3.08–3.24 (m, 2H), 3.74 (s, 2H), 5.66 (t, *J* = 5.9 Hz, 1H), 7.13–7.18 (m, 2H), 7.22–7.26 (m, 1H), 7.41 (dt, *J* = 0.9, 8.3 Hz, 1H), 7.56 (d, *J* = 7.9 Hz, 1H), 8.45 (s, 1H, NH). ^{1}H NMR (500 MHz, $CDCl_3$) for ***trans*-27**: δ 0.76 (d, *J* = 7.2 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) for ***cis*-27**: δ 13.95, 14.45, 25.53, 28.06, 30.24, 30.53, 33.61, 39.20, 42.39, 45.02, 48.37, 109.30, 111.53, 118.93, 120.22, 122.76, 123.88, 127.17, 136.58, 171.45.

***tert*-Butyl ((*S*)-3-(1-Methyl-1*H*-indol-3-yl)-1-oxo-1-((2-(2,3,3-trimethylcyclopentyl)ethyl)-amino)propan-2-yl) carbamate (28)**

Following *GP5* Prepared from alkene **23** (55.0 mg, 0.121 mmol), MeOH (20 mL), Pd–C (19.7 mg); column chromatography (EtOAc/petroleum ether = 1:2). The product was isolated and characterized as a diastereomer mixture in a ratio of 89:11. Yield: 38.4 mg (0.0843 mmol, 70%) of yellow oil. ESI-HRMS Calcd for $C_{27}H_{42}N_3O_3$: *m/z* 456.3221 (MH^+). Found: *m/z* 456.3222 (MH^+). IR ν_{max} 3429, 3305, 2950, 2868, 2243, 1651, 1523, 1501, 1472, 1365, 1325, 1244, 1166, 1047, 1013, 908, 860, 779, 734 cm^{-1}. $[\alpha]_D^{r.t.}$ = +7.6 (*c* = 2.4 mg/mL, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) for the major diastereomer: δ 0.41 (s, 3H), 0.75 (s, 3H), 0.80 (d, *J* = 6.9 Hz, 3H), 0.91–1.05 (m, 2H), 1.06–1.18 (m, 2H), 1.28–1.38 (m, 2H), 1.43 (s, 9H), 1.57–1.89 (m, 2H), 2.96–3.17 (m, 3H), 3.30 (dd, *J* = 5.2, 14.6 Hz, 1H), 3.74 (s, 3H), 4.39 (s, 1H), 5.19 (s, 1H), 5.61 (s, 1H), 6.92 (s, 1H), 7.10–7.14 (m, 1H), 7.20–7.25 (m, 1H), 7.29 (d, *J* = 8.2 Hz, 1H), 7.65 (d, *J* = 7.9 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) for the major diastereomer: δ 13.94, 14.47, 25.59, 28.03, 28.46, 28.65, 30.26, 30.36, 32.83, 39.18, 42.39, 44.98, 48.39, 55.42, 80.07, 109.36, 119.21, 119.41, 121.99, 127.97, 137.09, 155.57, 171.47 (two signals missing).

***N*-(2-((1*R*,3*R*)-2,2,3-Trimethylcyclopentyl) ethyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamide (*cis*-29), *N*-(2-((1*R*,3*S*)-2,2,3-Trimethylcyclopentyl) ethyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamide (*trans*-29) and *N*-(2-((1*R*,3*R*)-2,2,3-Trimethylcyclopentyl)ethyl)octahydro-1*H*-indole-2-carboxamide (*cis*-30), *N*-(2-((1*R*,3*S*)-2,2,3-Trimethylcyclopentyl)ethyl)octahydro-1*H*-indole-2-carboxamide (*trans*-30)**

Following *GP5* Prepared from alkene **26**[23] (148 mg, 0.499 mmol), MeOH (20 mL), Pd–C (45 mg); column chromatography (1. EtOAc/petroleum ether = 1:1 for the elution of the ***cis*-29**/***trans*-29** mixture; 2. EtOAc/MeOH = 1:1 for the elution of the ***cis*-30**/***trans*-30** mixture).

The mixture ***cis*-29**/***trans*-29** = 88:12 elutes first from the column. The aniline was isolated and characterized as a mixture of two diastereomers. Yield: 14.8 mg (0.0489 mmol, 10%) of dark orange oil. ESI-HRMS Calcd for $C_{19}H_{31}N_2O$: *m/z* 303.2431 (MH^+). Found: *m/z* 303.2429 (MH^+). IR ν_{max} 3231, 2930, 2865, 1614, 1585, 1539, 1465, 1411, 1365, 1322, 1266, 1246, 1148, 1132, 1058, 981, 929, 835, 816, 761, 711 cm^{-1}. $[\alpha]_D^{r.t.}$ = +15.1 (*c* = 1.1 mg/mL, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) for ***cis*-29**: δ 0.51 (s, 3H), 0.83 (d, *J* = 6.8 Hz, 3H), 0.86 (s, 3H), 1.13–1.36 (m, 4H), 1.38–1.55 (m, 2H), 1.63–1.92 (m, 6H), 2.49 (t, *J* = 6.0 Hz, 2H), 2.60 (t, *J* = 6.1 Hz, 2H), 3.27–3.48 (m, 2H), 5.72 (s, 1H), 6.26 (d, *J* = 2.4 Hz, 1H), 9.15 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) for ***cis*-29**: δ 14.00, 14.56, 22.89, 22.92, 23.19, 23.72, 25.72, 28.33, 30.36, 31.15, 39.06, 42.56, 45.14, 48.67, 107.42, 118.90, 123.97, 131.53, 161.45.

The mixture ***cis*-30**/***trans*-30** = 90:10 elutes second from the column. The product pyrrolidine was isolated and characterized as a mixture of diastereomers. Yield: 58.0 mg (0.189 mmol, 38%) of dark orange oil. ESI-HRMS Calcd for $C_{19}H_{35}N_2O$: *m/z* 307.2744 (MH^+). Found: *m/z* 307.2751 (MH^+). IR ν_{max} 3210, 3064, 2929, 2865, 2675, 1672, 1559, 1449, 1366, 1300, 1270, 1249, 1186, 1136, 1079, 1031, 981, 944, 917, 903, 844, 811, 729 cm^{-1}. $[\alpha]_D^{r.t.}$ = +25.8 (*c* = 1.9 mg/mL, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) for the mixture of diastereomers: δ 0.51 (s, 3H), 0.83 (d, *J* = 6.8 Hz, 3H), 0.86 (d, *J* = 2.6 Hz, 3H), 1.13–1.54 (m, 9H), 1.55–1.94 (m, 8H), 2.27–2.38 (m, 1H), 2.55–2.66 (m, 1H), 3.12–3.25 (m, 1H), 3.27–3.40 (m, 1H), 3.61–3.76 (m, 1H), 4.49 (s, 1H), 8.50 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) for the mixture of diastereomers: δ 13.99, 14.58, 22.00, 22.09, 25.73, 25.76, 26.06, 26.52, 28.09, 28.16, 30.31, 30.37, 30.51, 34.74, 37.79, 37.81, 39.70, 42.51, 42.53, 45.19, 45.22, 48.49, 48.59, 58.68, 58.72, 58.84, 58.86, 170.59.

2. 2. Biological Evaluation – Inhibition of Cholinesterases

The inhibitory potencies of the compounds against the ChEs were determined using the method of Ellman following the procedure described previously.[19] Briefly, compound stock solutions in DMSO were incubated with Ellman's reagent and the ChEs (final concentrations: 370 μM Ellman's reagent, 1 nM or 50 pM hBChE or murine (m) AChE, respectively) in 0.1 M phosphate buffer pH 8.0 for 5 min at 20 °C. mAChE was chosen as the surrogate for hAChE as they are structurally highly conserved in the composition of active site amino acid residues.[26] The reactions were started by the addition of the substrate (final concentration, 500 μM butyrylthiocholine iodide or acetylthiocholine iodide for hBChE and mAChE, respectively). The final content of DMSO was always 1%. The increase in absorbance at 412 nm was monitored for 2 min using a 96-well microplate reader (Synergy H4, BioTek Instruments, VT, USA). The initial velocities in the presence (v_i) and absence (v_o) of the test compounds were calculated. The inhibitory potencies were expressed as the residual activities, according to RA = (v_i – *b*) / (v_o – *b*), where *b* is the blank value using phosphate buffer without ChEs. For IC_{50} determinations, at least seven different concentrations of each compound were used. The IC_{50} values were obtained by plotting the residual ChE activities against the applied inhibitor concentrations, with the experimental data fitted

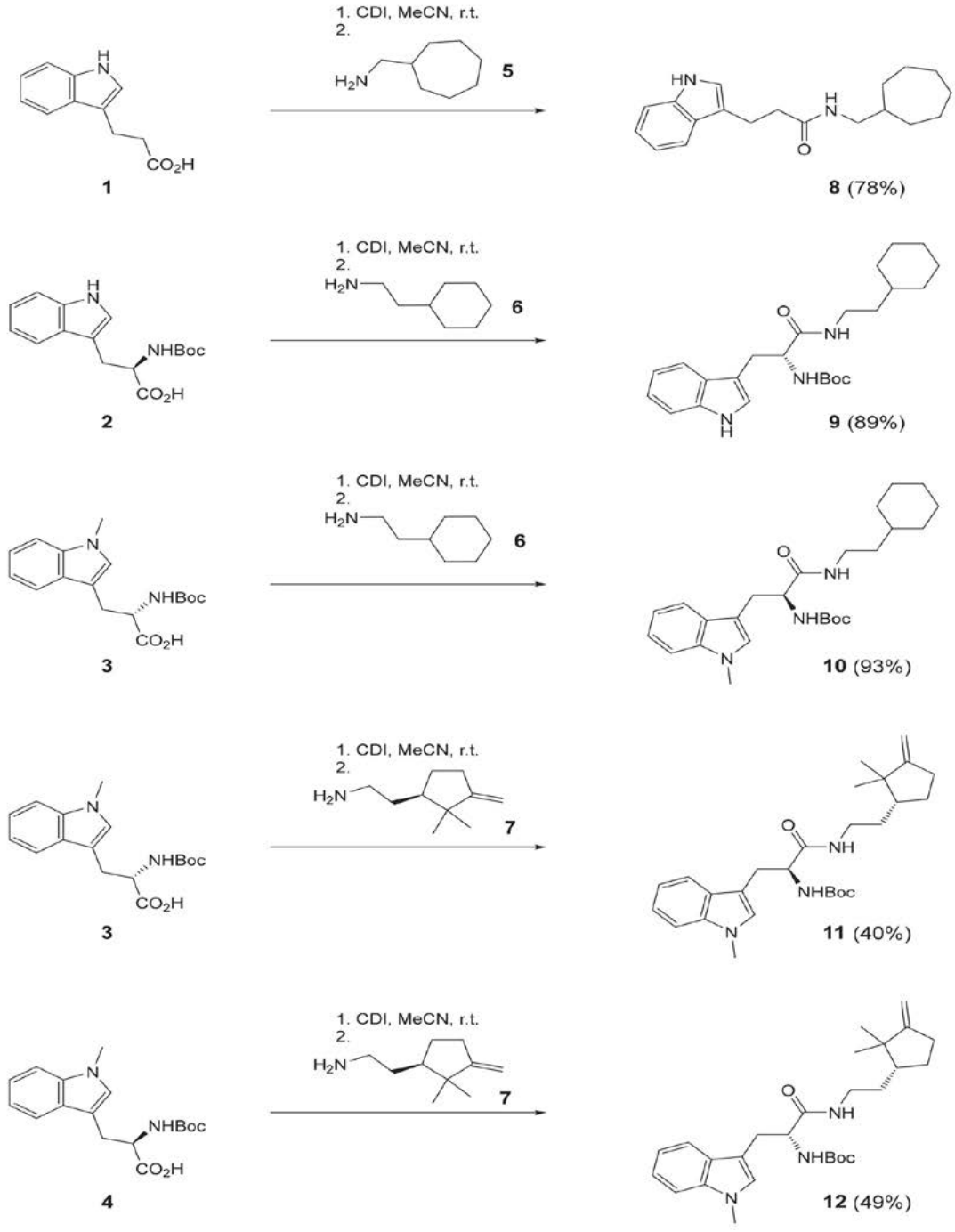
to a four-parameter logistic function (GraphPad Prism 8.0, GraphPad Software, Boston, MA, USA). Tacrine and donepezil were used as positive controls.

3. Results and Discussion

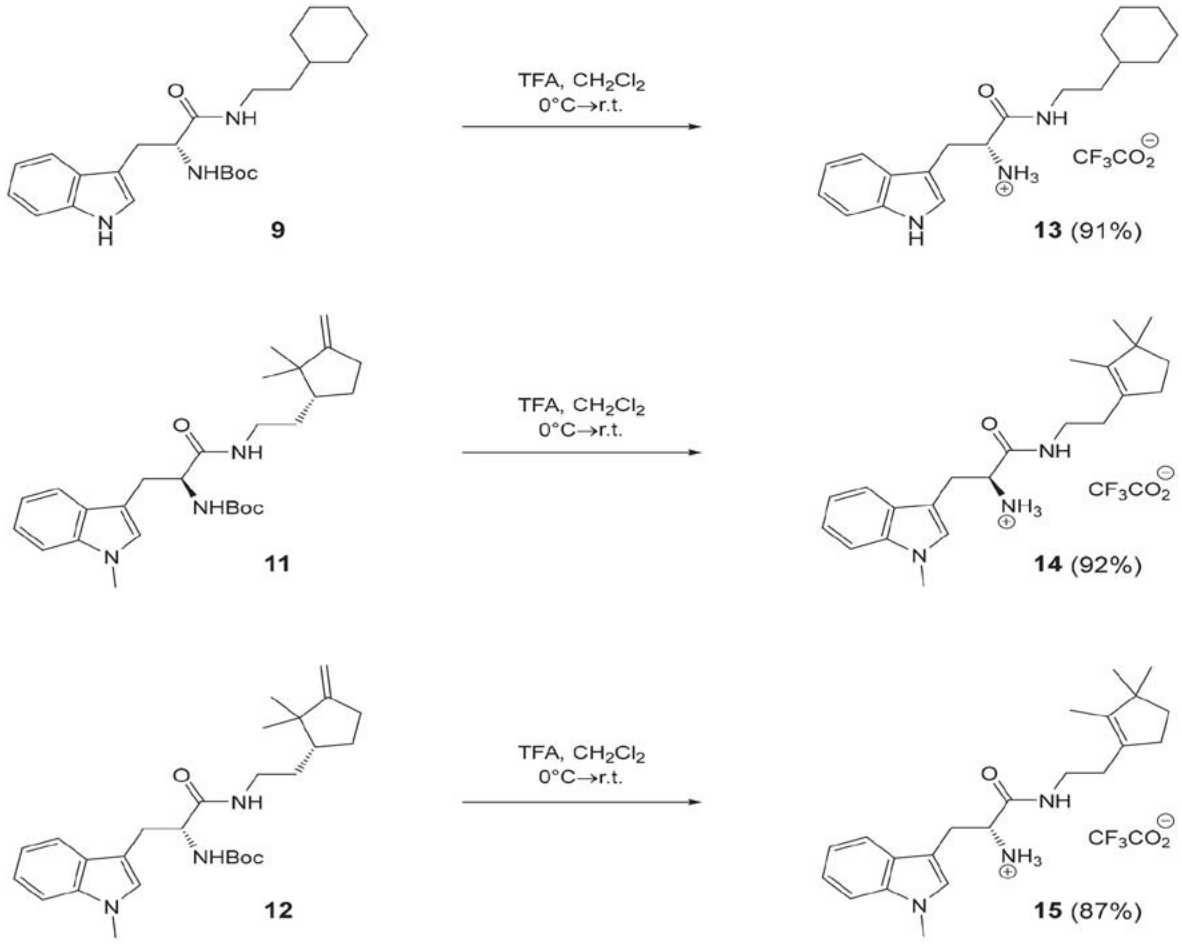
3. 1. Synthesis and ChE Inhibitory Activity

The synthesis of the products is not presented in a linear fashion, as the individual sequences range from one to four steps. Instead, the synthesis is divided into amidations with 1,1'-carbonyldiimidazole (CDI), *N*-Boc deprotection and isomerization with trifluoroacetic acid (TFA), *N*-Boc protection and acetylation, and catalytic hydrogenation (Schemes 1–4).

3-(1*H*-Indol-3-yl)propanoic acid (**1**) and tryptophan derivatives **2**–**4** were activated with CDI activating reagent before coupling with cycloalkyl-alkane-amines **5**–**7**. Amides **8**–**12** were obtained in 40–93% yields after isolation by column chromatography (Scheme 1).



Scheme 1. Synthesis of amides **8**–**12**.



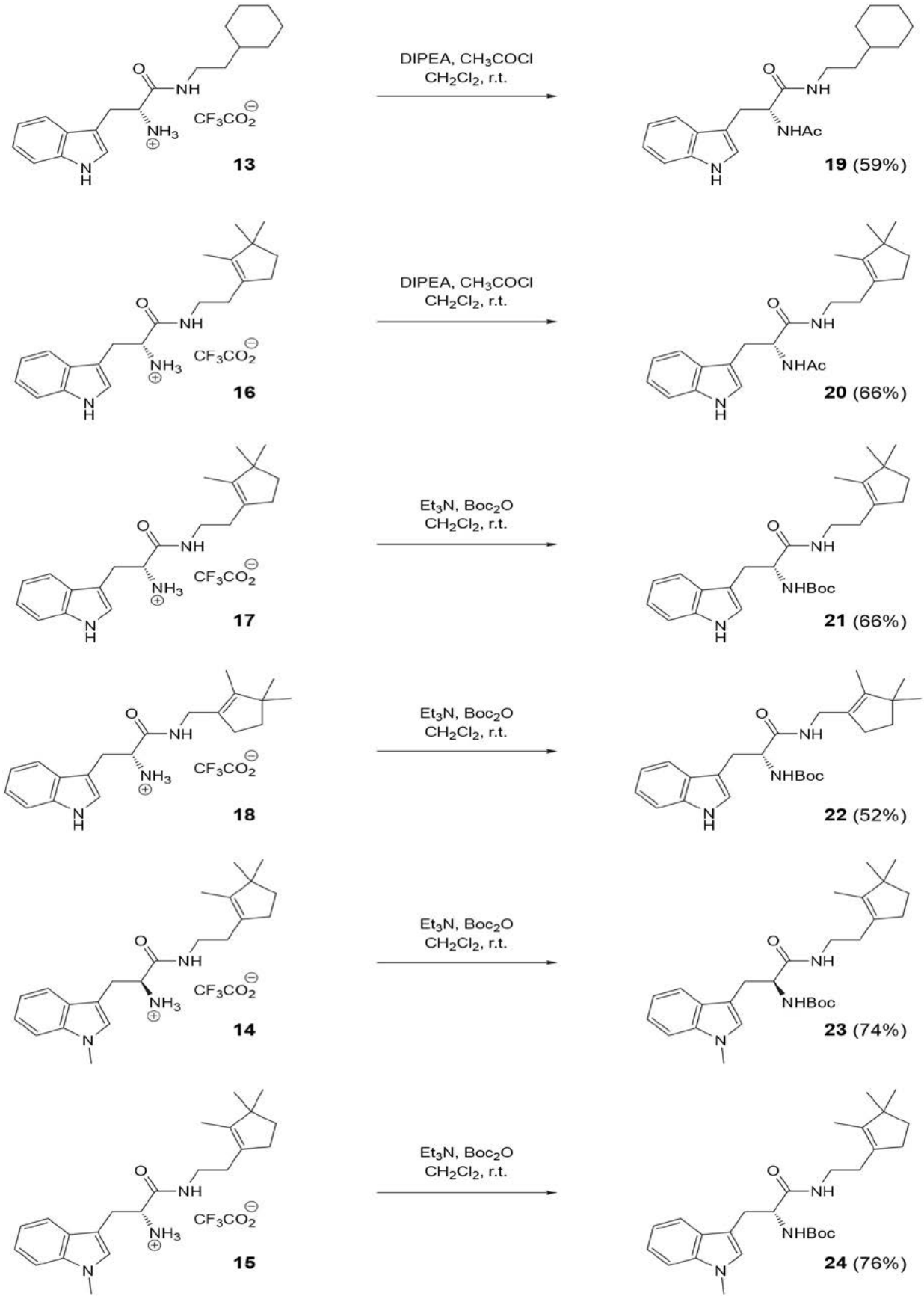
Scheme 2. *N*-Boc deprotection – synthesis of ammonium salts **13**–**15**.

TFA was used for the *N*-Boc protecting group cleavage of carbamates **9**, **11**, and **12**, and the corresponding trifluoroacetate salts **13**–**15** were obtained in 87–92% yields (Scheme 2). The Boc protecting group cleavage of carbamates **11** and **12** was accompanied by isomerization of the exocyclic double bond[22] by initial protonation of the double bond, 1,2-methyl shift, followed by deprotonation, giving cyclopentenes **14** and **15**.

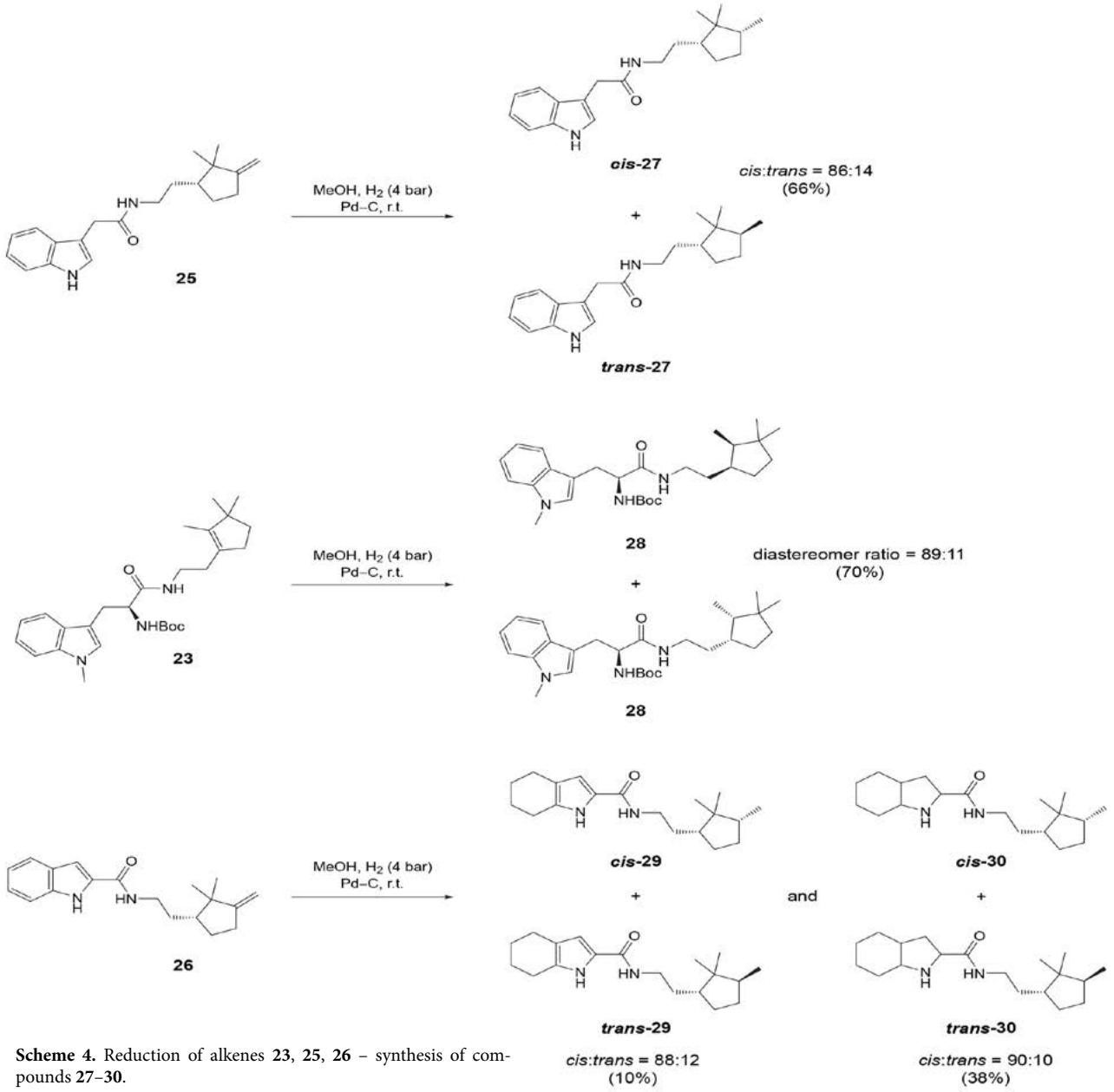
Treatment of trifluoroacetate salts **13** and **16** with acetyl chloride in the presence of DIPEA gave acetamides **19** and **20** in 59% and 66% yield, respectively. Similarly, treatment of **14**, **15**, **17**, and **18** with Boc_2O in the presence of Et_3N in dichloromethane gave Boc-protected amines **21**–**24** in 52–76% yield (Scheme 3).

Finally, catalytic hydrogenation of alkene **25** with Pd–C in methanol gave an inseparable mixture of cyclopentanes ***cis*-27** and ***trans*-27** in an 86:14 ratio and 66% yield (Scheme 4). The formation of the major cyclopentane ***cis*-27** was explained by the approach of the reagent from the less hindered side of the exocyclic double bond. Similarly, reduction of the endocyclic alkene **23** afforded an inseparable mixture of two diastereomers of product **28** in the relative ratio 89:11 in 70% yield. The absolute configuration of the newly formed stereocentres could not be determined; a relative *cis*-configuration on cyclopentane is shown for both isomers. Catalytic hydrogenation of the indole-2-carboxylic acid-derived amide **26** was not chemoselective and afforded a separable mixture of pyrrole derivatives ***cis*-29**/***trans*-29** and pyrrolidine derivatives ***cis*-30**/***trans*-30**. The pyrroles ***cis*-29**/***trans*-29** were isolated in 10% yield as an inseparable mixture of geometric isomers in the ratio 88:12. Similarly, the pyrrolidines ***cis*-30**/***trans*-30** were isolated in 38% yield as an inseparable mixture of several isomers, with a *cis*/*trans* cyclopentanes ratio of 90:10 (Scheme 4).

The structures of novel compounds were confirmed by spectroscopic methods (^1H and ^{13}C NMR, IR, and high-resolution mass spectrometry) and by elemental analyses for C, H, and N. Figure 2 shows the most typical proton shifts and multiplicities of compounds with substituted cyclopentene or cyclopentane structural motif. The germinal protons of the exocyclic alkene of compound **11** appear at 4.72 and 4.74 ppm as two triplets with a coupling constant of 2.2 and 2.5 Hz. The methyl groups of the cyclopentene moiety appear as singlet at 0.72 and 0.95 ppm. After acid-catalyzed rearrangement of the double bond and methyl group migration, a tetrasubstituted endocyclic bond is formed as shown in compound **20**. The two germinal methyl groups appear as singlet at 0.86 and 0.87 ppm,



Scheme 3. *N*-Boc protection and acetylation – synthesis of compounds **19**–**24**.



Scheme 4. Reduction of alkenes **23**, **25**, **26** – synthesis of compounds **27**–**30**.

while the methyl group on the endocyclic double bond appears as triplet at 1.33 ppm (J = 2.1 Hz). Similar chemical shifts and multiplicities are found for the related compounds **14**, **15**, **21**–**24**. Finally, catalytic hydrogenation of either the exocyclic or endocyclic double bond leads to substituted cyclopentane, yielding two diastereomers (see Scheme 4). As in compound ***cis*-29** (the major diastereomer), the two germinal methyl groups appear as singlet at 0.51 and 0.86 ppm, while the third methyl group appears as doublet at 0.83 ppm (J = 6.8 Hz). A very similar pattern of methyl groups is also observed for compound ***cis*-27** (Figure 2). The proton spectra of the compounds containing a substituted cyclopentene/cyclopentane structural motif are consistent with the previously reported compounds containing the same structural elements.[22]

All synthesized compounds were tested for inhibitory activity on human (h)BChE and murine (m)AChE (Table 1). Compounds **13**, **20**, **21**, and **24** showed selective submicromolar inhibition of hBChE, with compounds **13** (IC_{50} = 617 nM) and **21** (IC_{50} = 501 nM) being the most potent inhibitors of the series.

4. Conclusion

We report on 18 new compounds with indole structural motif that were synthesized and fully characterized. Additionally, inhibitory potencies of the synthesized compounds against hBChE (human butyrylcholinesterase) and mAChE (murine acetylcholinesterase)

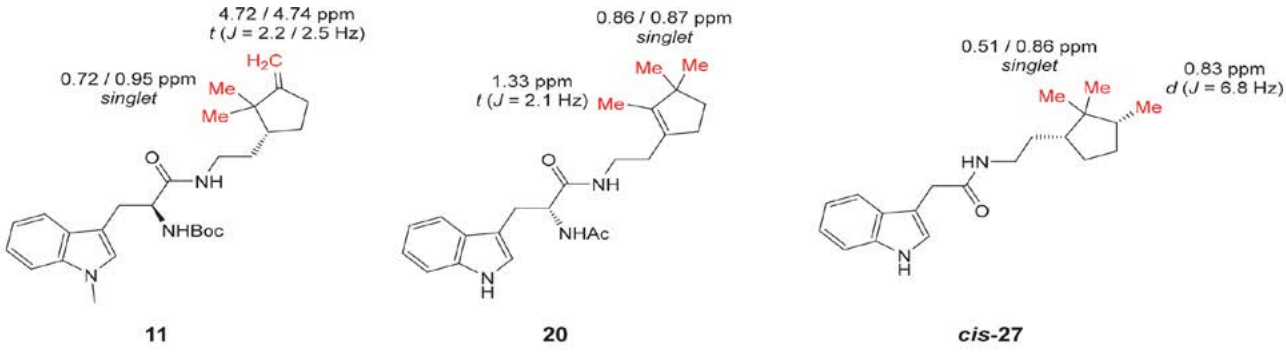
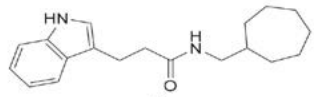
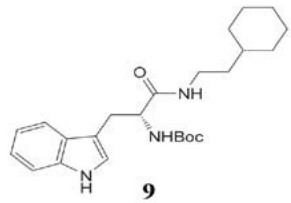
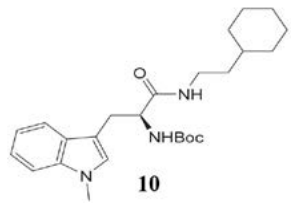
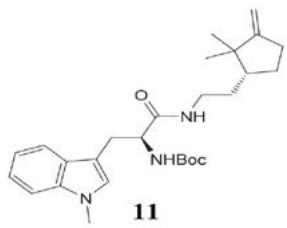
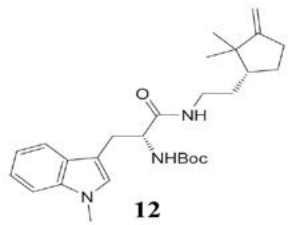
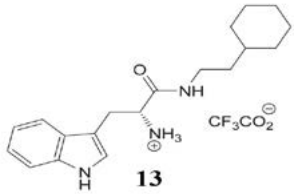
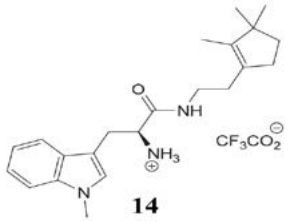
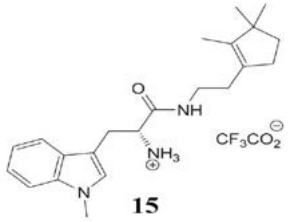
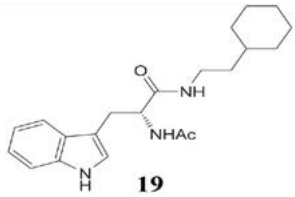
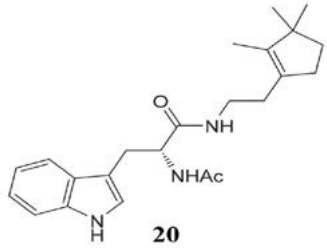
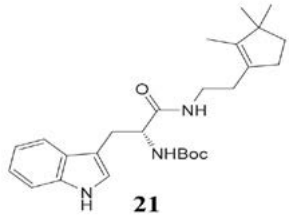
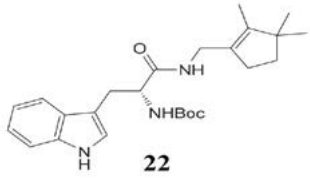
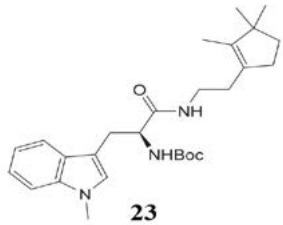
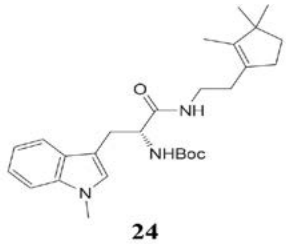
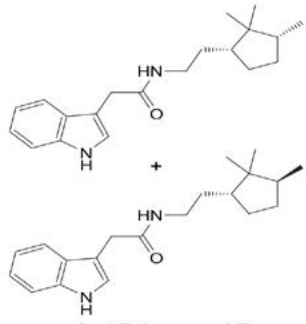
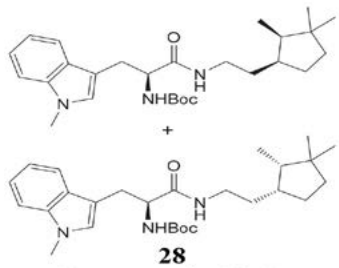
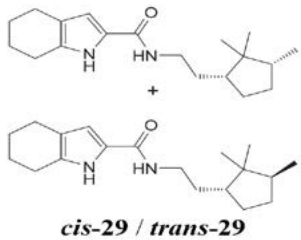


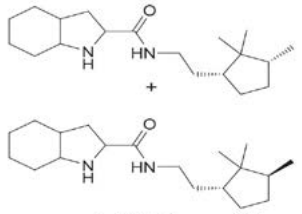
Figure 2. Representative proton shifts and multiplicities of products with substituted cyclopentene and cyclopentane motif.

Table 1. *In vitro* ChE inhibition.

Entry	Compound	hBChE RA at 100 μM [% ± SD] or IC_{50} [nM] ±SEM[a]	mAChE RA at 100 μM [% ± SD] or IC_{50} [nM] ±SEM[a]
1	 **8**	1687.6 ± 126.7	71.3 ± 8.7% Not active
2	 **9**	7653.3 ± 1141.1	87.4 ± 18.0% Not active
3	 **10**	54.8 ± 8.5% Not active	40981.3 ± 14812.5
4	 **11**	50.8 ± 2.7% Not active	11512.1 ± 2469.4
5	 **12**	9733.3 ± 2037.7	52.9 ± 8.5% Not active

Entry	Compound	hBChE RA at 100 μM [% ± SD] or IC_{50} [nM] ±SEM[a]	mAChE RA at 100 μM [% ± SD] or IC_{50} [nM] ±SEM[a]
6	 **13**	617.3 ± 11.0	99.1 ± 6.2% Not active
7	 **14**	1004.5 ± 103.2	81009.0 ± 9059.0
8	 **15**	1151.9 ± 150.1	72.3 ± 10.2% Not active
9	 **19**	4175.6 ± 245.1	96.7 ± 6.2% Not active
10	 **20**	988.4 ± 111.2	86.7 ± 4.5% Not active
11	 **21**	501.1 ± 46.7	51.4 ± 2.2% Not active
12	 **22**	3588.9 ± 715.5	35458.6 ± 7236.4

Entry	Compound	hBChE RA at 100 μM [% ± SD] or IC_{50} [nM] ±SEM[a]	mAChE RA at 100 μM [% ± SD] or IC_{50} [nM] ±SEM[a]
13	 **23**	42.6 ± 11.7% Not active	53.5 ± 0.6% Not active
14	 **24**	952.5 ± 228.5	67.6 ± 13.7% Not active
15[b]	 ***cis*-27 / *trans*-27** Isomers ratio: 85:15	1888.7 ± 123.2	7479.9 ± 2297.0
16[b]	 **28** Isomers ratio: 89:11	49.3 ± 13.5% Not active	12625.0 ± 3926.5
17[b]	 ***cis*-29 / *trans*-29** Isomers ratio: 88:12	2948.9 ± 653.3	21002.0 ± 4815.0

Entry	Compound	hBChE RA at 100 μM [% ± SD] or IC_{50} [nM] ±SEM[a]	mAChE
18[b,c]	 ***cis*-30 / *trans*-30** Mixture of stereoisomers	16179.7 ± 1108.0	84.7 ± 1.5% Not active

[a] RA – residual activity expressed as percentage ± standard deviation (SD) of one independent measurement performed in triplicate, SEM – standard error of the mean, IC_{50} values are average of two independent measurements; [b] prepared as an inseparable *cis*/*trans*-mixture; [c] obtained as a mixture of diastereomers.

was determined by the method of Ellman. The highest selective submicromolar inhibition of hBChE was achieved with compounds **13** (IC_{50} = 617 nM) and **21** (IC_{50} = 501 nM).

Supplementary Material

Copies of ^{1}H and ^{13}C NMR and MS spectra of the products are presented in the supporting information.

Acknowledgement

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Conflicts of interest

There are no conflicts to declare.

5. References

1. G. W. Gribble, *Indole Ring Synthesis: From Natural Products to Drug Discovery*, John Wiley & Sons Ltd., **2016,** Print. ISBN:9780470512180, Online ISBN:9781118695692. **DOI:**10.1002/9781118695692
2. Z. Li, Y. Liang, Y. Zhu, H. Tan, X. Li, W. Wang, Z. Zhang, N. Jiao, *Pyrroles and Their Benzo Derivatives: Reactivity* in *Comprehensive Heterocyclic Chemistry IV*, Eds.: D. StC Black, J. Cossy, C. V. Stevens, Elsevier, **2022,** 68–155, ISBN 9780128186565. **DOI:**10.1016/B978-0-12-409547-2.14853-X
3. U. Pindur, T. Lemster, *Curr. Med. Chem.* **2001**, *8*, 1681–1698. **DOI:**10.2174/0929867013371941
4. A. Aygun, U. Pindur, *Curr. Med. Chem.* **2003**, *10*, 1113–1127. **DOI:**10.2174/0929867033457511
5. C.-G. Yang, H. Huang, B. Jiang, *Curr. Org. Chem.* **2004**, *8*, 1691–1720. **DOI:**10.2174/1385272043369656
6. W. Gul, M. T. Hamann, *Life Sci.* **2005**, *78*, 442–453. **DOI:**10.1016/j.lfs.2005.09.007
7. D. S. Seigler, *Plant Secondary Metabolism*; Springer: New York, NY, USA, **2001;** p. 628.
8. S. M. Umer, M. Solangi, K. M. Khan, R. S. Z. Saleem, *Molecules* **2022**, *27*, 7586. **DOI:**10.3390/molecules27217586
9. V. Sharma, P. Kumar, D. Pathak, *J. Heterocycl. Chem.* **2010**, *47*, 491–502. **DOI:**10.1002/jhet.349
10. S. Kumar, *Future J. Pharm. Sci.* **2020**, *6*, 1–19. **DOI:**10.1186/s43094-020-00141-y
11. A. Dorababu, *RSC Med. Chem.* **2020**, *11*, 1335–1353. **DOI:**10.1039/D0MD00288G
12. Y. Wan, Y. Li, C. Yan, M. Yan, Z. Tang, *Eur. J. Med. Chem.* **2019**, *183*, 111691. **DOI:**10.1016/j.ejmech.2019.111691
13. W. Yin, M. S. Kabir, Z. Wang, S. K. Rallapalli, J. Ma, J. M. Cook, *J. Org. Chem.* **2010**, *75*, 3339–3349. **DOI:**10.1021/jo100279w
14. T. Matsuura, L. E. Overman, D. J. Poon, *J. Am. Chem. Soc.* **1998**, *120*, 6500–6503. **DOI:**10.1021/ja980788+
15. C. Sherer, T. J. Snape, *Eur. J. Med. Chem.* **2015**, *97*, 552–560. **DOI:**10.1016/j.ejmech.2014.11.007
16. https://go.drugbank.com/categories/DBCAT000528.
17. Z. Solárová, M. Kello, P. Solár, *Acta Chim. Slov.* **2021**, *68*, 151–158. **DOI:**10.17344/acsi.2020.6281
18. Alzheimer's Disease International, World Alzheimer Report 2019: Attitudes to Dementia, **2019**.
19. U. Košak, B. Brus, D. Knez, R. Šink, S. Žakelj, J. Trontelj, A. Pišlar, J. Šlenc, M. Gobec, M. Živin, L. Tratnjek, M. Perše, K. Sałat, A. Podkowa, B. Filipek, F. Nachon, X. Brazzolotto, A. Więckowska, B. Malawska, J. Stojan, I. M. Raščan, J. Kos, N. Coquelle, J.-P. Colletier, S. Gobec, *Sci. Rep.* **2016,** *6*, 39495. **DOI:**10.1038/srep39495
20. U. Košak, B. Brus, D. Knez, S. Žakelj, J. Trontelj, A. Pišlar, R. Šink, M. Jukič, M. Živin, A. Podkowa, F. Nachon, X. Brazzolotto, J. Stojan, J. Kos, N. Coquelle, K. Sałat, J.-P. Colletier, S. Gobec, *J. Med. Chem.* **2018,** *61*, 119–139. **DOI:**10.1021/acs.jmedchem.7b01086
21. S. Darvesh, *Curr. Alzheimer Res.* **2016,** *13*, 1173–1177.

DOI:10.2174/1567205013666160404120542
22. U. Grošelj, A. Golobič, D. Knez, M. Hrast, S. Gobec, S. Ričko and J. Svete, *Mol. Divers.* **2016**, *20*, 667–676. **DOI:**10.1007/s11030-016-9668-9
23. A. Meden, D. Knez, M. Jukič, X. Brazzolotto, M. Gršič, A. Pišlar, A. Zahirović, J. Kos, F. Nachon, J. Svete, S. Gobec, U. Grošelj, *Chem. Commun.* **2019**, *55*, 3765–3768. **DOI:**10.1039/C9CC01330J
24. A. Meden, D. Knez, N. Malikowska-Racia, X. Brazzolotto, F. Nachon, J. Svete, K. Sałat, U. Grošelj, S. Gobec, *Eur. J. Med. Chem.* **2020**, *208*, 112766. **DOI:**10.1016/j.ejmech.2020.112766
25. A. Meden, D. Knez, X. Brazzolotto, F. Nachon, J. Dias, J. Svete, J. Stojan, U. Grošelj, S. Gobec, *Eur. J. Med. Chem.* **2022,** *234*, 114248. **DOI:**10.1016/j.ejmech.2022.114248
26. G. Kryger, M. Harel, K. Giles, L. Toker, B. Velan, A. Lazar, C. Kronman, D. Barak, N. Ariel, A. Shafferman, *Acta Crystallogr. Sect. D* **2000**, *56*, 1385–1394. **DOI:**10.1107/S0907444900010659

Povzetek

V članku je opisana sinteza in antiholinesterazna aktivnost 18 doslej neobjavljenih spojin, derivatov indola in triptofana. Spojine, ki vsebujejo indolni strukturni fragment, izkazujejo selektivno submikromolarno zaviranje človeške butirilholin esteraze (hBChE). Strukture na novo sintetiziranih spojin so bile potrjene z ^{1}H in ^{13}C NMR, IR spektroskopijo in masno spektrometrijo visoke ločljivosti.