SYNTHESIS AND BIOLOGICAL EVALUATION OF 1,3-INDANDIONE DERIVATIVES AS ACETYLCHOLINESTERASE INHIBITORS

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Summary

The synthesis and activity of 1,3-indandione derivatives as acetylcholinesterase (AChE) inhibitors are reported. The synthetic key-step consists of the Knovenagel olefination between 1,3-indandione and a substituted (1-benzylpiperidin-4-yl)methanal followed by selective double bond reduction. AChE inhibitory activity was measured by a quick fluorimetric method. One of the new compounds showed a significant interaction with the enzyme. Molecular modelling studies were performed in order to propose the binding modes within the AChE gorge compared to the known inhibitor Donepezil.

Key words: Alzheimer's disease (AD), Acetylcholinesterase inhibitors, Knovenagel reaction, Molecular docking

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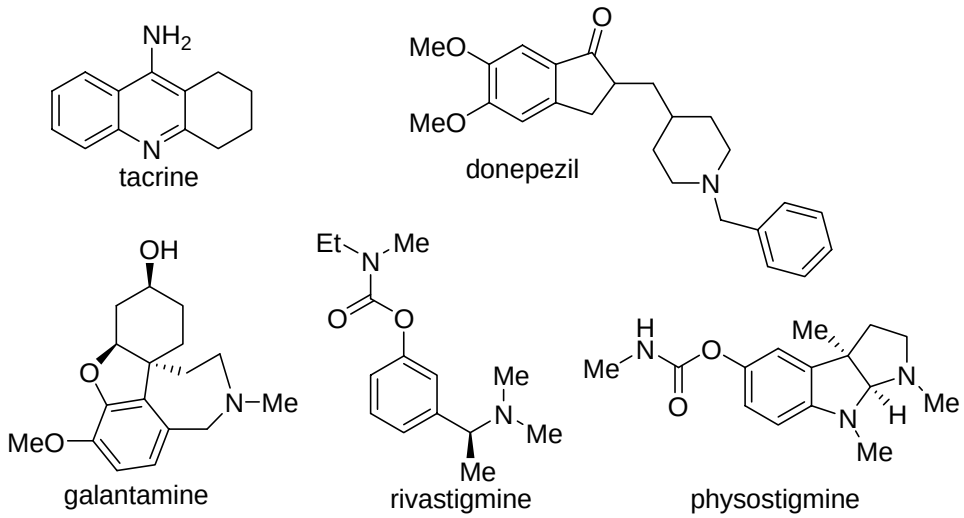
Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by premature mental deteriorations such as memory, reasoning and orientation impairments. AD is usually associated with aging and gradually lead to death in a few years. The loss of cortical cholinergic neurotransmission seems to be a main cause of the disease. A well-accepted hypothesis is that acetylcholinesterase (AChE) inhibitors can interfere with the progression of AD [1,2].

Nowadays several effective remedies are available for the treatment of this central nervous system dysfunction illness. Rivastigmine, Galantamine, Physostigmine and Donepezil (E2020) are commonly used as safe anti-AD agents because of their remarkable pharmacokinetics and pharmacodynamics properties, while the interest towards Tacrine is decreasing because of its hepatotoxicity (Figure 1). Donepezil appears as a safer agent due to its long-lasting and selective action associated with limited adverse effects [3], to be even used in multi-target-directed therapies, which seem to be the most promising approach for the management of AD [4]. In the attempt of obtaining analogs alternative to the Donepezil, we herein report a strategy for the synthesis of a new series of compounds with potential AChE inhibitory activity.

Figure 1: Chemical structure of the drugs available for AD treatment



Materials and methods

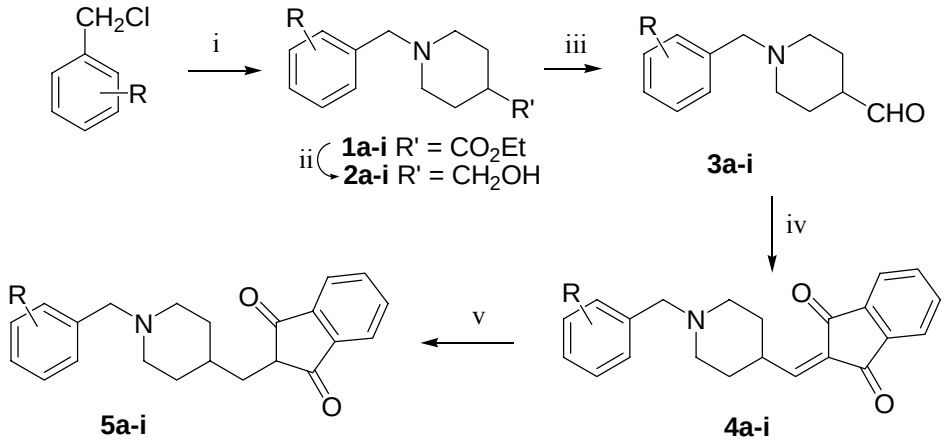
Chemicals

All chemicals of analytical grade were purchased from Aldrich Chemical Co. or Sigma Chemical Co. Thin layer chromatography (TLC) was performed on silica gel sheets with a fluorescent indicator (Statocrom SIF; Carlo Erba). Merck silica gel (Kieselgel 60/230-400 mesh) was used for column chromatography. Yields refer to purified products and are not optimized. All reactions were carried out under a nitrogen atmosphere.

Experimental

^{1}H-NMR spectra were recorded on a Brüker 300-MHz spectrometer with TMS as an internal standard: chemical shifts are expressed in δ values (ppm) and coupling constants (*J*) in Hz. IR spectra were recorded as thin films on a Perkin-Elmer 681 spectrophotometers. GC-MS (70 eV) and LC-MSD analyses were performed on a HP 5890 model and an Agilent 1100 series equipped with a Trap System VL Instruments, respectively. LC-MSD analyses on maleate salts were performed under cationic polarization. Elemental analyses were performed on a Perkin-Elmer 240C elemental analyzer and the results were within ± 0.4% of the theoretical values.

Synthetic scheme



Compounds	R
a	H
b	2-Me
c	3-Me
d	2-Cl
e	3-Cl
f	3-F
g	4-F
h	3-OMe
i	4-OMe

Scheme 1: Reagents: (i) INPE/K_2CO_3/EtOH; (ii) LAH/THF; (iii) $(COCl)_2$/DMSO/Et_3N/DCM; (iv) 1,3-indandione/piperidine/toluene; (v) TEAF/ $CHCl_3$

Synthesis

General procedure for the preparation of compounds 1a-i

The preparation of (1-benzylpiperidin-4-yl)ethylcarboxylate **1a** is reported as a representative example.

Benzyl chloride (1.94 g, 15.3 mmol) was added dropwise to a suspension of ethyl isonipecotate (2.00 g, 12.7 mmol) and K_2CO_3 (3.51 g, 25.4 mmol) in ethanol (65 mL). The resulting mixture was stirred at room temperature for 48 h. After this time H_2O (30 mL) was added and the mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure to give the expected compound as a yellow oil which was used in the next step without further purification (2.58 g, 82% yield). IR (KBr) 2944, 1720 cm^{-1}.^{1}H-NMR ($CDCl_3$) 1.23 (t, 3H, *J* = 7.1), 1.69-2.32 (m, 8H), 2.85 (m, 1H,), 3.48 (s, 2H), 4.11 (q, 2H, *J* = 7.1), 7.21-7.31 (m, 5H). m/z 247 [M^+]. Anal. ($C_{15}H_{21}NO_2$) C, H, N.

General procedure for the preparation of compounds 2a-i

The preparation of (1-benzylpiperidin-4-yl)methanol **2a** is reported as a representative example.
To a suspension of LAH (1.80 g, 47.4 mmol) in dry THF (40 mL) stirred at 0°C, a solution of **1a** (2.91 g, 11.8 mmol) in 20 mL of dry THF was added dropwise. The mixture was stirred at room temperature for 1 h and then heated under reflux for 4 h. After cooling to room temperature, H_2O (47 mL) and a solution of 2N NaOH (12 mL) were carefully added to the mixture. Ethyl acetate (250 mL) was then added and the organic layer separated. The organic solution was dried over anhydrous Na_2SO_4 and the solvent evaporated under reduced pressure to give the compound **2a** as a pale yellow oil (1.95 g, 81% yield). ^{1}H-NMR ($CDCl_3$) 1.20-1.38 (m, 4H), 1.70 (d, 2H, *J* = 11.8), 1.96 (td, 2H, *J* = 11.5, 2.6), 2.05 (bs, 1H), 2.91 (d, 1H, *J* = 11.5), 3.47 (d, 2H, *J* = 6.4), 3.50 (s, 2H), 7.21-7.40 (m, 5H). m/z 205 [M^+]. Anal. ($C_{13}H_{19}NO$) C, H, N.

General procedure for the preparation of compounds 3a-i

The preparation of (1-benzylpiperidin-4-yl)methanal **3a** is reported as a representative example.
To a solution of oxalyl chloride (1.52 g, 12.0 mmol) in dry CH_2Cl_2 (30 mL), cooled to –48 °C, dimethylsulfoxide (1,1 g, 14.0 mmol) was added dropwise and the reaction mixture was stirred at the same temperature for 10 min. A solution of **2a** (1.23 g, 6.0 mmol) in 3 mL of dry CH_2Cl_2 was added at –55 °C stirring for additional 15 min. Triethylamine (3.2 mL) was then added and the mixture was stirred at room temperature for 24 h. The organic solution was diluted with $CHCl_3$ (10 mL), shaken three times with H_2O, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a brown oil. Column chromatography purification on silica-gel (ethyl ether:petroleum ether, 3:2 as eluent) gave compound **3a** as a colourless oil (0.39 g, 32% yield). IR (KBr) 1740 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 1.62-1.75 (m, 2H), 1.80-1.95 (m, 4H), 2.11 (t, 2H, *J* = 10.7), 2.85 (m, 1H), 3.50 (s, 2H), 7.27-7.33 (m, 5H), 9.64 (s, 1H). m/z 203 [M^+]. Anal. ($C_{13}H_{17}NO$) C, H, N.

General procedure for the preparation of compounds 4a-i

The preparation of 2-[(1-benzylpiperidin-4-yl)methylene]-1*H*-indene-1,3(2*H*)-dione **4a** is reported as a representative example.
1,3-Indandione (0.41 g, 2.8 mmol) and piperidine (0.11 g, 1.6 mmol) were added subsequently to a solution of (1-benzylpiperidin-4-yl)methanal **3a** (0.57 g, 2.8 mmol) in dry toluene (10 mL). The reaction mixture was heated to reflux for 4 h. The volatiles were evaporated off and the residue was dissolved in $CHCl_3$ (10 mL). The organic layer was shaken with H_2O, then dried over anhydrous Na_2SO_4 and finally concentrated under

reduced pressure. Purification of the residue by column chromatography on silica-gel (ethyl ether:hexane, 8:2 as eluent) gave compound **4a** as an oil (0.32 g, 35% yield). The oil was added to a solution of maleic acid (0.11 g, 2.0 mmol) in ethanol (2 mL) and the solid maleate salt of **4a** was collected by filtration and recrystallized from ethanol to give a brick-red solid. Mp 127-132 (dec.) (ethanol). IR (KBr) 1690, 1710 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 0.97 (m, 2H), 1.32 (m, 4H), 1.70 (m, 1H), 2.05 (bs, 1H), 2.75 (bs, 1H), 3.50 (d, 1H, J = 6.5), 4.20 (m, 2H), 6.35 (s, 2H), 7.30-7.45 (m, 3H), 7.58 (m, 1H), 7.73 (m, 1H), 7.97 (m, 4H), 8.20 (bs, 1H), 10.60 (bs, 1H). m/z 332 [M^++1]. Anal. ($C_{22}H_{21}NO_2 \cdot C_4H_4O_4$) C, H, N.

2-{[1-(2-Methylbenzyl)piperidin-4-yl]methylene}-1H-indene-1,3(2H)-dione (4b)

Following the identical procedure to that described for **4a**, but using [1-(2-methylbenzyl)piperidine-4-yl]methanal **3b** (0.61 g, 2.8 mmol), **4b** was obtained as an oil (0.30 g, 31% yield) and transformed into the maleate salt. Mp 144-147 (dec.) (ethanol). IR (KBr) 1690, 1711 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 1.63-1.85 (m, 4H), 2.20 (m, 1H), 2.30 (s, 3H), 3.30-3.60 (m, 4H), 4.50 (m, 2H), 6.60 (s, 2H), 6.95-7.10 (m, 4H), 7.70 (m, 1H), 8.15-8.20 (m, 4H), 8.25 (bs, 1H), 10.45 (bs, 1H). m/z 346 [M^++1]. Anal. ($C_{23}H_{23}NO_2 \cdot C_4H_4O_4$) C, H, N.

2-{[1-(3-Methylbenzyl)piperidin-4-yl]methylene}-1H-indene-1,3(2H)-dione (4c)

Following the identical procedure to that described for **4a**, but using [1-(3-methylbenzyl)piperidine-4-yl]methanal **3c** (0.61 g, 2.8 mmol), **4c** was obtained as an oil (0.24 g, 25% yield) and transformed into the maleate salt. Mp 118-125 (dec.) (ethanol). IR (KBr) 1695, 1715 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 1.65-1.80 (m, 4H), 2.16 (m, 1H), 2.35 (s, 3H), 3.19-3.29 (m, 4H), 4.45 (m, 2H), 6.95-7.15 (m, 4H), 7.25-7.45 (m, 2H), 7.55 (m, 1H), 7.70-8.10 (m, 4H), 8.45 (bs, 1H), 10.65 (bs, 1H). m/z 346 [M^++1]. Anal. ($C_{23}H_{23}NO_2 \cdot C_4H_4O_4$) C, H, N.

2-{[1-(2-Chlorobenzyl)piperidin-4-yl]methylene}-1H-indene-1,3(2H)-dione (4d)

Following the identical procedure to that described for **4a**, but using [1-(2-chlorobenzyl)piperidine-4-yl]methanal **3d** (0.67 g, 2.8 mmol), **4d** was obtained as an oil (0.31 g, 30% yield) and transformed into the maleate salt. Mp 134-137 (dec.) (ethanol). IR (KBr) 1695, 1715 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 0.65-1.80 (m, 4H), 2.85 (m, 1H), 3.30-3.60 (m, 4H), 4.40 (m, 2H), 6.40 (s, 2H), 7.15-7.40 (m, 4H), 7.45 (m, 1H), 7.50-7.75 (m, 4H), 8.30 (bs, 1H), 10.35 (bs, 1H). m/z 367 [M^++1]. Anal. ($C_{22}H_{20}ClNO_2 \cdot C_4H_4O_4$) C, H, N.

2-{[1-(3-Chlorobenzyl)piperidin-4-yl]methylene}-1H-indene-1,3(2H)-dione (4e)

Following the identical procedure to that described for **4a**, but using [1-(3-chlorobenzyl)piperidine-4-yl]methanal **3e** (0.67 g, 2.8 mmol), **4e** was obtained as an oil (0.29 g, 28% yield) and transformed into the maleate salt. Mp 155-157 (dec.) (ethanol). IR (KBr) 1690, 1719 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 0.70-1.80 (m, 4H), 2.30 (m, 1H), 3.60-4.15 (m, 4H), 4.55 (m, 2H), 6.55 (s, 2H), 7.20-7.30 (m, 3H), 7.30-7.45 (m, 2H), 7.45-7.70 (m, 4H), 8.35 (bs, 1H), 10.40 (bs, 1H). m/z 367 [M^++1]. Anal. ($C_{22}H_{20}ClNO_2 \cdot C_4H_4O_4$) C, H, N.

2-{[1-(3-Fluorobenzyl)piperidin-4-yl]methylene}-1H-indene-1,3(2H)-dione (4f)

Following the identical procedure to that described for **4a**, but using [1-(3-fluorobenzyl)piperidine-4-yl]methanal **3f** (0.62 g, 2.8 mmol), **4f** was obtained as an oil (0.41 g, 42% yield) and transformed into the maleate salt. Mp 128-130 (dec.) (ethanol). IR (KBr) 1690, 1719 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 1.70-1.80 (m, 4H), 2.18 (m, 1H), 3.20-3.35 (m, 4H), 4.35 (m, 2H), 6.30 (s, 2H), 6.90-7.10 (m, 2H), 7.20-7.30 (m, 2H), 7.43 (m, 1H), 7.70-8.10 (m, 4H), 8.15 (bs, 1H), 10.90 (bs, 1H). m/z 350 [M^+ +1]. Anal. ($C_{22}H_{20}FNO_2 \cdot C_4H_4O_4$) C, H, N.

2-{[1-(4-Fluorobenzyl)piperidin-4-yl]methylene}-1H-indene-1,3(2H)-dione (4g)

Following the identical procedure to that described for **4a**, but using [1-(4-fluorobenzyl)piperidine-4-yl]methanal **3g** (0.62 g, 2.8 mmol), **4g** was obtained as an oil (0.36 g, 37% yield) and transformed into the maleate salt. Mp 109-113 (dec.) (ethanol). IR (KBr) 1695, 1719 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 1.65-1.80 (m, 4H), 2.22 (m, 1H), 3.15-3.45 (m, 4H), 4.65 (m, 2H), 6.35 (s, 2H), 7.05-7.20 (m, 2H), 7.30-7.40 (m, 2H), 7.53 (m, 1H), 7.70-8.00 (m, 4H), 8.25 (bs, 1H), 10.95 (bs, 1H). m/z 350 [M^+ +1]. Anal. ($C_{22}H_{20}FNO_2 \cdot C_4H_4O_4$) C, H, N.

2-{[1-(3-Methoxybenzyl)piperidin-4-yl]methylene}-1H-indene-1,3(2H)-dione (4h)

Following the identical procedure to that described for **4a**, but using [1-(3-methoxybenzyl)piperidine-4-yl]methanal **3h** (0.65 g, 2.8 mmol), **4h** was obtained as an oil (0.40 g, 40% yield) and transformed into the maleate salt. Mp 126-131 (dec.) (ethanol). IR (KBr) 1695, 1719 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 1.75-1.83 (m, 4H), 2.10 (m, 1H), 3.18-3.40 (m, 4H), 3.73 (s, 3H), 4.35 (m, 2H), 6.40 (s, 2H), 7.05-7.15 (m, 2H), 7.30-7.35 (m, 2H), 7.60 (m, 1H), 7.65-8.10 (m, 4H), 8.15 (bs, 1H), 11.10 (bs, 1H). m/z 362 [M^+ +1]. Anal. ($C_{23}H_{23}NO_3 \cdot C_4H_4O_4$) C, H, N.

2-{[1-(4-Methoxybenzyl)piperidin-4-yl]methylene}-1H-indene-1,3(2H)-dione (4i)

Following the identical procedure to that described for **4a**, but using [1-(4-methoxybenzyl)piperidine-4-yl]methanal **3i** (0.65 g, 2.8 mmol), **4i** was obtained as an oil (0.35 g, 35% yield) and transformed into the maleate salt. Mp 99-103 (dec.) (ethanol). IR (KBr) 1690, 1719 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 1.65-1.75 (m, 4H), 2.00 (m, 1H), 3.10-3.25 (m, 4H), 3.70 (s, 3H), 4.50 (m, 2H), 6.45 (s, 2H), 7.00-7.30 (m, 4H), 7.55 (m, 1H), 7.75-8.20 (m, 4H), 8.25 (bs, 1H), 10.93 (bs, 1H). m/z 362 [M^+ +1]. Anal. ($C_{23}H_{23}NO_3 \cdot C_4H_4O_4$) C, H, N.

General procedure for the preparation of compounds 5a-i

The preparation of 2-[(1-benzylpiperidin-4-yl)methyl]-1*H*-indene-1,3(2*H*)-dione **5a** is reported as a representative example.

A suspension of TEAF, obtained by mixing formic acid (0.41 g, 9.0 mmol) and triethylamine (0.36 g, 3.6 mmol) (3.52 mmol), was added to (2-[(1-benzylpiperidin-4-yl)methylen]-1*H*-inden-1,3-(2*H*)-dione **4a** (0.10 g, 0.3 mmol) under stirring. The resulting mixture was heated to reflux for 6 h. The volatiles were evaporated under reduced pressure and the residue was dissolved in $CHCl_3$. The organic layer was shaken with H_2O and

$NaHCO_3$ saturated solution, then dried (Na_2SO_4) and concentrated to give an oily residue. The purification of the residue by column chromatography on silica gel (ethyl ether:light petroleum, 95:5 as eluent) gave compound **5a** as a yellow oil (25 mg, 25% yield).
The oil was added to a solution of maleic acid (0.08 g, 1.5 mmol) in ethanol (1.5 mL) and the solid maleate salt of **5a** was collected by filtration and recrystallized from ethanol to give a brown solid. Mp 117-122 (dec.) (ethanol).. IR (KBr) 1695,1710 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 0.70-0.90 (m, 2H), 1.05-1.40 (m, 3H), 1.45-1.85 (m, 4H) 3.55 (t, 1H, *J* = 3.2), 4.15 (m, 1H), 4.45 (t, 1H, *J* = 3.2), 6.45 (s, 2H), 7.05-7.25 (m, 6H), 7.30 (m, 1H), 7.50-8.00 (m, 4H), 8.75 (bs, 1H), 11.0 (bs, 1H). m/z 334 [M^+ +1]. Anal. ($C_{22}H_{23}NO_2 \cdot C_4H_4O_4$) C, H, N.

2-{[1-(2-Methylbenzyl)piperidin-4-yl]methyl}-1H-indene-1,3(2H)-dione (5b)

Following the identical procedure to that described for **5a**, but using 2-{[1-(2-methylbenzyl)piperidin-4-yl]methylene}-1*H*-indene-1,3(2*H*)-dione **4b** (0.10 g, 0.3 mmol), **5b** was obtained as an oil (29 mg, 28% yield) and transformed into the maleate salt. Mp 141-144 (dec.) (ethanol). IR (KBr) 1716 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 1.00-1.35 (m, 2H), 1.40-1.85 (m, 3H), 1.90-2.05 (m, 4H), 2.10 (s, 3H), 3.45 (m, 1H), 3.60 (t, 1H, *J* = 3.2), 4.35 (t, 1H, *J* = 3.2), 6.30 (s, 2H), 7.10-7.40 (m, 4H), 7.50 (m,1H), 7.65 (m, 1H), 7.75-8.00 (m, 4H), 8.75 (bs, 1H), 11.0 (bs, 1H). m/z 348 [M^+ +1]. Anal. ($C_{23}H_{25}NO_2 \cdot C_4H_4O_4$) C, H, N.

2-{[1-(3-Methylbenzyl)piperidin-4-yl]methyl}-1H-indene-1,3(2H)-dione (5c)

Following the identical procedure to that described for **5a**, but using 2-{[1-(3-methylbenzyl)piperidin-4-yl]methylene}-1*H*-indene-1,3(2*H*)-dione (**4c**) (0.10 g, 0.3 mmol), **5c** was obtained as an oil (26 mg, 25% yield) and transformed into the maleate salt. Mp 159-164 (dec.) (ethanol). IR (KBr) 1724 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 1.05-1.45 (m, 2H), 1.50-1.80 (m, 3H), 1.85-2.00 (m, 4H), 2.30 (s, 3H), 2.80 (t, 1H, *J* = 3.5), 3.25 (m, 1H), 3.35 (t, 1H, *J* = 3.2), 6.20 (s, 2H), 7.00-7.30 (m, 4H), 7.40-7.50 (m,1H), 7.65-7.70 (m, 1H), 7.75-8.00 (m, 4H), 8.95 (bs, 1H), 10.9 (bs, 1H). m/z 348 [M^+ +1]. Anal. ($C_{23}H_{25}NO_2 \cdot C_4H_4O_4$) C, H, N.

2-{[1-(2-Chlorobenzyl)piperidin-4-yl]methyl}-1H-inden-1,3(2H)-dione (5d)

Following the identical procedure to that described for **5a**, but using 2-{[1-(2-chlorobenzyl)piperidin-4-yl]methylene}-1*H*-indene-1,3(2*H*)-dione **4d** (0.11 g, 0.3 mmol), **5d** was obtained as an oil (28 mg, 25% yield) and transformed into the maleate salt. Mp 128-132 (dec.) (ethanol). IR (KBr) 1722 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 0.70-1.15 (m, 2H), 1.60-1.85 (m, 3H), 1.95-2.20 (m, 4H), 3.15 (m, 1H), 3.60 (t, 1H, *J* = 3.3), 3.45 (t, 1H, *J* = 3.2), 6.20 (s, 2H), 7.20-7.30 (m, 4H), 7.45-7.50 (m,1H), 7.65-7.70 (m, 1H), 7.75-8.00 (m, 4H), 8.95 (bs, 1H), 12.2 (bs, 1H). m/z 369 [M^+ +1]. Anal. ($C_{22}H_{22}ClNO_2 \cdot C_4H_4O_4$) C, H, N.

2-{[1-(3-Chlorobenzyl)piperidin-4-yl]methyl}-1H-indene-1,3(2H)-dione (5e)

Following the identical procedure to that described for **5a**, but using 2-{[1-(3-chlorobenzyl)piperidin-4-yl]methylene}-1*H*-indene-1,3(2*H*)-dione **4d** (0.11 g, 0.3 mmol), **5e** was obtained as an oil (22 mg, 20% yield) and transformed into the maleate salt. Mp 170-173 (dec.) (ethanol). IR (KBr) 1728 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 0.75-1.25 (m, 2H), 1.65-

1.80 (m, 3H), 1.90-2.15 (m, 4H), 3.35 (m, 1H), 3.60 (t, 1H, *J* = 3.3), 4.35 (t, 1H, *J* = 3.2), 6.20 (s, 2H), 7.05-7.25 (m, 4H), 7.35-7.50 (m,1H), 7.70-7.80 (m, 1H), 7.90-8.05 (m, 4H), 8.90 (bs, 1H), 12.10 (bs, 1H). m/z 369 [M^+ +1]. Anal. ($C_{22}H_{22}ClNO_2 \cdot C_4H_4O_4$) C, H, N.

2-{[1-(3-Fluorobenzyl)piperidin-4-yl]methyl}-1H-indene-1,3(2H)-dione (5f)

Following the identical procedure to that described for **5a**, but using 2-{[1-(3-fluorobenzyl)piperidin-4-yl]methylene}-1*H*-indene-1,3(2*H*)-dione **4f** (0.10 g, 0.3 mmol), **5f** was obtained as an oil (25 mg, 25% yield) and transformed into the maleate salt. Mp 161-164 (dec.) (ethanol). IR (KBr) 1728 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 0.80-1.20 (m, 2H), 1.50-1.80 (m, 3H), 1.85-2.00 (m, 4H), 3.20 (m, 1H), 3.35 (t, 1H, *J* = 3.0), 3.45 (t, 1H, *J* = 3.0), 6.10 (s, 2H), 7.00-7.20 (m, 4H), 7.30-7.45 (m,1H), 7.50-7.60 (m, 1H), 7.65-7.90 (m, 4H), 8.80 (bs, 1H), 11.5 (bs, 1H). m/z 352 [M^+ +1]. Anal. ($C_{22}H_{22}FNO_2 \cdot C_4H_4O_4$) C, H, N.

2-{[1-(4-Fluorobenzyl)piperidin-4-yl]methyl}-1H-indene-1,3(2H)-dione (5g)

Following the identical procedure to that described for **5a**, but using 2-{[1-(4-fluorobenzyl)piperidin-4-yl]methylene}-1*H*-indene-1,3(2*H*)-dione **4g** (0.10 g, 0.3 mmol), **5g** was obtained as an oil (28 mg, 28% yield) and transformed into the maleate salt. Mp 167-171 (dec.) (ethanol). IR (KBr) 1728 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 0.70-1.15 (m, 2H), 1.30-1.70 (m, 3H), 1.75-1.95 (m, 4H), 3.30 (m, 1H), 3.35 (t, 1H, *J* = 3.0), 3.40 (t, 1H, *J* = 3.0), 6.15 (s, 2H), 7.15-7.25 (m, 4H), 7.30-7.40 (m,1H), 7.35-7.50 (m, 1H), 7.55-7.70 (m, 4H), 8.50 (bs, 1H), 11.8 (bs, 1H). m/z 369 [M^+ +1]. Anal. ($C_{22}H_{22}FNO_2 \cdot C_4H_4O_4$) C, H, N.

2-{[1-(3-Methoxybenzyl)piperidin-4-yl]methyl}-1H-indene-1,3(2H)-dione (5h)

Following the identical procedure to that described for **5a**, but using 2-{[1-(3-methoxybenzyl)piperidin-4-yl]methylene}-1*H*-indene-1,3(2*H*)-dione **4h** (0.11 g, 0.3 mmol), **5h** was obtained as an oil (34 mg, 31% yield) and transformed into the maleate salt. Mp 153-156 (dec.) (ethanol). IR (KBr) 1728 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 0.85-1.20 (m, 2H), 1.35-1.60 (m, 3H), 1.65-1.80 (m, 4H), 3.20 (m, 1H), 3.40 (t, 1H, *J* = 3.0), 3.55 (t, 1H, *J* = 3.0), 3.75 (s, 3H), 6.20 (s, 2H), 7.10-7.20 (m, 4H), 7.35- 7.50 (m, 1H), 7.60-7.75 (m, 1H), 7.85-8.00 (m, 4H), 8.60 (bs, 1H), 11.00 (bs, 1H). m/z 364 [M^+ +1]. Anal. ($C_{23}H_{25}NO_3 \cdot C_4H_4O_4$) C, H, N.

2-{[1-(4-Methoxybenzyl)piperidin-4-yl]methyl}-1H-indene-1,3(2H)-dione (5i)

Following the identical procedure to that described for **5a**, but using 2-{[1-(4-methoxybenzyl)piperidin-4-yl]methylene}-1*H*-indene-1,3(2*H*)-dione **4i** (0.11 g, 0.3 mmol), **5e** was obtained as an oil (32 mg, 29% yield) and transformed into the maleate salt. Mp 127-132 (dec.) (ethanol). Mp 137-142 (dec.) (ethanol). IR (KBr) 1728 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 0.80-1.20 (m, 2H), 1.45-1.70 (m, 3H), 1.70-2.00 (m, 4H), 3.30 (m, 1H), 3.45 (t, 1H, *J* = 3.0), 3.60 (t, 1H, *J* = 3.0), 3.70 (s, 3H), 6.25 (s, 2H), 7.15-7.30 (m, 4H), 7.40-7.50 (m,1H), 7.65-7.80 (m, 1H), 7.90-8.05 (m, 4H), 8.55 (bs, 1H), 11.30 (bs, 1H). m/z 364 [M^+ +1]. Anal. ($C_{23}H_{25}NO_3 \cdot C_4H_4O_4$) C, H, N.

AChE activity assay

Acetylcholinesterase (AChE) activity was measured by a fluorimetric assay using AmplexRed Acetylcholine / AChE assay kit (Molecular Probes Inc., USA). AChE (from human erytrocytes) was purchased from Sigma. Reaction was carried out at pH 8 and started adding 100 μL of AmplexRed reagent working solution (HRP 1U/mL, Choline oxidase 0.1U/mL, Ach 50 μM, AmpexRed reagent 200 μM, final concentrations) to 100 μL of enzyme working solution (200 mU/mL in Tris-Cl 50 mM, final concentration) in the absence (control) or presence of increasing concentration of inhibitors. Microplates were incubated at room temperature for 15 min protected from light, then fluorescence was measured in a fluorescence microplate reader (Perkin-Elmer HTS 7000PLUS) using excitation at 530-560 nm and emission detection at 590 nm. Background fluorescence, derived from no-enzyme control, was subtracted from each point. By comparison to 100% activity of the control assay, the percentage of enzyme inhibition was calculated at each inhibitor concentration and the concentration of drug that caused 50% inhibition of enzymatic activity (IC_{50}) was calculated by linear regression analysis of inhibition curves.

Results

Biological studies

Compounds **4a-i** and **5a-i** were tested for *in vitro* inhibition of human AChE by a commercially available enzyme fluorimetric assay (see Experimental section for details) [5]. The data reported represent means of at least three independent experiments. Reference standard compounds tacrine (IC_{50} = 0.29 μM) and eserine (IC_{50} = 0.019 μM) were tested, in order to validate the assay. Somewhat surprisingly, saturated compounds **5a-i** did not show any activity up to a 10 μM concentration. Amongst the unsaturated derivatives, only compounds **4a** and **4d** showed a detectable inhibitory activity (IC_{50} = 1.85 and 2.15 μM, respectively), even though weaker if compared to Donepezil, that, under similar conditions, showed an IC_{50} = 0.02 μM. However, substitution on the benzylic fragment of **4a** by a methyl group **4b**,**c**, a chlorine or fluorine atom **4e-g**, or a methoxy group **4h**,**i** resulted in almost complete loss of activity up to 10 μM.

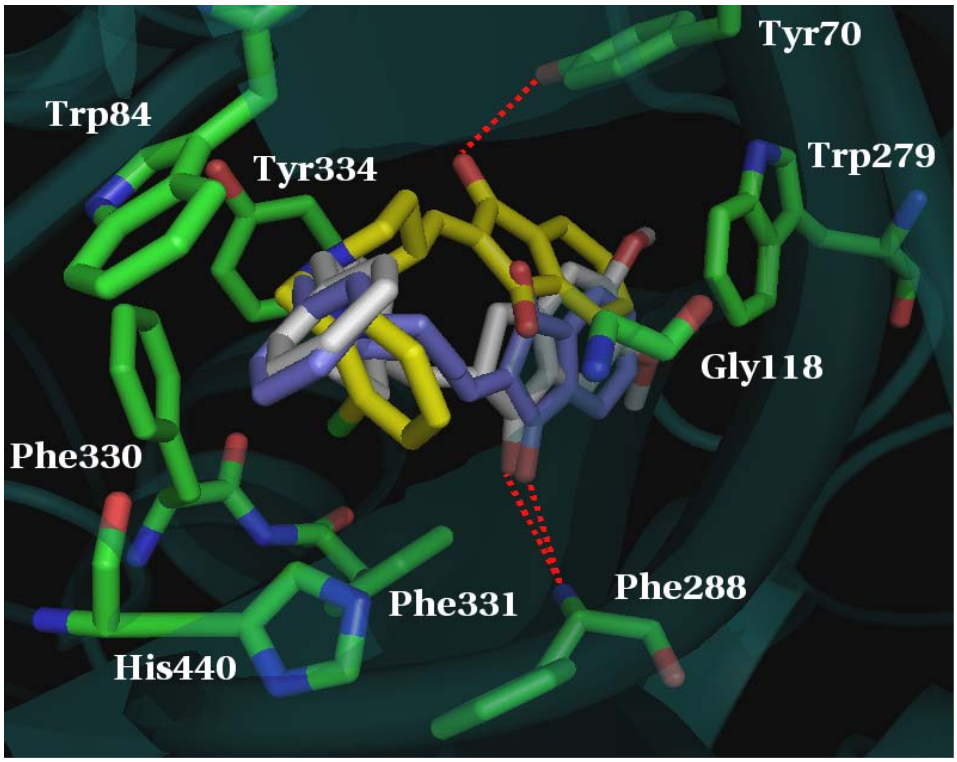
Molecular modelling

Compound **4a** and **4d**, the only two compounds showing a detectable AChE inihibitory activity, have been submitted to a molecular modelling study with the aim to investigate their binding modes into the enzyme gorge. In order to validate our approach, the crystal structure of AChE complexed with Donepezil has been considered as reference structural model. The structures of **4a** and **4d** have been built by means of Maestro GUI [6] onto the Donepezil scaffold extracted from the crystallographic model 1EVE [7] deposited into the Protein Data Bank [8].

Since all molecules showed an ionisable nitrogen atom, the first step has been directed to the investigation of the neutral/ionised form equilibriums. Such a task has been carried out by means of the pKa calculator plug-in [9-12] available into the JChem software [13] revealing a strongly larger population of the ionised form for all compounds at physiological pH values. This observation suggested to take into consideration all molecules with a net positive charge located onto the piperidine nitrogen. AChE

recognition of **4a** and **4d** has been carried out by means of docking simulation performed with Glide software [14]. The crystallographic structure 1EVE has been considered as receptor model after adding hydrogen atoms and removing co-crystallized compounds and water solvent molecules. In order to evaluate the accuracy of our approach, Donepezil has been re-docked into the enzyme model. In agreement with the Glide procedure the ligand binding site has been designed by means of a three-dimensional box, of 110,000 Å [3], centred onto the Donepezil crystallographic position. The ten best fitting configurations for each ligand have been submitted to the flexible docking algorithm. The Glide software has been able to remarkably reproduce the Donepezil binding mode, with an RMSd (Root Mean Square deviation) computed on the heavy atoms between the best pose and the crystallographic configuration equal to 0.14 Å. The recognition of **4a** and **4d** has been found quite similar to that reported for Donepezil (Figure 3).

Figure 3: AChE recognition of Donepezil, 4a and 4d. Interacting residues are labelled and reported in green stick carbon atoms. Dotted lines represent intermolecular hydrogen bond interactions



New compounds showed similar binding modes, interacting with almost the same aminoacids with different contact network especially concerning the electrostatic contributions. In particular all molecules, but less relevant for **4d**, displayed a π-π stacking between their indanone/indandione moiety and the Trp279, a hydrogen bond has been observed between Donepezil and **4a** sp^2 oxygen atoms and the Phe288 backbone amide. Such an interaction has been found particularly remarkable in the **4a** case and less relevant for Donepezil (Table 1).

Table 1: Molecular modelling summary. Theoretical affinity of Donepezil, 4a and 4d with respect to the AChE has been computed as wGscore. I.F. % = percentage of the ionised form at pH 7.4; El = electrostatic contributions energies; HB = hydrogen bond energies; vdW = hydrophobic interaction energies. * in µM; ** in kcal/mol

Inhibitors	*IC_{50}	pKa	I.F. %	wGscore	**El	**HB	**vdW
Donepezil	0.02	8.76	95.85	-8.96	-6.04	-0.48	-41.31
4a	1.85	8.11	83.59	-7,52	-4.43	-0,75	-29.67
4d	2.15	7.83	72.96	-6.43	-5.47	-0.66	-24.80

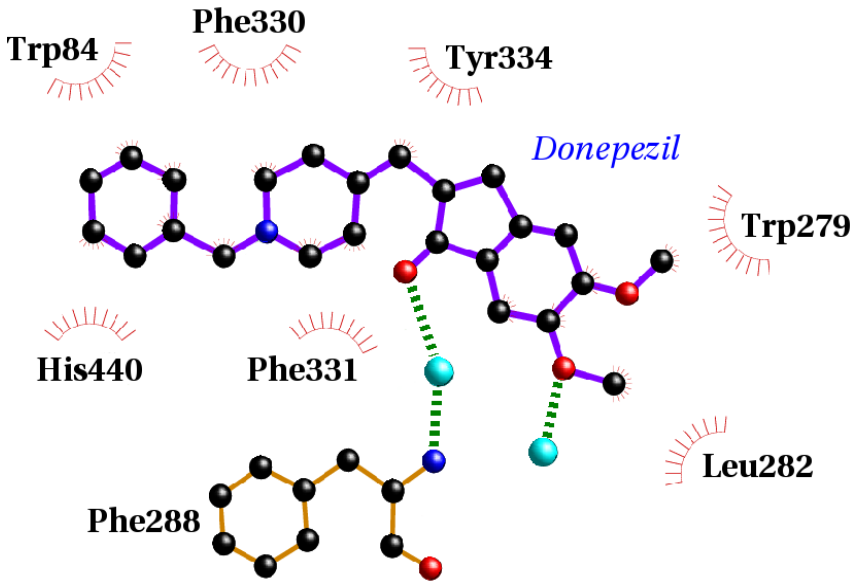
On the other hand, such kind of interaction can also be observed for **4d** with respect to the Tyr70 largely contributing to the different location of this compound, compared to Donepezil and **4a**, within the AChE. Van der Waals (vdW) contacts have been highlighted among the inhibitors piperidine ring and the Tyr334 and the Phe331. In particular a cation-π interaction has been addressed between the positively charged ligands nitrogen atom and the Phe330, in this case the closer position of the Donepezil allowed stronger contributions. Finally Donepezil and **4a** showed a π-π stacking with the Trp84. This interaction, for **4d**, has been reduced to few vdW contacts due to the chlorine substituent, which forced a rotation of the terminus benzyl moiety bringing it closer to the His440. Due to the hydrogen bond with the Tyr70, **4d** was involved in modest vdW contacts with the Gly118.
In order to obtain a quantitative scale related to the AChE affinity, the ionised form percentage of existence of each compound, has been considered coefficient for the Glide score allowing a good correlation with the Donepezil IC_{50} experimental data, which was in agreement with that previously reported by Rault [15]. A good agreement has been found also comparing the interaction energies, considered as the sum of the no-bonded contributions, to the IC_{50}. Molecular modelling results have been summarized in Table 1.

Discussion

Design

Figure 2 shows a LigPlot [16] analysis of Donepezil and essentially describes its binding mode to the AChE gorge derived from the crystallographic model 1EVE [7-8, 15, 17-19]. From the careful inspection of above-reported interaction and many other literature data, it was established that the contemporary presence of three fragments in the Donepezil structure is a prerequisite for the pharmacological activity. Thus the indanone moiety and the piperidine ring, linked by a methylene, and a *N*-appended benzyl group seem necessary to exert enzyme inhibition. As a first purpose, we planned to replace the indanone moiety of the reference compound with a 1,3-indandione. Even though the presence of the methoxy groups seemed to be important for biological activity, we decided to use a commercially available unsubstituted 1,3-indandione in order to explore the contribution of a second carbonyl group to the interaction with enzyme active site.

Figure 2: Schematic representation of the Donepezil AChE recognition. Dotted green lines represent hydrogen bond interactions. Water molecules are depicted as cyan spheres



The second planned structural modification consisted in the introduction of selected substituents on the benzylic fragment, leaving unaltered the piperidine moiety. A set of nine derivatives **5a-i** were then prepared and tested for their anti AChE activity (Scheme 1). The introduction of a double bound between the position 2 of the indandione and the carbon linking the piperidine ring was also taken into consideration. Intermediates **4a-i** were therefore subjected to biological tests as well. All these new analogs, based on a backbone similar to that of Donepezil, should maintain a molecular arrangement still consistent with the enzyme interaction.

Chemistry

The synthetic pathway for the above-mentioned compounds consists of five steps (Scheme 1).
In the first step ethyl isonipecotate (INPE) was alkylated with a substituted benzyl chloride in the presence of potassium carbonate to give the ethyl esters **1a-i** [20]. Lithium aluminum hydride (LAH) reduction of **1a-i** in dry THF afforded primary alchols **2a-i**, which were in turn subjected to Swern oxidation to give aldehydes **3a-i** [21,22]. The condensation of aldehydes **3a-i** with 1,3-indandione was achieved by a Knovenagel reaction, *via* Mannich base, in the presence of a catalytic amount of piperidine in toluene to produce the olefinic compounds **4a-i** in variable yield [23-25]. Triethylamine formate (TEAF) was used for the final double-bond reduction to give compounds **5a-i** [6].
Compounds **4a-i** and **5a-i** were converted into the corresponding maleate salts, which were purified by crystallization from ethanol. In this form, both sets of derivatives were quickly subjected to biological tests.

Conclusion

We designed and synthesized a new class of 1,3-indandione derivatives as potential inhibitors of acetylcholinesterase enzyme. Amongst the tested compounds, only unsaturated derivative **4a** showed a significant interaction with the enzyme although its inhibitory activity resulted at best 90-fold lower than that observed with Donepezil. This result would confirm that methoxy groups in the reference compound play a crucial role in improving AChE binding, while the presence of the second carbonyl did not conclusively prevent a correct interaction. Surprisingly, the corresponding saturated analog **5a** did not inhibit the enzyme at a similar concentration. As a result, the rigidity conferred to the molecule by the presence of the double bond may thus be a favorable structural requisite for a correct orientation during interaction. Finally, the general loss of activity showed by compounds bearing a substituent on the benzylic portion would confirm what already stated about interaction of the reference model compound Donepezil and its benzyl-substituted analogs [17,18].

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