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Synthesis and Anticholinesterase Evaluation of Cassine, Spectaline and Analogues

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Abstract: In this work, twelve analogues of piperidine alkaloids (-)-cassine and (-)-spectaline were synthesized, as well as the racemic forms of these natural products. The compounds were evaluated for their inhibition of electric eel acetylcholinesterase ($AChE_{ee}$) and human butyrylcholinesterase ($BChE_{hu}$) by on-flow mass-spectrometry-based dual-enzyme assay, and the inhibition mechanisms for the most potent analogues were also determined. Our results showed a preference for $BChE_{hu}$ inhibition with compounds **10c** (Ki = 5.24 μM), **12b** (Ki = 17.4 μM), **13a** (Ki = 13.2 μM) and **3** (Ki = 11.3 μM) displaying the best inhibitory activities.

Keywords: cassine; spectaline; synthesis; analogues; anticholinesterase activity

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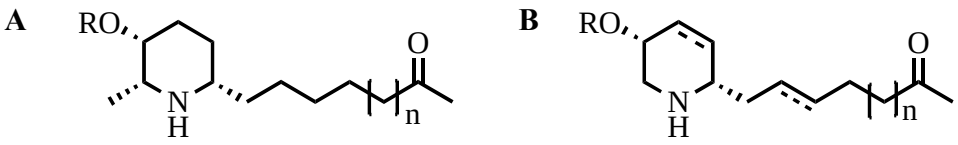
1. Introduction

(-)-Cassine (**1**) is a piperidine alkaloid first isolated from *Cassia excelsa* leaves in 1964, [1] and later from *Senna spectabilis* (syn *Cassia spectabilis*), species occurring mainly in tropical and subtropical areas of the planet [2]. Among the alkaloids obtained from *S. spectabilis*, (-)-cassine (**1**) was isolated as the major component, together with (-)-spectaline (**3**) [3]. Due to the difficulties associated with the separation of these homologous structures, many biological studies were initially undertaken on this alkaloid mixture or on their semi-synthetic analogues [3–5]. The reported biological activities of mixtures of (-)-cassine (**1**) and (-)-spectaline (**3**) include antimalarial, schistosomicidal, antiproliferative, antinociceptive, antiviral, anti-inflammatory, analgesic, leishmanicidal and cholinesterase-inhibitory [4–14].

An estimated 40–50 million people live with dementia worldwide, and Alzheimer's disease (AD) is the most common cause [15,16]. AD has complex pathophysiology, and knowledge about this disease is constantly evolving. However, some of its characteristics are well-established, including: (i) decreased acetylcholine levels in synaptic clefts of most regions of the hippocampus (cholinergic hypothesis); (ii) amyloid beta peptide (Aβ) accumulation; and (iii) tau protein hyperphosphorylation. In addition, other mechanisms such as oxidative stress, energy metabolism dysregulation and inflammation play a role in the disease process [16,17]. Acetylcholinesterase (AChE) inhibition prevents acetylcholine (ACh) conversion into choline (Ch), thus increasing ACh levels in the synaptic clefts. In fact, AChE inhibition is the targeted mechanism currently available to treat AD [16]. Although selective AChE inhibition restores the cholinergic system to some extent, studies have demonstrated that butyrylcholinesterase (BChE) can rescue the cholinesterasic function when AChE is absent. Therefore, dual AChE and BChE inhibition has been described as a more-beneficial treatment for AD patients [18]. Besides playing a role in ACh cleavage, AChE could participate in the amyloidogenic pathway through the interaction of Aβ with a hydrophobic environment that is close to the AChE peripheral anionic site [19,20]. BChE is potentially a better target than the well-known AChE for the treatment of later-stage cognitive decline in AD [21].

The anticholinesterase activity of **3** and its derivatives was first identified in 2005 [11], and a deeper investigation identified the mechanism of action and in vivo effects of these compounds [12]. Although (-)-cassine (**1**) is the main piperidine alkaloid in the *S. spectabilis* flower, its anticholinesterase activity was only evaluated by bioautography and microplate screening assays [22], while the activity of (-)-spectaline (**3**) and the corresponding O-acetyl derivatives (**2** and **4**) were separately also assessed (Figure 1A). The authors suggested that the 3-OH group had a role in establishing more important interactions with the enzyme than the acetyl group in compounds **2** and **4,** and the docking studies pointed out that the length of the side chain had an effect in the inhibition of AChE. [23].



R=H, n = 6, (-)-cassine (1)
R= Ac, n = 6, *O*-acetylcassine (2)
R= H, n = 8, (-)-spectaline (3)
R= Ac, n = 8, *O*-acetylspectaline (4)

R= H or Ac
n= 1, 6 or 8

Figure 1. (**A**): Chemical structures of natural products (-)-cassine (**1**), (-)-spectaline (**3**), O-acetylspectaline (**4**) and semi-synthetic derivative O-acetylcassine (**2**). (**B**): General structures of 3-hydroxypiperidines investigated in this work.

Encouraged by these previous studies which suggested that the size of the side chain may play a role in AChE inhibition and that the methyl group in the piperidine ring does not interact with the active site, here we investigate the potential biological effects of synthetic (±)-cassine (**1**), (±)-spectaline (**3**) and analogues thereof on cholinesterase inhibition, and evaluate the impact of structural simplification by removal of the methyl group present at C-2 in the structure of these natural products, as well as the length and the presence of unsaturation in the alkyl side chain and in the piperidine ring, as depicted in Figure 1B.

2. Materials and Methods

2.1. Synthetic Methods

General: Dichloromethane (DCM) and triethylamine (Et_3N) were pretreated with calcium hydride and distilled before use. Ethyl acetate, acetonitrile, methanol, chloroform and toluene were treated with 4 Å molecular sieves for at least 24 h before use and stored under nitrogen-purged atmosphere. BF_3·OEt was distilled prior to use. All other solvents and commercial reagents were used as supplied without further purification unless stated otherwise. Reactions were monitored by thin-layer chromatography (silica gel 60 F254 in aluminum foil), and visualization was achieved under UV light (254 nm) followed by staining in potassium permanganate ($KMnO_4$), Dragendorff stain or *p*-anisaldehyde stain (*p*-ASD). Silica gel 60 (200–400 Mesh) was used for purifications by standard flash column chromatography. NMR spectra were recorded on a Bruker Avance DPX 250 MHz (250 MHz ^{1}H, 63 MHz ^{13}C), Bruker Avance III 400 (400 MHz ^{1}H, 101 MHz ^{13}C) or Bruker Avance III 500 (500 MHz ^{1}H, 126 MHz ^{13}C) unit (Bruker Co., Billerica, Massachusetts, USA). The chemical shifts are expressed in parts per million (ppm) relative to the residual solvent signal as an internal reference: (1) $CDCl_3$ ^{1}H RMN = 7.26, ^{13}C RMN = 77.16; (2) methanol-d4: ^{1}H RMN = 3.31, ^{13}C RMN = 49.00. Multiplicities are reported with the following symbols: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and multiples thereof. High-resolution mass spectra (ESI) were acquired on an Xevo Q-Tof mass spectrometer (Waters, Manchester, UK) equipped with a nanoESI-type ionization source. IR spectra were recorded using a Thermo Scientific Nicolet IS5 spectrometer, using Thermo Scientific ID3 ATR (Thermo Fisher Scientific, Waltham, Massachusetts, USA) Melting points were

recorded on a MP50 Mettler Toledo (Columbus, Ohio, USA) melting point apparatus and are uncorrected.

***N*-(furan-2-ylmethyl)-2-nitrobenzenesulfonamide (5a)** [24]. Commercially available furfurylamine (0.70 mL, 8.0 mmol, 1.0 eq) was dissolved in THF:H_2O (*v*/*v* 1:1, 80.0 mL), followed by the addition of $NaHCO_3$ (2.00 g, 24.0 mmol, 3.00 eq) and 2-nitrobenzenesulfonyl chloride (NsCl) (2.20 g, 9.60 mmol, 1.20 eq) at room temperature. The reaction mixture was stirred for 2 h and extracted with EtOAc (3 × 50.0 mL). The combined organic phases were washed with saturated NaCl solution, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (SiO_2, hexanes/EtOAc 0% to 30%, 10% increases) yielding **5a** as a white solid (2,0g, 7,3 mmol, 92% yield).

TLC: (hexanes: EtOAc = 7:3), *Rf* = 0,4 (UV, $KmnO_4$ or *p*-ASD)

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 - 7.99 (m, 1 H), 7.86 - 7.79 (m, 1 H), 7.72 - 7.63 (m, 2 H), 7.07 (dd, *J* = 0.9, 1.8 Hz, 1 H), 6.16 - 6.07 (m, 2 H), 5.86 (t, *J* = 5.8 Hz, 1 H), 4.35 (d, *J* = 6.1 Hz, 2 H)

^{13}C NMR (126 MHz, $CDCl_3$) δ 149.2, 147.8, 142.7, 134.1, 133.5, 132.9, 131.2, 125.4, 110.4, 108.7, 40.8

IR (cm^{-1}, thin film, ATR) 3298, 1530, 1360, 1323, 1157, 1044, 856, 700

HRMS (ESI) calculated for $C_{11}H_{10}N_2O_5Sna$ $[M+Na]^+$: 305.0208; found 305.0226

***N*-(1-(furan-2-yl)ethyl)-2-nitrobenzenesulfonamide (5b):**

Commercially available furfurylamine (1.00 mL, 12.0 mmol, 1.20 eq) and benzophenone (1.80 g, 10.0 mmol, 1.00 eq) were dissolved in toluene (24.0 mL). Then, $BF_3{\cdot}OEt_2$ (123 µL, 1.00 mmol, 0.10 eq) was added, the round bottom flask was adapted with a Dean–Stark trap and the reaction mixture was heated under reflux overnight. After this period, the solvent was removed under reduced pressure, producing a brown-yellow solid. Recrystallization with methanol (heat to 45 °C and cool to 0 °C) furnished *N*-(diphenylmethylene)-1-(furan-2-yl)methanamine (**S-I**, CAS: 56542-90-6) as white crystals (1.85 g, 7.00 mmol, 71%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 - 7.63 (m, 2 H), 7.53 - 7.44 (m, 3 H), 7.42 - 7.30 (m, 4 H), 7.24 (s, 2 H), 6.33 (br s, 1 H), 6.23 (br s., 1 H), 4.55 (s, 2 H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2, 153.9, 141.8, 139.7, 136.5, 130.4, 128.8, 128.7, 128.2, 128.0, 110.4, 106.5, 51.2

S-I (1.0 g, 3.8 mmol, 1.0 eq) was solubilized in THF (38 mL, 0.1 M) under a nitrogen atmosphere and the mixture was cooled to −78 °C. A solution of *n*-BuLi 1.6 M in hexanes (2.50 mL, 5.75 mmol, 1.50 eq) was added dropwise and the solution was stirred for 40 min. Iodomethane (360 µL, 5.75 mmol, 1.50 eq) was added to the dark red solution which was allowed to stir for 1 h at 0 °C. After this period, the reaction turned a dark yellow color and it was quenched with saturated aqueous $NaHCO_3$ solution and extracted with Et_2O. The combined organic phases were washed with saturated NaCl solution, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was used without purification in the next step.

The residue from the previous step was dissolved in a mixture of acetone (19 mL) and HCl 1M (19 mL) at 0 °C. The mixture was stirred overnight and then extracted with Et_2O. The aqueous phase was neutralized with solid K_2CO_3 until pH 7–8 and THF (19 mL), $NaHCO_3$ (968 mg, 11.5 mmol, 3.00 eq) and 2-nitrobenzenesulfonyl chloride (1.0 g, 4.6 mmol, 1.2 eq) were added. The reaction was stirred for 5 h or until TLC showed complete conversion of the starting material. The reaction mixture was extracted with EtOAc (3 × 20.0 mL) and the combined organic phases were washed with saturated NaCl solution, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The material was purified by flash column chromatography (SiO_2, hexanes/EtOAc 0% to 40%, 10% increases) yielding **5b** as a white solid (795 mg, 2.68 mmol, 70% yield).

TLC: (hexanes: EtOAc = 7:3), *Rf* = 0.5 (UV, *p*-ASD)

MP: 62.5–64.6 °C

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.05 - 7.89 (m, 1 H), 7.86 - 7.75 (m, 1 H), 7.71 - 7.55 (m, 2 H), 7.01 - 6.91 (m, 1 H), 6.11 - 5.96 (m, 2 H), 5.81 (d, *J* = 8.8 Hz, 1 H), 4.83 - 4.60 (m, 1 H), 1.53 (d, *J* = 7.0 Hz, 3 H)

^{13}C NMR (63 MHz, $CDCl_3$) δ 153.4, 147.5, 142.0, 134.3, 133.3, 132.9, 131.0, 125.3, 110.1, 106.6, 48.3, 20.9

IR (cm^{-1}, thin film, ATR) 1538, 1415, 1355, 1338, 1167, 1157, 735

HRMS (ESI) calculated for $C_{12}H_{12}N_2O_5sNa$ $[M+Na]^+$: 319.0365; found 319.0309

6-allyl-1-((2-nitrophenyl)sulfonyl)-1,6-dihydropyridin-3(2H)-one (7a): To a solution of compound **5a** (1.4 g, 5.0 mmol, 1.0 eq) in THF:H_2O (*v*/*v* 4:1, 50.0 mL), was added $NaHCO_3$ (842 mg, 10.0 mmol, 2.00 eq), NaOAc (410 mg, 5.00 mmol, 1.00 eq) and *N*-bromosuccinimide (899 mg, 5.00 mmol, 1.00 eq) at 0 °C. The reaction was kept at this temperature under magnetic stirring for 30 min or until total consumption of starting material was achieved (TLC analysis). After this period, the reaction was quenched by addition of saturated $NaHCO_3$ solution (20.0 mL), saturated with $Na_2S_2O_3$ (20.0 mL) and extracted with EtOAc (3 × 20.0 mL). The combined organic phases were washed with saturated NaCl solution, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was subjected to the next step without purification.

The material obtained previously was dissolved in dry MeCN (50 mL, 0,1 M) under an N_2 atmosphere and cooled to −30 °C. Then, allyltrimethylsilane (2.45 mL, 15.0 mmol, 3.00 eq) was added followed by $Sn(oTf)_2$ (322 mg, 0.750 mmol, 0.150 eq), and the reaction was kept at this temperature under magnetic stirring for 60 min or until total consumption of starting material was achieved (TLC analysis). After this period, the reaction was quenched by the addition of saturated $NaHCO_3$ solution (20 mL) and extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with saturated NaCl solution, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The material was purified by column chromatography (SiO_2, hexanes/EtOAc 0% to 50%, 10% increases) yielding **7a** as a light yellow solid (972 mg, 3.00 mmol, 60% yield, 2 steps).

TLC: (hexanes: EtOAc = 7:3), *Rf* = 0,33 (UV, $kMnO_4$)

MP: 108–111 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (dd, *J* = 1.7, 7.6 Hz, 1 H), 7.69 (dquin, *J* = 1.7, 7.5 Hz, 2 H), 7.65 - 7.60 (m, 1 H), 7.04 (dd, *J* = 5.1, 10.5 Hz, 1 H), 6.03 - 5.93 (m, 1 H), 5.82 (tdd, *J* = 7.2, 10.0, 17.1 Hz, 1 H), 5.24 - 5.10 (m, 2 H), 4.80 - 4.71 (m, 1 H), 4.32 (d, *J* = 18.6 Hz, 1 H), 4.02 (d, *J* = 18.6 Hz, 1 H), 2.64 - 2.51 (m, 2 H)

^{13}C NMR (126 MHz, $CDCl_3$) δ 191.0, 149.8, 147.9, 134.3, 132.5, 132.4, 132.2, 131.2, 127.0, 124.6, 119.7, 54.0, 49.7, 37.4

IR (cm^{-1}, thin film, ATR) 1693, 1542, 1439, 1358, 1261, 1165, 1126, 1048, 993, 920, 852, 778, 743, 730, 675

HRMS (ESI) calculated for $C_{14}H_{15}N_2O_5S$ $[M+H]^+$: 323.0702; found 323.0699

6-allyl-2-methyl-1-((2-nitrophenyl)sulfonyl)-1,6-dihydropyridin-3(2H)-one (7b): To a solution of compound **5b** (593 mg, 2.00 mmol, 1.00 eq) in THF:H_2O (*v*/*v* 4:1, 20 mL) was added $NaHCO_3$ (337 mg, 4.00 mmol, 2.00 eq), NaOAc (164 mg, 2.00 mmol, 1.00 eq) and *N*-bromosuccinimide (360 mg, 2.00 mmol, 1.00 eq) at 0 °C. The reaction was kept at this temperature under magnetic stirring for 30 min or until total consumption of starting material was achieved according to TLC. After this period, the reaction was quenched by addition of saturated $NaHCO_3$ solution (10 mL), saturated with $Na_2S_2O_3$ (10mL) and extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with saturated NaCl solution, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was subjected to the next step without purification.

The material obtained in the previous step was solubilized in dry MeCN (20 mL, 0,1 M) under an N_2 atmosphere and cooled to −30 °C. Then, allyltrimethylsilane (1.3 mL, 8.0 mmol, 4.0 eq) was added followed by $Sn(oTf)_2$ (125 mg, 0.30 mmol, 0.15 eq), and the reaction was kept at this temperature under magnetic stirring for 60 min or until total consumption of starting material was achieved (TLC analysis). After this period, the reaction was quenched by addition of saturated $NaHCO_3$ solution (20 mL) and extracted with EtOAc (3 × 20 mL).

The combined organic phases were washed with saturated NaCl solution, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The material was purified by column chromatography (SiO_2, hexanes/EtOAc 0% to 50%, 10% increases) yielding **7b** as a solid (414 mg, 1.23 mmol, 61% yield, 2 steps).

TLC: (hexanes: EtOAc = 7:3), *Rf* = 0,5 (UV, *p*-ASD)

MP: 90.8–91.8 °C

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (d, *J* = 7.5 Hz, 1 H), 7.76 - 7.65 (m, 2 H), 7.62 (d, *J* = 8.8 Hz, 0 H), 7.06 (dd, *J* = 5.0, 10.7 Hz, 1 H), 5.98 (dd, *J* = 1.3, 10.7 Hz, 0 H), 5.94 - 5.84 (m, 1 H), 5.26 - 5.14 (m, 2 H), 4.77 - 4.64 (m, 1 H), 4.37 (q, *J* = 7.3 Hz, 1 H), 2.79 (td, *J* = 6.8, 13.4 Hz, 1 H), 2.65 - 2.50 (m, 1 H), 1.59 (d, *J* = 7.5 Hz, 4 H)

^{13}C NMR (126MHz, $CDCl_3$) δ 194.1, 148.9, 148.1, 134.2, 133.0, 132.6, 132.1, 131.4, 124.8, 124.8, 119.5, 57.4, 54.1, 42.1, 21.6

IR (cm^{-1}, thin film, ATR) 1675, 1535, 1358, 1170

6-allyl-1-((2-nitrophenyl)sulfonyl)-1,2,3,6-tetrahydropyridin-3-ol (8a): Compound **7a** (754 mg, 2.35 mmol, 1.00 eq) was dissolved in methanol (45 mL), then $CeCl_3{\cdot}7H_2O$ (1,2 g, 3.0 mmol, 1.3 eq) was added. After a homogeneous solution was formed, the reaction mixture was cooled to −78 °C and $NaBH_4$ (148 mg, 3.50 mmol, 1.50 eq) was added. After 20 min, TLC analysis showed complete conversion of starting material and the reaction mixture was allowed to reach room temperature. The reaction was quenched by addition of HCl 0,5M solution (20 mL) and extracted with DCM (3 × 20 mL). The combined organic phases were washed with saturated NaCl solution, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The material was purified by column chromatography (SiO_2, hexanes/EtOAc 0% to 50%, 10% increases) yielding **8a** as a white solid (560 mg, 2,20 mmol, 74% yield).

TLC: (hexanes: EtOAc = 1:1), *Rf* = 0,46 (UV, kMnO4 or *p*-ASD)

MP: 82–85 °C

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (dd, *J* = 1.7, 7.5 Hz, 1 H), 7.69 (dquin, *J* = 1.7, 7.4 Hz, 2 H), 7.64 - 7.61 (m, 1 H), 5.84 - 5.78 (m, 1 H), 5.78 - 5.66 (m, 2 H), 5.06 (dd, *J* = 1.6, 17.1 Hz, 1 H), 5.04 - 4.97 (m, 1 H), 4.39 (br s., 1 H), 4.17 - 4.09 (m, 1 H), 4.05 (dd, *J* = 6.1, 13.7 Hz, 1 H), 2.98 (dd, *J* = 9.9, 13.7 Hz, 1 H), 2.45 - 2.35 (m, 2 H)

^{13}C NMR (126 MHz, $CDCl_3$) δ 147.9, 134.0, 133.8, 133.5, 132.0, 130.8, 130.3, 129.0, 124.4, 118.6, 62.9, 54.0, 45.3, 38.9

IR (cm^{-1}, thin film, ATR) 3496, 1539, 1330, 1163, 1151, 939, 741

HRMS (ESI) calculated for $C_{14}H_{15}N_2O_5SK$ $[M+K]^+$: 363.0417; found 363.0411

6-allyl-2-methyl-1-((2-nitrophenyl)sulfonyl)-1,2,3,6-tetrahydropyridin-3-ol (8b): Compound **7b** (414 mg, 1.23 mmol, 1.00 eq) was dissolved in methanol (25 mL), then $CeCl_3{\cdot}7H_2O$ (602 mg, 1.60 mmol, 1.30 eq) was added. After a homogeneous solution was formed, the reaction mixture was cooled to −78 °C and $NaBH_4$ (78 mg, 1.8 mmol, 1.5 eq) was added. After 1 h, TLC analysis showed complete conversion of starting material and the reaction mixture was allowed to reach room temperature. The reaction was quenched by addition of HCl 0,5M solution (10 mL) and extracted with DCM (3 × 15 mL). The combined organic phases were washed with saturated NaCl solution, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The material was purified by column chromatography (SiO_2, hexanes/EtOAc 0% to 70%, 10% increases) yielding **8b** as an oil (302 mg, 0.89 mmol, 72% yield).

TLC: (hexanes: EtOAc = 1:1), *Rf* = 0,40 (UV and *p*-ASD)

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.11 - 7.96 (m, 1 H), 7.75 - 7.65 (m, 2 H), 7.65 - 7.54 (m, 1 H), 5.97 - 5.74 (m, 2 H), 5.64 - 5.50 (m, 1 H), 5.20 - 5.11 (m, 1 H), 5.09 (s, 1 H), 4.40 - 4.24 (m, 1 H), 4.24 - 4.12 (m, 1 H), 4.09 (br s, 1 H), 2.78 - 2.57 (m, 1 H), 2.40 (ddd, *J* = 8.7, 9.5, 13.5 Hz, 1 H), 1.75 (d, *J* = 5.1 Hz, 1 H), 1.28 (d, *J* = 6.7 Hz, 3 H)

^{13}C NMR (63 MHz, $CDCl_3$) δ 148.0, 134.4, 133.8, 133.8, 131.9, 131.2, 127.2, 126.8, 124.5, 118.5, 65.6, 53.5, 50.6, 42.2, 14.8

IR (cm^{-1}, thin film, ATR) 3530, 1542, 1371, 1169, 1139, 1125, 1020, 996, 757

HRMS (ESI) calculated for $C_{15}H_{19}N_2O_5S$ $[M+H]^+$: 339.1009; found 339.1001

Methyl ketones synthesis

General procedure A (alkylation)

Acetyl acetoacetate (0.61 mL, 4.8 mmol, 1.2 eq) was added to a solution of NaOEt, prepared from ethanol (4.0 mL) and Na (110 mg, 4.80 mmol, 1.20 eq). Bromo alkene (4.0 mmol, 1.0 eq) was added to the solution and kept stirring under reflux for 12 h. After this period, the reaction was allowed to reach room temperature, neutralized with HCl 6 M and, after addition of water, extracted with EtOAc (3×). The combined organic phases were washed with saturated NaCl solution, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The material was purified by column chromatography (SiO_2, hexanes/EtOAc 0% to 4%, 2% increases).

Ethyl 2-acetyldec-9-enoate (S-IV): The title compound was prepared according to general procedure A using 8-bromo-1-octene (0.7 mL, 4.0 mmol, 1.0 eq). Yield of 71%, colorless oil.

TLC: (hexanes: EtOAc = 9:1), *Rf* = 0,43 (*p*-ASD)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.78 (tdd, *J* = 6.7, 10.3, 17.0 Hz, 1 H), 4.97 (qd, *J* = 1.6, 17.1 Hz, 1 H), 4.91 (dd, *J* = 1.4, 10.2 Hz, 1 H), 4.23 - 4.14 (m, 2 H), 3.38 (t, *J* = 7.4 Hz, 1 H), 2.20 (s, 3 H), 2.07 - 1.96 (m, 2 H), 1.90 - 1.75 (m, 2 H), 1.39 - 1.28 (m, 6 H), 1.28 - 1.23 (m, 5 H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 203.3, 169.9, 139.0, 114.2, 61.2, 59.9, 33.6, 29.1, 28.7 (3x) 28.1, 27.3, 14.1)

IR: (cm^{-1}, thin film, ATR) 2928, 2856, 1740, 1716, 1241, 1148, 909

Ethyl 2-acetyldodec-11-enoate (S-V): The title compound was prepared according to general procedure A using 10-bromo-decene (0.83 mL, 4.00 mmol, 1.00 eq). Yield was 72%, colorless oil.

TLC: (hexanes: EtOAc = 9:1), *Rf* = 0,43 (*p*-ASD)

^{1}H NMR 1H NMR (500MHz, $CDCl_3$) δ 5.77 (tdd, *J* = 6.7, 10.3, 17.0 Hz, 1 H), 4.95 (qd, *J* = 1.7, 17.1 Hz, 1 H), 4.89 (td, *J* = 1.1, 10.1 Hz, 1 H), 4.21 - 4.12 (m, 2 H), 3.36 (t, *J* = 7.5 Hz, 1 H), 2.19 (s, 3 H), 2.04 - 1.95 (m, 2 H), 1.88 - 1.73 (m, 2 H), 1.39 - 1.29 (m, 2 H), 1.29 - 1.18 (m, 13 H)

^{13}C NMR 13C NMR (126 MHz, $CDCl_3$) δ 203.2, 169.9, 139.0, 114.1, 61.2, 59.9, 33.7, 29.2, 29.2, 29.2, 29.0, 28.8, 28.6, 28.1, 27.3, 14.0

General procedure B (Krapcho decarboxylation)

A solution of ketoester (1.00 eq) in DMSO (sufficient for 0.05 M) was treated with ground NaCl (3 eq) and H_2O (32 eq) and heated between 170–180 °C for 18 h. After this period, the reaction mixture was cooled, water was added and extracted with EtOAc. The combined organic phases were washed with saturated NaCl solution, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The material was purified by column chromatography (SiO_2, hexanes/EtOAc 5% to 10%, 1% increases).

Undec-10-en-2-one (B) (CAS 36219-73-5) [25]: the title compound was prepared according to general procedure B using **S-IV** (103 mg, 0.43 mmol, 1.00). Yield was 69% (50.0 mg, 0.23 mmol), colorless oil.

TLC: (hexanes: EtOAc = 9:1), *Rf* = 0,53 (*p*-ASD)

^{1}H NMR 1H NMR (500MHz, $CDCl_3$) δ 5.79 (dd, *J* = 10.3, 17.1 Hz, 1 H), 5.01 - 4.89 (m, 2 H), 2.40 (t, *J* = 7.5 Hz, 2 H), 2.12 (s, 3 H), 2.05 - 1.99 (m, 2 H), 1.59 - 1.51 (m, 2 H), 1.39 - 1.34 (m, 2 H), 1.33 (br s., 1 H), 1.31 - 1.21 (m, 6 H)

^{13}C NMR (126 MHz, $CDCl_3$) δ 209.2, 139.0, 114.1, 43.7, 33.7, 29.8, 29.2, 29.1, 28.9, 28.8, 23.8

Tridec-12-en-2-one (C) (CAS 60437-21-0) [26]: the title compound was prepared according to general procedure B using **S-V** (609 mg, 2.57 mmol, 1.00). Yield was 80% (609 mg, 2.57 mmol), colorless oil.

TLC: (hexanes: EtOAc = 9:1), *Rf* = 0,50 (*p*-ASD)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (tdd, *J* = 6.7, 10.3, 17.0 Hz, 1 H), 4.97 (qd, *J* = 1.7, 17.1 Hz, 1 H), 4.94 − 4.87 (m, 1 H), 2.40 (t, *J* = 7.5 Hz, 2 H), 2.12 (s, 3 H), 2.07 - 1.97 (m, 2 H), 1.61 - 1.49 (m, 2 H), 1.42 - 1.30 (m, 3 H), 1.26 (s, 11 H)

^{13}C NMR (101MHz, $CDCl_3$) δ 209.3, 139.2, 114.1, 43.8, 33.8, 29.8, 29.4, 29.4, 29.3, 29.1, 29.1, 28.9, 23.8

General Procedure C (Cross-metathesis reaction)

To a mixture of hydroxypiperidine (**8a** or **8b**, 1.0 eq) and unsaturated methyl ketone (5.0 eq) in DCM (sufficient for 0.05 M) was added Hoveyda–Grubbs II catalyst 7.5 mol%, portion-wise. The reaction mixture was kept under reflux for 24 h, allowed to reach room temperature and treated with DMSO (3,75 eq) for 12 h under magnetic stirring. The solvent was removed under reduced pressure and the residue was purified by column chromatography (SiO_2, hexanes/Et_2O 0% to 100%, 10% increases).

7-(5-hydroxy-1-((2-nitrophenyl)sulfonyl)-1,2,5,6-tetrahydropyridin-2-yl)hept-5-en-2-one (*E*/*Z* mixture) (9a): the title compound was prepared according to general procedure C using **8a** (130 mg, 0.40 mmol, 1.00 eq), commercially available 5-hexen-2-one (234 μL, 2.00 mmol, 5.00 eq) and Hoveyda–Grubbs II catalyst (19.40 mg, 0.030 mmol, 0.075 eq). Yield was 81% (128 mg, 0.324 mmol).

TLC: (hexanes: EtOAc = 1:1), *Rf* = 0,17 (UV or *p*-ASD)

^{1}H NMR (400MHz, $CDCl_3$) δ 8.06 - 8.00 (m, 1 H), 7.73 - 7.65 (m, 2 H), 7.65 - 7.60 (m, 1 H), 5.82 - 5.74 (m, 1 H), 5.74 - 5.67 (m, 1 H), 5.47 - 5.33 (m, 2 H), 4.41 - 4.33 (m, 1 H), 4.06 - 3.95 (m, 2 H), 2.56 - 2.44 (m, 2 H), 2.44 - 2.19 (m, 5 H), 2.16 - 2.10 (m, 3 H) (Major isomer)

^{13}C NMR (101MHz, $CDCl_3$) δ 209.2, 148.0, 134.1, 133.7, 132.6, 132.0, 130.9, 130.7, 128.8, 126.2, 124.4, 62.8, 54.1, 45.5, 42.7, 37.8, 30.3, 26.6

IR (cm^{-1}, thin film, ATR) 3421, 1708, 1543, 1371, 1164, 971

HRMS (ESI) calculated for $C_{18}H_{22}N_2O_6SK$ $[M+K]^+$: 433.0836; found 433.0804

12-(5-hydroxy-1-((2-nitrophenyl)sulfonyl)-1,2,5,6-tetrahydropyridin-2-yl)dodec-10-en-2-one (*E*/*Z* mixture) (9b): the title compound was prepared according to general procedure C using **8a** (114 mg, 0.35 mmol, 1.00 eq), methyl ketone B (294 mg, 1.75 mmol, 5.00 eq) and Hoveyda–Grubbs II catalyst (17.0 mg, 0.026 mmol, 0.075 eq). Yield was 68% (111.0 mg, 0.2380 mmol).

TLC: (Et_2O, 100%), *Rf* = 0,43 (UV or *p*-ASD

^{1}H NMR (400MHz, $CDCl_3$) δ 8.03 - 7.97 (m, 1 H), 7.71 - 7.58 (m, 3 H), 5.82 - 5.68 (m, 2 H), 5.48 - 5.37 (m, 1 H), 5.36 - 5.24 (m, 1 H), 4.32 (br s, 1 H), 4.14 - 3.93 (m, 2 H), 3.04 - 2.89 (m, 1 H), 2.45 - 2.27 (m, 4 H), 2.12 (s, 3 H), 1.99 - 1.85 (m, 2 H), 1.61 - 1.45 (m, 2 H), 1.34 - 1.28 (m, 1 H), 1.25 (br s, 7 H)

^{13}C NMR (101MHz, $CDCl_3$) δ 210.0, 147.8, 134.7, 134.0, 133.6, 131.9, 130.6, 130.2, 128.9, 124.6, 124.3, 62.7, 54.2, 45.2, 43.8, 37.7, 32.4, 29.9, 29.1(×2), 29.0, 23.8, 23.8 (×2)

IR (cm^{-1}, thin film, ATR) 3427, 2928, 2855, 1706, 1543, 1371, 1165, 970, 851, 745

HRMS (ESI) calculated for $C_{23}H_{32}N_2O_6SNa$ $[M+Na]^+$: 487.1879; found 487.1858

14-(5-hydroxy-1-((2-nitrophenyl)sulfonyl)-1,2,5,6-tetrahydropyridin-2-yl)tetradecadec-12-en-2-one (*E*/*Z* mixture) (9c): the title compound was prepared according to general procedure C using **8a** (97 mg, 0.3 mmol, 1.0 eq), methyl ketone C (297 mg, 1.5 mmol, 5.0 eq) and Hoveyda–Grubbs II catalyst (9.700 mg, 0.015 mmol, 0.075 eq). Yield was 82% (121.0 mg, 0.246 mmol).

TLC: (Et_2O, 100%), *Rf* = 0,43 (UV or *p*-ASD

^{1}H NMR (400MHz, $CDCl_3$) δ 8.03 - 7.97 (m, 1 H), 7.71 - 7.58 (m, 3 H), 5.82 - 5.68 (m, 2 H), 5.48 - 5.37 (m, 1 H), 5.36 - 5.24 (m, 1 H), 4.32 (br s, 1 H), 4.14 - 3.93 (m, 2 H), 3.04 - 2.89 (m, 1 H), 2.45 - 2.27 (m, 4 H), 2.12 (s, 3 H), 1.99 - 1.85 (m, 2 H), 1.61 - 1.45 (m, 2 H), 1.34 - 1.28 (m, 1 H), 1.25 (Br. s., 7 H)

^{13}C NMR (101MHz, $CDCl_3$) δ 210.1, 147.6, 134.6, 133.8, 133.5, 131.8, 130.4, 130.1, 128.6, 124.4, 124.1, 62.5, 54.1, 45.0, 43.6, 37.5, 32.3, 29.7 (×2), 29.2, 29.1, 29,0, 28.9 (×2), 23.7

IR (cm^{-1}, thin film, ATR) 3417, 2923, 1706, 1544, 1370, 1165, 970, 744

HRMS (ESI) calculated for $C_{25}H_{37}N_2O_6SK$ $[M+H]^+$: 493.2372; found 493.2291

12-(5-hydroxy-6-methyl-1-((2-nitrophenyl)sulfonyl)-1,2,5,6-tetrahydropyridin-2-yl) dodec-10-en-2-one (*E*/*Z* mixture) (9d): the title compound was prepared according to general procedure C using **8b** (102 mg, 0.30 mmol, 1.00 eq), methyl ketone B (255 mg, 1.50

mmol, 5.0 eq) and Hoveyda–Grubbs II catalyst (14.00 mg, 0.023 mmol, 0.075 eq). Brown oil, 57% yield (115.0 mg, 0.227 mmol).

TLC: (hexanes: EtOAc = 1:1), *Rf* = 0,23 (UV or *p*-ASD)

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.09 - 8.00 (m, 1 H), 7.75 - 7.57 (m, 3 H), 5.87 - 5.76 (m, 1 H), 5.63 - 5.35 (m, 3 H), 4.33 - 4.20 (m, 1 H), 4.20 - 4.12 (m, 1 H), 4.09 (d, *J* = 4.9 Hz, 1 H), 2.68 - 2.55 (m, 1 H), 2.42 (t, *J* = 7.4 Hz, 2 H), 2.13 (s, 3 H), 2.08 - 1.92 (m, 2 H), 1.69 - 1.48 (m, 4 H), 1.42 - 1.17 (m, 11 H)

^{13}C NMR (63 MHz, $CDCl_3$) δ 209.9, 148.0, 134.7, 133.8, 133.7, 131.9, 131.1, 127.0, 126.8, 125.6, 124.4, 65.5, 54.0, 50.6, 43.9, 41.1, 32.6, 30.0, 29.3, 29.3, 29.2, 29.0, 23.9, 14.8

IR (cm^{-1}, thin film, ATR) 2926, 2853, 1705, 1543, 1370, 1170, 1138, 757

HRMS (ESI) calculated for $C_{24}H_{35}N_2O_6S$ $[M+H]^+$: 479.2210; found 479.2207

14-(5-hydroxy-6-methyl-1-((2-nitrophenyl)sulfonyl)-1,2,5,6-tetrahydropyridin-2-yl) tetradec-12-en-2-one (*E*/*Z* mixture) (9e): the title compound was prepared according to general procedure C using **8b** (117 mg, 0.350 mmol, 1.00 eq), methyl ketone C (343 mg, 1.73 mmol, 5.00 eq) and Hoveyda–Grubbs II catalyst (17.0 mg, 0.026 mmol, 0.075 eq). Yield was 66% (115 mg, 0.227 mmol).

TLC: (hexanes: EtOAc = 7:3), *Rf* = 0,33 (UV or *p*-ASD)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 - 8.00 (m, 1 H), 7.70 - 7.65 (m, 2 H), 7.62 - 7.58 (m, 1 H), 5.80 (td, *J* = 2.8, 10.6 Hz, 1 H), 5.60 - 5.36 (m, 3 H), 4.24 (m, 1 H), 4.15 (m, 1 H), 4.08 (br s, 1 H), 2.63 - 2.54 (m, 1 H), 2.40 (t, *J* = 7.5 Hz, 2 H), 2.32 (ddd, *J* = 7.9, 10.2, 13.2 Hz, 1 H), 2.12 (s, 3 H), 2.08 - 1.94 (m, 3 H), 1.55 (t, *J* = 6.8 Hz, 2 H), 1.39 - 1.18 (m, 15 H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 209.8, 148.0, 134.8, 133.8, 133.7, 131.9, 131.1, 127.0, 126.8, 125.6, 124.4, 65.6, 54.0, 50.6, 43.9, 41.1, 32.6, 30.0, 29.5, 29.5, 29.3, 29.3, 29.2, 29.2, 24.0, 14.8

IR (cm^{-1}, thin film, ATR) 3463, 2926, 2853, 1708, 1544, 1370, 1170, 1138, 1020, 778, 757

HRMS (ESI) calculated for $C_{26}H_{38}N_2O_6SNa$ $[M+Na]^+$: 529.2343; found 529.2339

General Procedure D (*N*-deprotection)

To a solution of *N*-nosylpiperidine in MeCN (sufficient for 0.05 M) was added K_2CO_3 (5 eq) and benzenethiol (3 eq). The resulting yellow solution was stirred for 45 min at room temperature or until total consumption of starting material, then filtered. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (SiO_2, hexanes 100% to eliminate yellow compounds, then DCM:MeOH 0% to 10%, with 0,5% Et_3N as additive).

7-(5-hydroxy-1,2,5,6-tetrahydropyridin-2-yl)hept-5-en-2-one (10a, *E*/*Z* mixture**)**: the title compound was prepared according to general procedure D using **9a** (66.00 mg, 0.096 mmol, 1.000 eq), K_2CO_3 (115 mg, 0.84 mmol, 5.00 eq) and benzenethiol (53 μL, 0.5 mmol, 3.0 eq). Yield was 82% (29.00 mg, 0.138 mmol).

TLC: (DCM:MeOH = 8:2), *Rf* 0.33= ($KMnO_4$ or Dragendorff)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.97 −-5.91 (m, 1 H), 5.79 - 5.73 (m, 1 H), 5.57 - 5.46 (m, 1 H), 5.46 - 5.36 (m, 1 H), 3.98 - 3.91 (m, 1 H), 3.36 - 3.25 (m, 1 H), 3.13 (d, *J* = 12.8 Hz, 1 H), 3.01 (br s, 2 H), 2.92 (dd, *J* = 2.9, 12.8 Hz, 1 H), 2.51 (q, *J* = 6.8 Hz, 2 H), 2.29 (q, *J* = 6.9 Hz, 2 H), 2.25 - 2.15 (m, 2 H), 2.13 (s, 2 H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 208.6, 133.6, 132.3, 128.1, 126.6, 62.2, 54.0, 50.4, 43.0, 38.2, 30.0, 26.7

IR (cm^{-1}, thin film, ATR) 3353, 2917, 1708, 1436, 1362, 1041, 971, 734

HRMS (ESI) calculated for $C_{12}H_{20}NO_2$ $[M+H]^+$: 210. 1494; found 210. 1477

12-(5-hydroxy-1,2,5,6-tetrahydropyridin-2-yl)dodec-10-en-2-one (10b, *E*/*Z* mixture**):** the title compound was prepared according to general procedure D using **9b** (110 mg, 0.24 mmol, 1.00 eq), K_2CO_3 (164 mg, 1.19 mmol, 5.00 eq) and benzenethiol (75 μL, 0.7 mmol, 3.0 eq). Yield was 84% (56.0 mg, 0.20 mmol).

TLC: (DCM: MeOH = 9:1), *Rf* = 0,33 ($KMnO_4$ or Dragendorff)

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.01 - 5.90 (m, 1 H), 5.77 (d, *J* = 10.1 Hz, 1 H), 5.60 - 5.51 (m, 1 H), 5.40 - 5.28 (m, 1 H), 4.69 (br s, 2 H), 4.01 (br s, 1 H), 3.54 (q, *J* = 7.2 Hz, 1 H), 3.44 - 3.33 (m, 1 H), 3.33 - 3.14 (m, 1 H), 3.05 - 2.91 (m, 1 H), 2.45 - 2.31 (m, 2 H), 2.11 (s, 3 H), 2.06 - 1.90 (m, 2 H), 1.62 - 1.49 (m, 2 H), 1.49 - 1.39 (m, 1 H), 1.37 - 1.14 (m, 7 H)

^{13}C NMR (126 MHz, $CDCl_3$) δ 209.4, 135.2, 131.2, 128.0, 124.5, 61.6, 53.9, 52.8, 50.0, 43.7, 37.4, 32.5, 29.8, 29.2, 29.0, 28.9, 23.7

IR (cm^{-1}, thin film, ATR) 3330, 2925, 2853, 1711, 1438, 1361, 1038, 970, 749

HRMS (ESI) calculated for $C_{17}H_{30}NO_2$ $[M+H]^+$: 280.2277; found 280.2264

14-(5-hydroxy-1,2,5,6-tetrahydropyridin-2-yl)tetradec-12-en-2-one (10c, *E/Z* mixture**):** the title compound was prepared according to general procedure D using **9c** (202 mg, 0.41 mmol, 1.00 eq), K_2CO_3 (283 mg, 2.05 mmol, 5.00 eq) and benzenethiol (130 μL, 1.2 mmol, 3.0 eq). Yield was 85% (107 mg, 0.35 mmol).

TLC: (DCM: MeOH = 9:1), *Rf* = 0,33 ($KMnO_4$ or Dragendorff)

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.94 - 5.84 (m, 1 H), 5.74 (d, *J* = 10.1 Hz, 1 H), 5.56 - 5.45 (m, 1 H), 5.39 - 5.26 (m, 1 H), 3.93 (br s, 1 H), 3.48 (br s, 2 H), 3.23 (t, *J* = 6.1 Hz, 1 H), 3.09 (d, *J* = 12.6 Hz, 1 H), 2.88 (dd, *J* = 2.8, 12.7 Hz, 1 H), 2.37 (t, *J* = 7.5 Hz, 2 H), 2.16 (t, *J* = 6.9 Hz, 1 H), 2.09 (s, 3 H), 2.03 - 1.90 (m, 2 H), 1.52 (t, *J* = 6.9 Hz, 2 H), 1.36 - 1.26 (m, 2 H), 1.22 (br s, 9 H)

^{13}C NMR (126 MHz, $CDCl_3$) δ 209.3, 134.6, 133.1, 127.8, 125.1, 77.3, 76.8, 62.1, 54.1, 50.2, 43.6, 38.2, 32.5, 29.7, 29.3, 29.2, 29.1, 29.0, 23.7

IR (cm^{-1}, thin film, ATR) 3318, 2923, 2852, 1713, 1436, 1360, 1020, 969, 720

HRMS (ESI) calculated for $C_{19}H_{34}NO_2$ $[M+H]^+$: 308.2589; found 308.2502

12-(5-hydroxy-6-methyl-1,2,5,6-tetrahydropyridin-2-yl)dodec-10-en-2-one (10d, *E/Z* mixture**):** the title compound was prepared according to general procedure D using **9d** (52.0 mg, 0.10 mmol, 1.00 eq), K_2CO_3 (70.0 mg, 0.51 mmol, 5.00 eq) and benzenethiol (33.0 μL, 0.31 mmol, 3.00 eq). Light yellow oil, 81% yield (26 mg, 0.09 mmol).

TLC: (DCM:MeOH = 9:1), *Rf* = 0.5 (*p*-ASD)

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.96 (ddd, *J* = 2.3, 5.1, 9.9 Hz, 1 H), 5.74 (d, *J* = 9.9 Hz, 1 H), 5.55 −-5.46 (m, 1 H), 5.39 - 5.30 (m, 1 H), 3.66 (d, *J* = 5.0 Hz, 1 H), 3.35 (t, *J* = 6.6 Hz, 1 H), 2.85 (dq, *J* = 2.0, 6.5 Hz, 1 H), 2.39 (t, *J* = 7.5 Hz, 2 H), 2.29 - 2.12 (m, 3 H), 2.11 (s, 3 H), 2.05 - 1.95 (m, 2 H), 1.54 (t, *J* = 6.9 Hz, 2 H), 1.38 - 1.19 (m, 8 H), 1.19 - 1.11 (m, 3 H)

^{13}C NMR (126 MHz, $CDCl_3$) δ 209.5, 134.5, 133.8, 128.7, 125.7, 65.9, 55.8, 53.4, 43.9, 39.0, 32.7, 30.0, 29.5, 29.3, 29.2, 29.1, 23.9, 17.7

IR (cm^{-1}, thin film, ATR) 337, 2925, 2853, 1713, 1359, 972

HRMS (ESI) calculated for $C_{18}H_{32}NO_2$ $[M+H]^+$: 294.2428; found 294.2426

14-(5-hydroxy-6-methyl-1,2,5,6-tetrahydropyridin-2-yl)tetradec-12-en-2-one (10e, *E/Z* mixture**):** the title compound was prepared according to general procedure D using **9e** (90.0 mg, 0.18 mmol, 1.00 eq), K_2CO_3 (123 mg, 0.89 mmol, 5.00 eq) and benzenethiol (57.0 μL, 0.54 mmol, 3.00 eq). Light yellow solid, 92% yield (56 mg, 0.16 mmol).

TLC: (DCM: MeOH: NH_4OH (27%) = 88:10:2), *Rf* = 0,5 (*p*-ASD)

^{1}H NMR (250 MHz, $CDCl_3$) δ 5.72 (dd, *J* = 1.4, 10.0 Hz, 1 H), 5.58 - 5.42 (m, 1 H), 5.41 - 5.23 (m, 1 H), 3.67 - 3.61 (m, 1 H), 3.32 (t, *J* = 6.7 Hz, 1 H), 2.82 (dq, *J* = 2.1, 6.5 Hz, 1 H), 2.38 (t, *J* = 7.4 Hz, 2 H), 2.22 - 2.06 (m, 6 H), 1.97 (q, *J* = 6.7 Hz, 2 H), 1.52 (t, *J* = 7.0 Hz, 2 H), 1.36 - 1.18 (m, 11 H), 1.14 (d, *J* = 6.6 Hz, 3 H)

^{13}C NMR (63 MHz, $CDCl_3$) δ 209.4, 134.4, 133.9, 128.7, 125.7, 77.7, 76.7, 65.9, 55.8, 53.4, 43.9, 39.0, 32.7, 29.9, 29.5, 29.4, 29.4, 29.2, 23.9, 17.7

IR (cm^{-1}, thin film, ATR) 3402, 2923, 2852, 1714, 1462, 1359, 971, 718, 640

HRMS (ESI) calculated for $C_{20}H_{36}NO_2$ $[M+H]^+$: 322.2741; found 322.2736

General Procedure E (synthesis of compounds 11a, 11b, 11c)

To a solution of compound **10a–c** (1.0 eq) in EtOAc (sufficient for 0.1 M) at 0 °C, was added Boc_2O (1.3 eq), and it was allowed to reach room temperature. After total consumption of starting material, according to TLC, Ac_2O (2.0 eq), Et_3N (4.00 eq) and DMAP (0.05 eq) were added. The reaction mixture was stirred for 2 h, then diluted with EtOAc and washed with a citric acid 5% solution. The aqueous phase was extracted with EtOAc and washed with saturated NaCl solution, dried over Na_2SO_4, filtered and concentrated under reduced pressure. To the residue was added a HCl 4 M solution in EtOAc. After total consumption of starting material, the reaction was treated with saturated $NaHCO_3$ solution and extracted with EtOAc (3×). The combined organic phases

were washed with saturated NaCl solution, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The material was purified by column chromatography (SiO_2, DCM/MeOH 0% to 10%, 2% increases).

6-(6-oxohept-2-en-1-yl)-1,2,3,6-tetrahydropyridin-3-yl acetate (11a, *E*/*Z* mixture): the title compound was prepared according to general procedure E using 10a (21 mg, 0.1 mmol, 1.0 eq), Boc_2O (30.0 µL, 0.13 mmol, 1.30 eq), Ac_2O (19 µL, 0.2 mmol, 2.0 eq), Et_3N (56 µL, 0.4 mmol, 4.0 eq) and DMAP (0.600 mg, 0.005 mmol, 0.050 eq). Yield was 48% (12 mg, 0.05 mmol).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.01 - 5.93 (m, 1 H), 5.93 - 5.86 (m, 1 H), 5.61 - 5.51 (m, 1 H), 5.50 - 5.39 (m, 1 H), 5.09 - 5.01 (m, 1 H), 3.32 (dt, *J* = 1.7, 6.5 Hz, 1 H), 3.25 - 3.18 (m, 1 H), 3.08 - 2.98 (m, 1 H), 2.90 (br s, 1 H), 2.56 - 2.48 (m, 2 H), 2.39 - 2.20 (m, 4 H), 2.17 - 2.12 (m, 3 H), 2.10 - 2.05 (m, 3 H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 208.2, 170.7, 136.5, 132.6, 126.3, 123.7, 64.7, 53.5, 47.1, 43.1, 37.9, 29.9, 26.7, 21.3

IR (cm^{-1}, thin film, ATR) 2920, 1728, 1715, 1370, 1238, 1024, 971

HRMS (ESI) calculated for $C_{14}H_{22}NO_3$ $[M+H]^+$: 252.1600; found 252.1616

6-(11-oxododec-2-en-1-yl)-1,2,3,6-tetrahydropyridin-3-yl acetate (11b, *E/Z* mixture**):** the title compound was prepared according to general procedure E using 10b (56.0 mg, 0.16 mmol, 1.0 eq), Boc_2O (51.0 µL, 0.18 mmol, 1.30 eq), Ac_2O (39 µL, 0.3 mmol, 2.0 eq), Et_3N (113 µL, 0.65 mmol, 4.0 eq) and DMAP (1.200 mg, 0.008 mmol, 0.050 eq), resulting in 29% yield (18 mg, 0.06 mmol).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.00 (d, *J* = 10.3 Hz, 1 H), 5.92 - 5.84 (m, 1 H), 5.60 - 5.49 (m, 1 H), 5.45 - 5.31 (m, 1 H), 5.01 (br s, 1 H), 3.33 - 3.23 (m, 1 H), 3.17 (d, *J* = 13.9 Hz, 1 H), 2.99 (dd, *J* = 3.4, 14.0 Hz, 1 H), 2.40 (t, *J* = 7.5 Hz, 2 H), 2.32 —-2.16 (m, 2 H), 2.16 - 2.09 (m, 3 H), 2.09 - 2.03 (m, 4 H), 2.03 - 1.90 (m, 2 H), 1.62 - 1.48 (m, 2 H), 1.38 - 1.20 (m, 8 H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 209.3, 170.7, 137.3, 134.5, 125.3, 123.5, 65.1, 53.7, 47.4, 43.7, 38.2, 32.5, 29.8, 29.3, 29.2, 29.1, 28.9, 23.8, 21.3

IR (cm^{-1}, thin film, ATR) 2926, 2853, 1731, 1715, 1433, 1369, 1237, 1026, 968

HRMS (ESI) calculated for $C_{19}H_{32}NO_3$ $[M+H]^+$: 322.2382; found 322.2375

6-(13-oxotetradec-2-en-1-yl)-1,2,3,6-tetrahydropyridin-3-yl acetate (11c, *E/Z* mixture**):** the title compound was prepared according to general procedure E using 10c (60.0 mg, 0.16 mmol, 1.0 eq), Boc_2O (50.0 µL, 0.18 mmol, 1.30 eq), Ac_2O (38 µL, 0.3 mmol, 2.0 eq), Et_3N (110 µL, 0.65 mmol, 4.0 eq) and DMAP (1.200 mg, 0.008 mmol, 0.050 eq), resulting in 34% yield (18 mg, 0.06 mmol).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.99 (d, *J* = 10.1 Hz, 1 H), 5.93 - 5.83 (m, 1 H), 5.61 - 5.49 (m, 1 H), 5.43 - 5.31 (m, 1 H), 5.00 (br s, 1 H), 3.32 - 3.21 (m, 1 H), 3.16 (d, *J* = 14.1 Hz, 1 H), 2.99 (dd, *J* = 3.3, 14.1 Hz, 1 H), 2.40 (t, *J* = 7.5 Hz, 2 H), 2.30 - 2.16 (m, 2 H), 2.12 (s, 3 H), 2.09 - 2.02 (m, 3 H), 1.99 (q, *J* = 7.0 Hz, 2 H), 1.92 - 1.80 (m, 1 H), 1.65 - 1.48 (m, 2 H), 1.39 - 1.29 (m, 2 H), 1.26 (br s, 10 H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 209.3, 170.7, 137.5, 134.5, 125.4, 123.7, 123.5, 65.2, 53.7, 47.5, 43.7, 38.3, 32.6, 29.8, 29.4, 29.3, 29.2, 29.1, 29.1, 23.8, 21.3

IR (cm^{-1}, thin film, ATR) 2918, 2849, 1731, 1716, 1369, 1238, 1025, 968, 719

HRMS (ESI) calculated for $C_{21}H_{36}NO_3$ $[M+H]^+$: 350.2695; found 350.2684

General Procedure F (catalytic hydrogenation)

To a solution of compound 10a–e (1 eq) in AcOEt (sufficient for 0.1 M) under N_2 atmosphere was added $Pd(OH)_2$ 20%/C (20 mol%). Then, the atmosphere was changed to H_2 (1 atm) and the reaction was left stirring overnight. After this period, the mixture was filtered through a pad of Celite and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (SiO_2, isocratic DCM: MeOH: NH_4OH, 88:10:2).

7-(5-hydroxypiperidin-2-yl)heptan-2-one (12a): the title compound was prepared according to general procedure F using 10a (31.0 mg, 0.15 mmol, 1.00 eq) and $Pd(OH)_2$ (4.00 mg, 0.03 mmol, 0.20 eq). Isolated in 47% yield (15.0 mg, 0.07 mmol).

TLC: (DCM: MeOH = 8:2), Rf = 0,33 (p-ASD)

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.89 (br s, 1 H), 3.77 (br s, 2 H), 3.12 (d, *J* = 12.1 Hz, 1 H), 2.81 (d, *J* = 12.1 Hz, 1 H), 2.55 (Br. s., 1 H), 2.42 (t, *J* = 7.3 Hz, 2 H), 2.16 - 2.08 (m, 3 H), 1.85 (br s, 1 H), 1.59 - 1.45 (m, 5 H), 1.43 - 1.24 (m, 5 H)

^{13}C NMR (126 MHz, $CDCl_3$) δ 209.2, 63.7, 56.7, 51.9, 43.5, 35.9, 30.6, 29.9, 29.1, 26.0, 25.4, 23.6

IR (cm^{-1}, thin film, ATR) 3353, 2915, 2851, 1704, 1448, 1163, 1074

HRMS (ESI) calculated for $C_{12}H_{24}NO_2$ $[M+H]^+$: 214.1807; found 214.1793

12-(5-hydroxypiperidin-2-yl)dodecan-2-one (12b): the title compound was prepared according to general procedure F using 10b (62.0 mg, 0.22 mmol, 1.00 eq) and $Pd(OH)_2$ (18.0 mg, 0.13 mmol). Isolated in 38% yield (24.0 mg, 0.08 mmol).

TLC: ($CHCl_3$: MeOH = 9:1), Rf = 0,16 (p-ASD)

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.83 (Br. s., 1 H), 3.03 (d, *J* = 12.1 Hz, 1 H), 2.77 (d, *J* = 11.9 Hz, 1 H), 2.51 - 2.44 (m, 1 H), 2.41 (t, *J* = 7.5 Hz, 2 H), 2.24 (d, *J* = 19.6 Hz, 2 H), 2.13 (s, 3 H), 1.87 - 1.79 (m, 1 H), 1.61 - 1.47 (m, 4 H), 1.47 - 1.38 (m, 1 H), 1.38 - 1.29 (m, 4 H), 1.27 (br s, 11 H)

^{13}C NMR (126 MHz, $CDCl_3$) δ 209.4, 63.9, 56.8, 52.0, 43.8, 36.3, 30.8, 29.8, 29.6, 29.5, 29.5, 29.4, 29.3, 29.1, 26.2, 25.6, 23.8

IR (cm^{-1}, thin film, ATR) 3397, 2915, 2848, 1718, 1445, 1152, 962

HRMS (ESI) calculated for $C_{17}H_{34}NO_2$ $[M+H]^+$: 284.2589; found 284.2581

14-(5-hydroxypiperidin-2-yl)tetradecan-2-one (12c): the title compound was prepared according to general procedure F using 10c (107 mg, 0.35 mmol, 1.00 eq) and $Pd(OH)_2$ (21.0 mg, 0.15 mmol). Isolated in 46% yield (50.0 mg, 0.16 mmol).

TLC: (DCM:MeOH = 8:2), Rf = 0,33 (p-ASD)

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.80 (br s, 1 H), 3.01 (d, *J* = 12.1 Hz, 1 H), 2.75 (d, *J* = 11.9 Hz, 1 H), 2.54 (br s, 2 H), 2.49 - 2.42 (m, 1 H), 2.39 (t, *J* = 7.5 Hz, 2 H), 2.11 (s, 3 H), 1.81 (d, *J* = 13.3 Hz, 1 H), 1.57 - 1.40 (m, 4 H), 1.39 - 1.27 (m, 5 H), 1.23 (br s, 15 H)

^{13}C NMR (126 MHz, $CDCl_3$) δ 209.4, 64.3, 56.8, 52.4, 43.8, 36.8, 31.0, 29.8, 29.7, 29.5 (×3), 29.4, 29.3, 29.1, 26.8, 25.7, 23.8

IR (cm^{-1}, thin film, ATR) 3329, 2923, 2852, 1715, 1439, 1358, 1163, 753

HRMS (ESI) calculated for $C_{19}H_{38}NO_2$ $[M+H]^+$: 312.2903; found 312.2885

12-(5-hydroxy-6-methylpiperidin-2-yl)dodecan-2-one [1, (±)-cassine]: the title compound was prepared according to general procedure F using **10d** (25.0 mg, 0.08 mmol, 1.00 eq) and $Pd(OH)_2$ (5.00 mg, 0.04 mmol). Light yellow solid, 87% yield (23.0 mg, 0.08 mmol).

TLC: (DCM:MeOH = 8:2), *Rf* = 0,4 (*p*-ASD)

MP: 66.6–67.9 °C

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.53 (br s, 1 H), 2.75 (q, J = 6.4 Hz, 1 H), 2.57 - 2.49 (m, 1 H), 2.40 (t, J = 7.4 Hz, 2 H), 2.12 (s, 3 H), 1.91 - 1.85 (m, 1 H), 1.59 - 1.49 (m, 2 H), 1.49 - 1.46 (m, 1 H), 1.45 (dd, J = 2.2, 4.4 Hz, 1 H), 1.36 - 1.22 (m, 18 H), 1.09 (d, J = 6.4 Hz, 3 H)

^{13}C NMR (126 MHz, $CDCl_3$) δ 209.5, 68.1, 57.3, 55.9, 43.9, 37.1, 32.2, 30.0, 29.9, 29.7, 29.6, 29.5, 29.5, 29.3, 26.2, 25.9, 24.0, 18.8

IR (cm^{-1}, thin film, ATR) 2919, 2850, 1708, 1472, 1425, 1357, 1161, 993

HRMS (ESI) calculated for $C_{18}H_{36}NO_2$ $[M+H]^+$: 298.2741; found 298.2739

14-(5-hydroxy-6-methylpiperidin-2-yl)tetradecan-2-one [3, (±)-spectaline]: the title compound was prepared according to general procedure F using **10e** (53 mg, 0.16 mmol, 1.00 eq) and $Pd(OH)_2$ (10.0 mg, 0.07 mmol). Light yellow solid, 90% yield (48.0 mg, 0.15 mmol).

TLC: (DCM:MeOH = 8:2), *Rf* = 0,4 (*p*-ASD)

^{1}H NMR (500 MHz, MeOD(d4)) δ 3.60 - 3.57 (m, 1 H), 2.76 (dq, J = 1.4, 6.7 Hz, 1 H), 2.60 - 2.53 (m, 1 H), 2.47 (t, J = 7.4 Hz, 2 H), 2.13 (s, 1 H), 2.12 - 2.10 (m, 1 H), 1.94 - 1.87 (m, 1 H), 1.67 - 1.59 (m, 1 H), 1.59 —-1.46 (m, 4 H), 1.44 - 1.32 (m, 6 H), 1.30 (br s, 15 H), 1.11 (d, J = 6.6 Hz, 3 H)

^{13}C NMR (126 MHz, MeOD(d4)) δ 212.4, 68.2, 57.9, 56.4, 44.5, 37.6, 32.8, 31.0, 30.9, 30.8, 30.8, 30.8, 30.7, 30.4, 29.9, 27.0, 26.2, 25.0, 18.4

IR (cm^{-1}, thin film, ATR) 2917, 2849, 1712, 1470, 1261, 1090, 993

HRMS (ESI) calculated for $C_{20}H_{40}NO_2$ $[M+H]^+$: 326.3054; found 326.3049

General Procedure G (synthesis of compounds 13a-c)

To a solution of compound **12a–c** in EtOAc (sufficient for 0.1 M) was added 0.1 mL HCl 4 M in dioxane. After 18 h the solvent was removed under reduced pressure, the residue was suspended in 1 mL of DCM and acetyl chloride (1.8 eq), freshly distilled, was added. The mixture was kept under reflux for 18 h. After this period was added $NaHCO_3$ saturated solution and extracted with EtOAc. The combined organic phases were washed with saturated NaCl solution, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The material was purified by column chromatography (SiO_2, DCM/MeOH 0% to 10%, 2% increases).

6-(6-oxoheptyl)piperidin-3-yl acetate (13a): the title compound was prepared according to general procedure G using 12a (24.0 mg, 0,06 mmol, 1.00 eq) and AcCl (8.0 µL, 0,1 mmol, 1.8 eq). Yield was 63% (10.0 mg, 0.04 mmol).

TLC: (DCM:MeOH = 9:1), Rf = 0,26 (*p*-ASD)

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.85 (br s, 1 H), 3.22 - 3.08 (m, 1 H), 2.84 (d, J = 13.8 Hz, 1 H), 2.51 (br s, 1 H), 2.41 (t, J = 7.4 Hz, 2 H), 2.12 (s, 2 H), 2.11 - 2.02 (m, 3 H), 1.96 (d, J = 14.5 Hz, 1 H), 1.69 - 1.51 (m, 4 H), 1.45 - 1.27 (m, 6 H), 1.24 (s, 2 H)

^{13}C NMR (101 MHz, $CDCl_{3)}$ δ 208.8, 170.3, 67.0, 55.6, 48.8, 43.3, 35.9, 29.5, 28.8, 27.9, 26.9, 25.3, 23.3, 21.1

IR (cm^{-1}, thin film, ATR) 2915, 2850, 1738, 1716, 1465, 1376, 1235, 1087, 1022, 668

HRMS (ESI) calculated for $C_{14}H_{26}NO_3$ $[M+H]^+$: 256.1913; found 256.1910

6-(11-oxododecyl)piperidin-3-yl acetate (13b): the title compound was prepared according to general procedure G using 12b (15.0 mg, 0,05 mmol, 1.00 eq) and AcCl (6.0 µL, 0,08 mmol, 1.80 eq). Yield was 58% (15.0 mg, 0.03 mmol).

TLC: (DCM:MeOH = 9:1), Rf = 0,4 (p-ASD)

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.88 (br s, 1 H), 3.20 (d, J = 13.8 Hz, 1 H), 2.87 (d, J = 13.7 Hz, 1 H), 2.55 (br s, 1 H), 2.42 (t, J = 7.5 Hz, 2 H), 2.14 (s, 3 H), 2.11 (s, 3 H), 2.02 - 1.94 (m, 1 H), 1.70 - 1.51 (m, 4 H), 1.51 - 1.31 (m, 5 H), 1.27 (br s, 13 H)

^{13}C NMR (126 MHz, $CDCl_3$) δ 209.4, 170.7, 67.4, 56.1, 49.2, 43.8, 36.5, 29.9, 29.7, 29.5, 29.5, 29.4, 29.4, 29.2, 28.2, 27.2, 25.9, 23.9, 21.5

IR (cm^{-1}, thin film, ATR) 2923, 2850, 1733, 1716, 1372, 1240, 1022, 668

HRMS (ESI) calculated for $C_{19}H_{36}NO_3$ $[M+H]^+$: 326.2695; found 326.2712

6-(13-oxotetradecyl)piperidin-3-yl acetate (13c): the title compound was prepared according to general procedure G using 12c (23.0 mg, 0,07 mmol, 1.00 eq) and AcCl (10.0 µL, 0,14 mmol, 1.80 eq). Yield was 57% (15.0 mg, 0.04 mmol).

TLC: (DCM:MeOH = 9:1), Rf = 0,46 (p-ASD)

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.88 - 4.81 (m, 2 H), 3.15 (td, J = 2.3, 13.8 Hz, 2 H), 2.84 (dd, J = 2.0, 13.8 Hz, 2 H), 2.59 - 2.44 (m, 2 H), 2.40 (t, J = 7.5 Hz, 3 H), 2.12 (s, 5 H), 2.10 - 2.07 (m, 5 H), 2.00 −-1.90 (m, 3 H), 1.73 - 1.61 (m, 2 H), 1.61 - 1.51 (m, 6 H), 1.47 - 1.30 (m, 8 H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 209.5, 170.9, 67.9, 56.2, 49.7, 44.0, 37.0, 30.0, 29.9, 29.7(×3), 29.6 (×2), 29.5, 29.3, 28.6, 27.8, 26.1, 24.0, 21.6

IR (cm^{-1}, thin film, ATR) 2924, 2852, 1734, 1717, 1436, 1373, 1240, 1022, 668

HRMS (ESI) calculated for $C_{21}H_{40}NO_3$ $[M+H]^+$: 354.3008; found 354.3005

2.2. Biological Assays

Di- and trisubstituted piperidine derivatives (**10a–c, 11a–c, 12a–c, 13a–c, 1** and **3**) were submitted to cholinesterase-inhibition screening assays based on the simultaneous on-flow dual parallel enzyme assay system. The approach included immobilization of AChE from Electrophorus Electricus ($AChE_{ee}$, Sigma-Aldrich, S. Louis, MO, USA) and BChE from human serum ($BChE_{hu}$) in order to obtain $AChE_{ee}$-ICER and $BChE_{hu}$-ICER, respectively. The LC–MS configuration and the mass spectrometer (MS) parameters have been previously described [27,28]. The on-flow dual parallel enzyme assay was carried out on an Nexera LC system (Shimadzu, Kyoto, Japan) system consisting of three LC 20AD pumps, an SIL-20A auto-sampler, a DGU-20A degasser, a CTO-20A oven and a CBM-20A system controller.

The LC system was coupled with an AmaZon Speed Ion Trap (IT) mass spectrometry (MS) instrument (Bruker Daltonics, Bremen, Germany) equipped with an electrospray ionization (ESI) interface source, operating in the positive mode (scan 50–250 m/z).

The two immobilized capillary enzyme reactors (ICERs) and the MS instrument were interfaced through two 10-port two-position high-pressure switching valves (Valco Instruments Co. Inc., Houston, USA) [27].

The dual-system assay comprised three steps. Briefly, after the sample was injected, with both valves (A and B) in position 1, the reactive content of each ICER was transferred to the storage (step 1). In step 2, with both valves (A and B) in position 2, pump B directed the $AChE_{ee}$-ICER enzymatic reaction for analysis in the MS. Meanwhile, the $BChE_{hu}$-ICER reactive content was held in storage. In Step 3, while valve A was switched to position 1 again, valve B was kept in position 2. In this position, the $BChE_{hu}$-ICER enzymatic reaction content that had been held in storage was flushed by Pump B and finally analyzed in the MS [27].

The data were acquired by using the Bruker Data Analysis Software (version 4.3, Bruker Daltonics Inc., Billerica, United States). All the analyses were performed at room temperature (21 °C). The enzymatic reaction was monitored by directly quantifying the acetylcholine hydrolysis product, choline (Ch) [M + H]+ m/z 104 [27,28].

The racemic form of the piperidine derivatives **10a–c**, **11a–c**, **12a–c**, **13a–c** and (±)-cassine (**1**) and (±)-spectaline (**3**) were solubilized in methanol to a stock solution of 1.00 mM for each compound. Galantamine was used as standard cholinesterase inhibitor.

Initially, the inhibition assay was conducted with the compounds at a fixed concentration of 100 µM, prepared with 10 µL of stock solution of the tested compound, 20 µL of ACh solution (final concentration of 70 µM) and 70 µL of ammonium acetate solution (15.0 mM, pH 8.0). The solutions were prepared in duplicate and vortex-mixed for 10 s, and 20-µL aliquots were used for injection. The negative (absence of ACh) and positive (presence of ACh and absence of the tested compound) controls were analyzed between each sample. The percentage of inhibition provided by each sample was calculated by comparing the area of enzymatic activity in the presence (*Pi*) and absence (P_0) of the inhibitor, according to the equation below, where (P_i) is the peak area of Ch that was produced in the presence of the tested compound and in the absence of the tested compound (P_0), and *Sb* corresponds to Ch that was quantified during spontaneous ACh hydrolysis.

$$\%\ inhibition = \left[1 - \left(\frac{Pi - Sb}{P_0 - Sb}\right)\right] \times 100$$

Sb was determined by injecting the reaction mixture into an empty open tubular silica capillary (blank analysis to quantify spontaneous ACh hydrolysis).

The half maximum inhibitory concentration (IC_{50}), the mechanism of action, and its steady-state inhibition constant (Ki) were determined for the compounds with %I ≥ 65% at 100 µM.

To obtain the IC_{50} value of each compound, stock solutions of compounds **10c** (2.5–1000 µM), **12b** (2.5–1.500 µM), **12c** (2.5–2000 µM), **13a** (2.5–1000 µM), **1** (2.5–1.500 µM) and **3** (2.5–2000 µM) were prepared in methanol. The reaction solutions were prepared by mixing 10 µL of compound **10c** (final concentration 0.25–100 µM), **12b** (0.25–150 µM), **12c** (0.25–200 µM), **13a** (0.25–100 µM), **1** (0.25–150 µM) and **3** (0.25–200 µM) with 20 µL of 70 µM ACh; the final volume of 100 µL was reached with ammonium acetate solution (15.0 mM, pH 8.0). The solutions were prepared in duplicate and vortex-mixed for 10 s, and 20 µL aliquots were injected into the system. The percentage of inhibition (%I) was calculated by using the equation above.

To determine Ki, 20 µL of different $AChE_{ee}$ solutions (10, 20, 50, 60 or 100 µM) containing 10 µL of one of the tested compounds at a fixed concentration (**10c** at 5, 10 or 20 µM, **12b** at 10, 20 or 30 µM, **12c** at 50, 60 or 70 µM, **13a** at 7, 15 or 20 µM, **1** at 10, 30 or 40 µM or **3** at 10, 30 or 40 µM) were mixed. The final volume of 100 µL was reached with ammonium acetate solution (15.0 mM, pH 8.0). The solutions were prepared in duplicate

and vortex-mixed for 10 s, and 20 µL aliquots were injected into the system. Positive controls (presence of ACh at 10, 20, 50, 60 or 100 µM and absence of compound) were also analyzed.

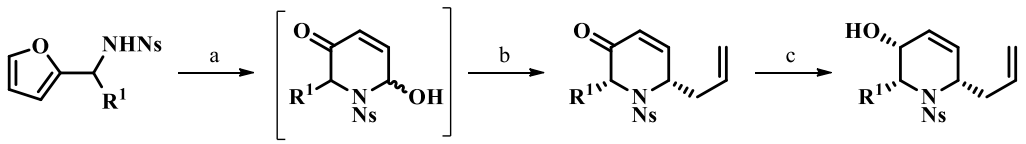
To verify the inhibition mechanism, reciprocal plots of 1/[product choline] versus 1/[ACh] were constructed, and Ki was determined from the re-plots of the primary reciprocal plot data.

3. Results and Discussion

3.1. Chemistry

Due to its varied biological properties and the difficulties associated with its isolation in pure-form from natural sources, several different synthetic methodologies have been reported for the alkaloid (-)-cassine (**1**) [29]. To achieve the construction of the piperidine core, we explored the approach reported by Zhou and colleagues [30,31], and later employed by Padwa and colleagues, in the synthesis of *epi*-indolizidine 223A [32] and (±)-cassine (**1**) [33] which allowed the synthesis of natural products **1** and **3** as well as their analogues **10a-3**, **11a-c**, **12a-c** and **13a-c**.

Starting from protected furfurylamine **5a**, an aza-Achmatowicz rearrangement [34–37] provided hemiaminal **6a**, which was not isolated but immediately submitted to a Hosomi–Sakurai allylation reaction catalyzed by $Sn(OTf)_2$ [38], to yield piperidinone **7a**. Next, Luche reduction [39] of piperidinone **7a** stereoselectively furnished the key intermediate **8a**. The same sequence was employed to prepare intermediate **8b** (Scheme 1).



5a, R^1= H **6a, R^1= H** **7a, R^1= H, 60%** **8a, R^1= H, 74%**
5b, R^1= CH_3 **6b, R^1= CH_3** **7b, R^1= CH_3, 61%** **8b, R^1= CH_3, 72%**

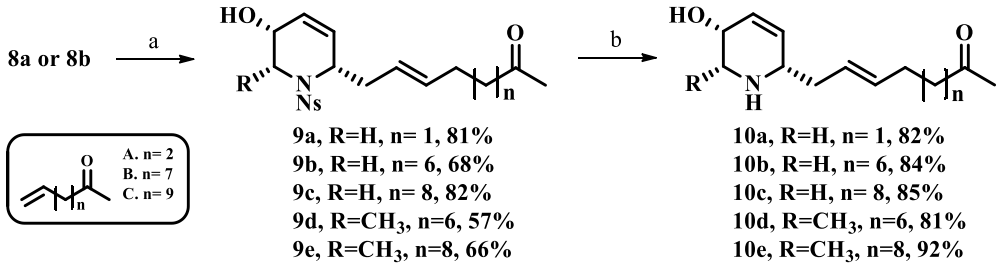
a) NBS, $NaHCO_3$, NaOAc, THF:H_2O, 0 °C; b) CH_2=$CHCH_2TMS$, 0.15 mol% $Sn(OTf)_2$, MeCN, -30 °C; c) $CeCl_3 \cdot 7\ H_2O$, $NaBH_4$, MeOH -78 °C

Scheme 1. Synthesis of key intermediates **8a** and **8b**.

The entirely *cis* configuration of **8a** was initially assigned by ^{1}H NMR with $^3J_{H5\text{-}H6ax}$ = 9.9 Hz consistent with axial orientation of H-5, and further confirmed by NOESY correlations between $H6_{ax}$ and the allyl substituent, as well as between H2 and the *ortho* hydrogen of the nosyl (Ns) protecting group (Figure S1A). This assignment was later corroborated by X-ray diffraction crystallography analysis of **8a** (Figure S1B). The disfavored $A^{1,3}$-strain involving the nosyl group and the C-2 substituent in 1,2,3,6-tetrahydropyridine **8b** explains the pseudo-axial orientation of the allyl substituent at C-2. The exceptional stereospecificity in the reduction of **7a** is rationalized by the axial attack of the incoming hydride reagent controlled by the steric hindrance of the substituents at C-2 and C-6. (Figure S1C) [33].

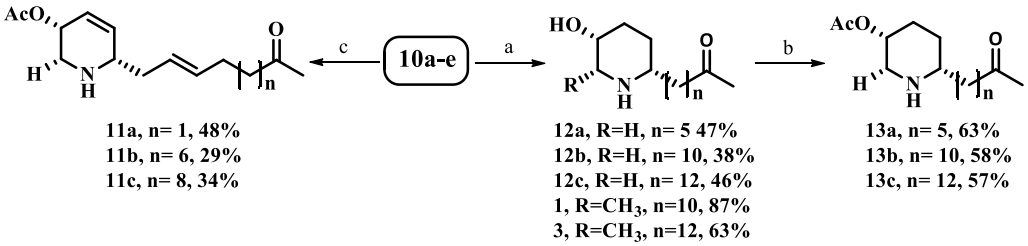
From hydroxypiperidine **8a**, a cross-metathesis reaction catalyzed by Hoveyda–Grubbs II catalyst with different unsaturated methyl ketones [40] provided compounds **9a–e**. Under mild *N*-deprotection conditions, compounds **9a–e** yielded *nor*-cassine and *nor*-spectaline analogues **10a–c** with 7, 12 and 14 carbons in the alkyl side chain, respectively. Natural product precursors **10d,e** were synthesized accordingly [Scheme 2].

To prepare the remaining analogues, intermediates **10a–c** were subjected to a stepwise procedure to achieve selective *O*-acetylation, which yielded analogues **11a–c.** In parallel, intermediates **10a–e** underwent catalytic hydrogenation to provide saturated analogues **12a–c** and natural products (±)-cassine **(1)** and (±)-spectaline **(3).** Finally, analogues **13a–c** were obtained by selective *O*-acetylation of compounds **12a–c,** respectively (Scheme 3).



a) A, B or C, Hoveyda-Grubbs II (7.5 mol%), DCM, reflux; b) Thiophenol, K_2CO_3, MeCN

Scheme 2. Final steps in the synthesis of compounds **10a–10e**.



a) $Pd(OH)_2/C$, H_2, EtOAc, b) (i) HCl 3M, (ii) AcCl, $CHCl_3$, reflux, (iii) $NaHCO_3$ (satd.sol.); c) (i) Boc_2O, (ii) Ac_2O, Et_3N, DMAP, (iii) HCl 3M, (iv) $NaHCO_3$ (satd. sol.)

Scheme 3. Final steps in the synthesis of compounds **12a–c, 13a–c, 1** and **3**.

A total of 12 analogues were synthesized in six to eight steps, with overall yields ranging from 9 to 28% for the analogues bearing the 5-hydroxypiperidine moiety and from 2% to 13% for the acetylated analogues.

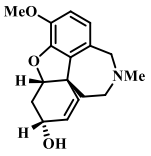
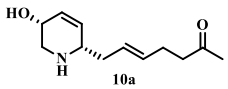
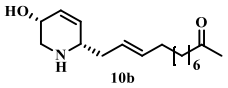
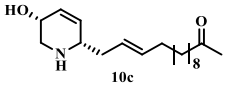
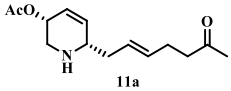
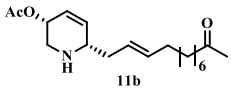
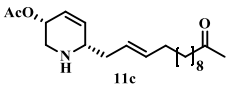
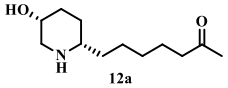
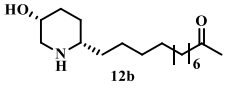
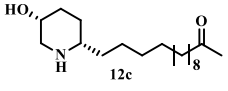
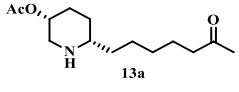
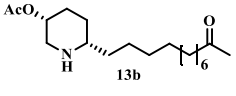
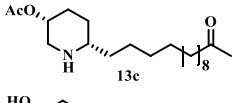
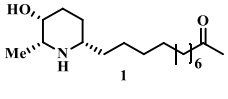
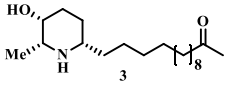
3.2. Cholinesterase-Inhibition Screening Assays Results

In this study, the capacity for cholinesterase inhibition ($AChE_{ee}$ and $BChE_{hu}$) of compounds **10a-c**, **11a-c**, **12a-c**, **13a-c**, **1** and **3** was investigated by the recently developed on-flow mass-spectrometry-based dual-enzyme assay detailed in supporting information [27,28].

The preliminary inhibition data at 100 μM showed that the compounds tended to have higher affinity for $BChE_{hu}$ than for $AChE_{ee}$, including the racemic form of the natural products cassine (**1**) and spectaline (**3**), with both exhibiting a mixed-type mechanism of inhibition. These results are in accordance with those reported by Suciati and colleagues who also observed higher $BChE_{hu}$ inhibition, compared to $AChE_{ee}$, for the ethanolic extract of *S. spectabilis* [41]. Piperidine derivatives **12b** and **12c**, lacking the methyl group, displayed a reduction in the % of inhibition of $AChE_{ee}$ in comparison to **1** and **3**, which was less significant for $BChE_{hu}$. The presence of a methyl group did not seem to be essential for the inhibitory activity of $BChE_{hu}$ when the percentage of inhibition was considered, but its presence enhanced the anti-$AChE_{ee}$ activity (Table 1).

On the basis of these preliminary results, the effect of the alkyl chain length on the inhibition activity was unclear, although five out of six compounds with inhibition ≥65% for $BChE_{hu}$ have longer alkyl chains (n = 12 or 14; compounds **10c, 12b,c, 1 and 3**) with only **13a** displaying the same range of inhibition with a shorter side chain**.** It is noteworthy that the *O*-acetylated analogue **13a** with a shorter side chain at C-2 displayed higher inhibition than the corresponding *O*-acetylated *nor*-cassine (**13b**) and *nor*-spectaline (**13c**) analogues regarding both $AChE_{ee}$ and $BChE_{hu}$, with a striking difference being observed for the former. As for the other derivatives bearing a seven-carbon side chain, i.e., **10a**, **11a** and **12a**, their inhibitory activity was shown to be lower (or at most, equipotent) when compared to the other analogues in the same series.

Table 1. Results of the studies about the inhibition of $AChE_{ee}$-ICER and $BChE_{hu}$-ICER activities by heterocyclic compounds **10a-c**, **11a-c**, **12a-c**, **13a-c**, **1** and **3**.

Chemical Structures	$AChE_{ee}$	$BChE_{hu}$			
	% Inhibition at 100 μM	% Inhibition at 100 μM	$IC_{50} \pm SEM$ [1] (μM)	K_i (μM)	Mechanism Type
 Galantamine*	100	100	0.227 ± 0.002 **	0.25 **	Competitive **
 10a	3.61	27.9	ND	ND	ND
 10b	14.8	52.2	ND	ND	ND
 10c	29.1	78.4	3.89 ± 1	5.24	Mixed
 11a	13.5	11.9	ND	ND	ND
 11b	27.1	57.4	ND	ND	ND
 11c	53.3	50.2	ND	ND	ND
 12a	32.2	18.4	ND	ND	ND
 12b	35.3	74.4	23.2 ± 3	17.4	Non-competitive
 12c	32.6	65.1	111 ± 16	54.7	Mixed
 13a	60.3	85.9	29 ± 4	15.2	Non-competitive
 13b	0.42	24.0	ND	ND	ND
 13c	19.2	63.8	ND	ND	ND
 1	58.2	78.7	18.1 ± 3	30.3	Mixed
 3	42.4	69.8	49.8 ± 4	11.3	Mixed

* $AChE_{ee}$ and $BChE_{hu}$ standard inhibitor ** value reported in reference [6]; [1] SEM: standard error of the mean; IC_{50}: required compound concentration to achieve 50%; ND: not determined.

The incorporation of two unsaturated compounds in these piperidine derivatives (**10a-c** compared to **12a**–**c**) seems to be detrimental to their anti-$AChE_{ee}$ properties, while the picture for the anti-$BChE_{hu}$ activity is much less clear, as within the *O*-acetylated series (**11a**–**c** vs. **13a**–**c**).

Comparison of the bis-unsaturated piperidine derivatives **10a**–**c** and **11a**–**c** shows that *O*-acetylation appears to be beneficial regarding anticholinesterase activity, with **11a**–**c** inhibiting $AChE_{ee}$ more extensively than **10a**–**c**; however, the same does not hold true for the inhibition of $BChE_{hu}$. The *O*-acylation in the series of saturated piperidine derivatives (**12a**–**c** vs. **13a**–**c**) does not translate into a significant increase in the anti-cholinesterase activity for both enzymes (**12b** vs. **13b** and **12c** vs. **13c**), except when one compares the % of inhibition of **13a** and **12a.**

Furthermore, the combined characteristics of unsaturation and hydroxyl-group acetylation at C-3 reduced the activity of the compounds. For example, compounds **11a**–**c** had both modifications and did not reach the minimum inhibition of 65% for either $AChE_{ee}$ or $BChE_{hu.}$ (Table 1).

All the compounds that presented inhibition ≥65% (**10c, 12b–c, 13a, 1, 3)** had their IC_{50} values determined for $BChE_{hu}$. Compound **10c** was the most active (IC_{50} 3.89± 1 μM) (Figure 2A), followed by the racemic natural product (±)-cassine **(1)** (IC_{50} 18.1 ± 3 μM) (Figure 3A), **12b** (IC_{50} 23.3 ± 3 μM) (Figure 4A) and **13a** (IC_{50} 29.0 ± 4 μM) (Figure 5A). The least-active compounds were (±)-spectaline **(3)** (Figure S73A) and its analogue **12c** (IC_{50} 111 ± 16 μM) (Figure S72A), which was surprising because analogue **10c**, which also displayed 14 carbons in the alkyl side chain, was the most active, suggesting that unsaturation played a role for this compound.

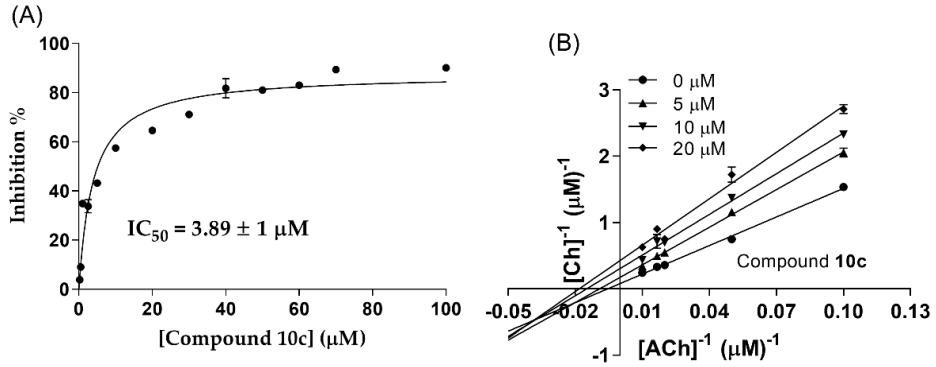


Figure 2. Dose-response inhibition curve (**A**) and Lineweaver−Burk reciprocal plots (**B**) for compound **10c** $BChE_{hu}$-ICER using the on-flow dual parallel enzyme assay. Results obtained from three independent experiments (n = 3) expressed by mean ± SEM.

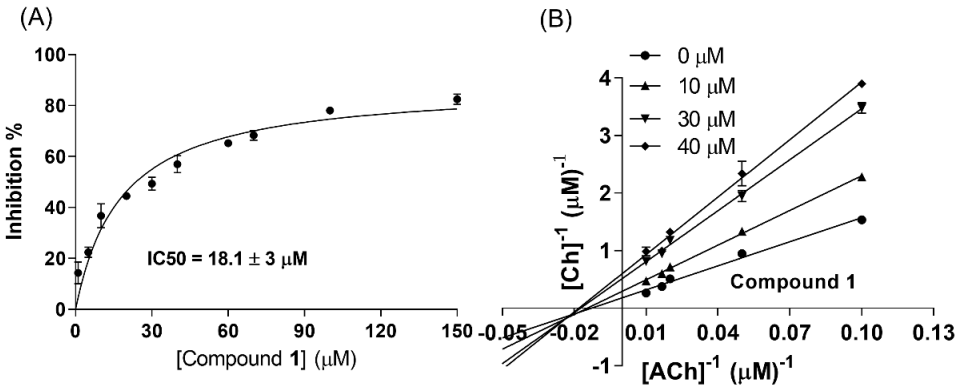


Figure 3. Dose-response inhibition curve (**A**) and Lineweaver−Burk reciprocal plots (**B**) for compound **1** $BChE_{hu}$-ICER using the on-flow dual parallel enzyme assay. Results obtained from three independent experiments (n = 3) expressed by mean ± SEM.

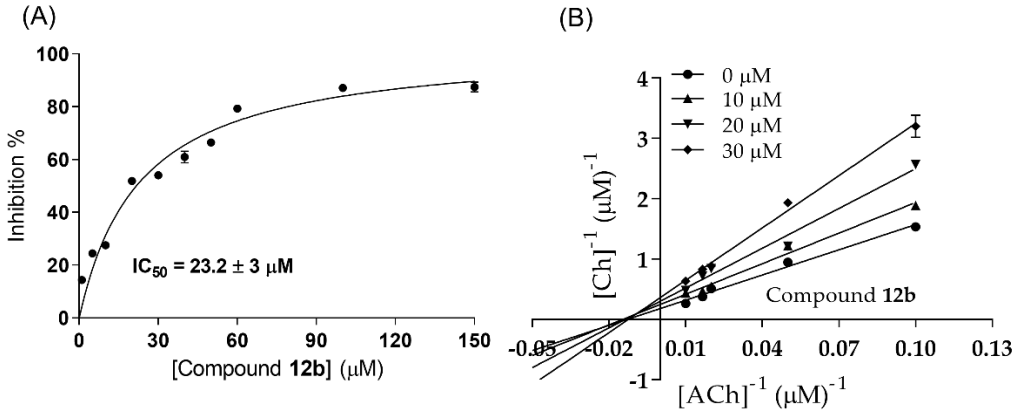


Figure 4. Dose-response inhibition curve (**A**) and Lineweaver−Burk reciprocal plots (**B**) for compound **12b** $BChE_{hu}$-ICER using the on-flow dual parallel enzyme assay. Results obtained from three independent experiments (n = 3) expressed by mean ± SEM.

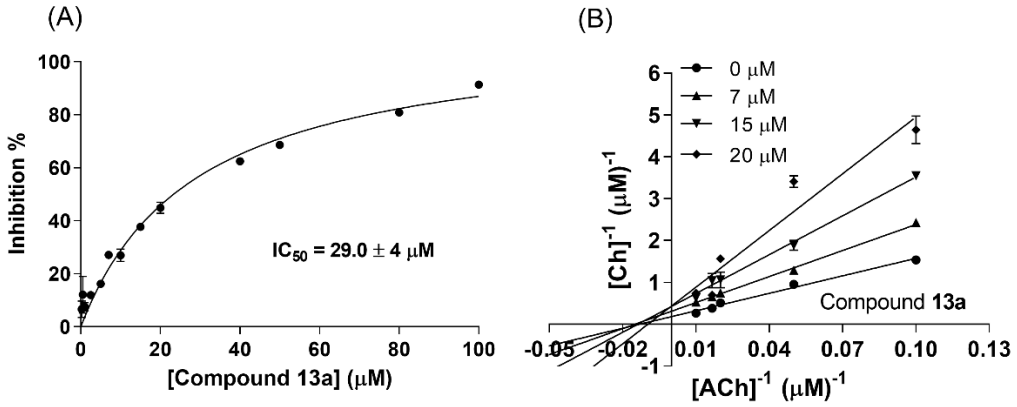


Figure 5. Dose-response inhibition curve (**A**) and Lineweaver−Burk reciprocal plots (**B**) for compound **13a** $BChE_{hu}$-ICER using the on-flow dual parallel enzyme assay. Results obtained from three independent experiments (n = 3) expressed by mean ± SEM.

To further understand the inhibition activity of these compounds, the type of inhibition mechanism was determined (Figures 2, 3, 4 and 5B and Figures S72B and S73B).

The results of our studies on the inhibition mechanisms indicate that cassine (**1**) and spectaline (**3**) inhibit butyrylcholinesterase via a mixed mechanism, the same pattern observed for 5-hydroxy piperidines **10c** and **12c** both displaying a 14-carbon alkyl chain at C-2. A non-competitive mechanism was observed for compounds **12b** and **13a**, the latter a 5-hydroxy piperidine bearing a seven-carbon side chain.

For mixed-type inhibition, V_{Max} and K_M values are affected. K_M increases and V_{Max} decreases since the inhibitor binds to the enzyme at a location distinct from the substrate binding site. Binding affinity for the substrate is decreased when the inhibitor is present. For non-competitive inhibition, K_M value remains unchanged but V_{Max} decreases. Here, the inhibitor binds to a site other than the active site. Binding causes a change in the structure of the enzyme so the substrate cannot bind, and no catalysis occurs [42,43].

The inhibitor constant, Ki, relates to the binding affinity, and the values for each compound were determined by replotting the primary reciprocal plot data. The slope and 1/v-axis intercept of each complex can be replotted against its corresponding inhibitor concentration.

Compounds **10c** (K_i = 5.24 µM), **3** (K_i = 11.3 µM), **12b** (K_i = 17.4 µM) and **13a** (K_i = 15.2 µM) substantially reduced the rate of the enzymatic reaction and showed higher binding activity, which illustrates a characteristic behavior of non-competitive and mixed-type inhibitors as observed in the double-reciprocal plots (Figures 1, 2, 3 and 4B, Figures

S72B and S73B). In the early stages of drug discovery studies, the evaluation of inhibition mode is a significant assessment since the interaction mode could be affected by the physiological environments to which the enzyme is exposed. Competitive inhibitors bind exclusively to the free enzyme form, while non-competitive or mixed-type inhibitors bind with some affinity to both forms, e.g., the free enzyme and the enzyme–substrate complex. While mixed-type inhibitors bind to the enzyme and the enzyme–substrate complex with different affinity, non-competitive inhibitors bind equally well to the enzyme and enzyme–substrate complex.

Therefore, the non-competitive and mixed-type mechanisms of inhibition can be a significant advantage in vivo when the physiological environment exposes the enzyme to high substrate concentrations. Although the clinical advantage of non-competitive inhibition has been recognized, the historical approaches for drug discovery have been focused on active-site-directed inhibitors as is the principal model of drugs in clinical use today [44].

4. Conclusions

As $BChE_{hu}$ is potentially a better target than the well-known AChE for the treatment of later-stage cognitive decline in AD, the discovery of $BChE_{hu}$ inhibitors that can act selectively and reversibly or pseudo-irreversibly in vivo is desirable because they will provide not only drug candidates, but also chemical probes to investigate the potential of $BChE_{hu}$ to serve as a therapeutic target. Our results indicate that none of the analogues of cassine (1) and spectaline (3) prepared performed better than the parent compounds in the inhibition assay of $AChE_{ee}$ at 100 μM. On the other hand, compounds 10c, 12b and 13a displayed smaller inhibition constants than cassine (1) while only 10c stood as a more potent inhibitor than spectaline (3) for $BChE_{hu}$, pointing to the fact that deletion of the methyl group at C-2 (spectaline numbering) and the unsaturation in the side chain are beneficial for $BChE_{hu}$ inhibition, a feature that should be taken into consideration for future development of $BChE_{hu}$ inhibitors and structure–activity relationship studies.

Supplementary Materials: The following are available at: https://www.mdpi.com/article/10.3390/scipharm90040063/s1. Figure S1. NOESY correlations for **8a** and **8b** (S1A and S1C) and coupling constant H5–H6 (S1A) for **8a** observed by 1H RMN spectroscopy. B: Crystal structure of intermediate **8a**. 1H- and 13C-NMR spectra of compounds **5a**, **S-I**, **5b**, **7a**, **7b**, **8a**, **8b**, **S-IV**, **S-V**, **B**, **C**, **9a-9e**, **10a-e**, **11a-c**, **12a-c**, **1**, **3** and **13a-c** and HSQC, COSY and NOESY NMR spectra of compound **8a** and **8b** shown in Figure S2–S71. Dose-response inhibition curve (**A**) and Lineweaver−Burk reciprocal plots (**B**) for compound **12c** and **3**, respectively, in Figures S72 and S73, respectively.

Author Contributions: Conceptualization: M.C.R.S., C.L.C. and R.A.P.; Methodology: M.C.R.S., A.F.L.V., C.L.C. and R.A.P.; Investigation: M.C.R.S., A.F.L.V., C.L.C. and R.A.P.; Writing: M.C.R.S., C.L.C. and R.A.P.; Funding acquisition: C.L.C. and R.A.P. All authors have read and agreed to the published version of the manuscript.

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