Design, Synthesis and Bioevaluation of Tacrine Hybrids with Cinnamate and Cinnamylidenacetate Derivatives as Potential Anti-Alzheimer Drug Candidates

Catarina Quintanova, Rangappa S. Keri, Sérgio M. Marques, Maria G-Fernandes, Sandra M. Cardoso, M. Luísa Serralheiro and M. Amélia Santos

Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1, 1049-001 Lisboa, Portugal.

Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.

Centre for Nano and Material Sciences, Jain University, Jain Global Campus, Bangalore, Karnataka, 562112, India

CNC–Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal.

Faculty of Medicine, University of Coimbra, Coimbra, Portugal.

Supplemental

EXPERIMENTAL SECTION

General methods and materials

Analytical grade reagents were purchased from Sigma, Aldrich, Fluka, Merck and Alfa Aesar, and used as supplied. An Aβ peptide fragment 1–42 (Aβ_{42}) was obtained from American Peptide Company Inc. (USA). Solvents were dried according to standard methods. The chemical reactions were monitored by TLC using aluminum plates coated with silica gel 60 F254 (Merck). Column flash chromatography separations were performed using silica gel Merck 230–400 mesh ASTM. The ^1H and ^13C NMR spectra were recorded on a Bruker ADVANCE III 300 or 400 MHz spectrometer at room temperature. Chemical shifts (δ) are reported in ppm from the internal reference TMS (tetramethylsilane), for organic solvents. Assignments given for the NMR spectra of new
compounds were based on DEPT, COSY, HSQC experiments. The following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet, bs = broad singlet.

Melting points were measured with a Leica Galen III hot stage apparatus and are uncorrected. The electro spray ionization mass spectra (ESI-MS) were obtained on a 500 MS LC Ion Trap (Varian Inc., Palo Alto, CA, USA) mass spectrometer equipped with an ESI ion source, operated in the positive or negative ion mode. Microanalyses were performed using a Fisons EA1108 CHNF/O instrument.

**Synthesis of the compounds**

**General procedure for the synthesis of the tacrine–hybrid compounds (5a–9a):** To a stirred mixture of \( N \)-(1,2,3,4-tetrahydroacridin-9-yl)etane-1,2-diamine (4a), equivalent amount of cinnamic acid derivatives (or the corresponding homologous 5-phenyl-2,4-pentadienoic acid derivatives) and \( N \)-methylmorpholine (NMM, 2.5 equiv) in DCM under nitrogen atmosphere, was added dropwise 2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphorinane-2,4,6-trioxide (T3P, 1.5 equiv). The reaction mixture was left stirring at room temperature for 4 h; then the reaction mixture was diluted with water and extracted with CH\(_2\)Cl\(_2\); afterwards the combined organic phases were washed with aqueous solutions of 0.1M of HCl, 1% NaOH and water. The organic layer was dried over anhydrous sodium sulfate, the solvent was evaporated and the residue was recrystalized from different solvents affording the subtitled compounds (5a–9a).

**\( N \)-(2-(1,2,3,4-tetrahydroacridin-9-ylamino)ethyl)cinnamamide (5a):** Compound 4a and cinnamic acid afforded the title compound as a yellow solid, with recrystallization from n-hexan; yield 50%; M.p. 86-88 °C. \( ^1\)H NMR (400 Hz, MeOD), \( \delta \) (ppm): 8.12 (d, \( J = 8.4 \) Hz, 1H, CHC-CN), 7.76 (d, \( J = 8.4 \)Hz, 1H, CHC-N), 7.56-7.51 (m, 4H, ArH), 7.38-7.35 (m, 4H, ArH), 6.51 (d, \( J = 15.8 \) Hz, 1H, NHCOCH=CH), 3.73 (t, \( J = 6.0 \) Hz, 2H, CH\(_2\)CH\(_2\)NHCO), 3.58 (t, \( J = 6.0 \) Hz, 2H, CH\(_2\)CH\(_2\)NHCO), 2.95 (2H, bs, CH\(_2\)-C=N), 2.78 (2H, bs, CH\(_2\)-CCN), 1.89 (4H, m, (CH\(_2\)_2CH-CN). \( ^{13}\)C NMR (MeOD), \( \delta \) (ppm): 169.45, 159.30, 153.14, 147.94, 142.11, 136.21, 130.96), 130.00, 129.80, 128.88, 128.02, 124.97, 124.35, 121.56, 121.34, 117.29, 41.65, 34.23, 26.26, 24.15, 23.70; \( m/z \) (ESI-MS): 372.2 (M+H)+. M.p. 86-88 °C. Elemental analysis calc: (C\(_{24}\)H\(_{25}\)N\(_3\)O\(_12\)H\(_2\)O): C 73.42, H 7.02, N 10.70%; found: C 73.42, H 6.80, N 10.61%.
5-Phenyl-N-(2-(1,2,3,4-tetrahydroacridin-9-ylamino)ethyl)penta-2,4-dienamide (6a): Compound 4a and 5-phenyl-2,4-pentadienoic acid afforded the title compound as a yellow solid, after recrystallization from n-hexan; yield: 31%; M.p. 87-89 °C; 

$^1$H NMR (DMSO), $\delta$ (ppm): 8.12 (d, $J = 8.5$ Hz, 1H, CH=CCNH), 7.71 (d, $J = 9.4$ Hz, 1H, CH=CN), 7.57-7.50 (m, 3H, H arom), 7.39-7.30 (m, 4H, H arom), 7.25-7.18 (m, 1H, H arom), 7.10-6.94 (m, 2H, CH=CH), 6.13 (d, $J = 14.9$ Hz, 1H, CH-CO), 5.56 (s, 1H, NH-CO), 3.56-3.51 (m, 3H, NHCH$_2$CH$_2$), 2.90 (t, $J = 5.8$ Hz, 2H, CH$_2$-CN), 2.72 (t, $J = 5.8$ Hz, 2H, CH$_2$-CCNH), 1.80 (bs, 4H, (CH$_2$)$_2$CH$_2$CN); 

$^{13}$C NMR (DMSO), $\delta$ (ppm): 165.82, 157.79, 150.12, 146.78, 139.39, 138.12, 136.17, 128.69, 128.49, 127.86, 126.87, 125.03, 123.23, 122.97, 119.93, 115.75, 48.19, 33.50, 24.97, 22.77, 22.43; 

m/z (ESI MS): 398.3 (M+H)$^+$. Elem. analysis calc: (C$_{26}$H$_{27}$N$_3$O.1H$_2$O.0.5Et$_2$O): C 74.31, H 7.57, N 9.28%; found: C 74.36, H 7.10, N 9.28%.

3-(Benzo[d][1,3]dioxol-5-yl)-N-(2-(1,2,3,4-tetrahydroacridin-9-ylamino)ethyl)acrylamide (7a): Compound 4a and 3,4-(methylenedioxy)cinnamic acid afforded the title compound as a yellow solid, after recrystallization from diethylether; yield: 32%; M.p. 89-91°C. 

$^1$H NMR (DMSO), $\delta$ (ppm): 8.12 (d, $J = 8.0$ Hz, 1H, CH-CCNH), 7.71 (d, $J = 8.4$Hz, 1H, CH-CN), 7.52 (t, $J = 7.6$ Hz, 1H, CH-CHCN), 7.40-7.31 (m, 2H, H arom), 7.15 (d, $J = 1.6$ Hz, 1H, CH-COCH$_3$), 7.07 (d, $J = 8.1$ Hz, 1H, CH=CHOCH$_3$), 6.94 (d, $J = 8.0$ Hz, 1H, CH-COCH$_3$), 6.43 (d, $J = 15.7$ Hz, 1H, CH-COH), 6.06 (s, 2H, OCH$_2$O), 5.60 (s, 1H, NH), 3.57-3.51 (m, 3H, NHCH$_2$CH$_2$NH), 2.89 (t, $J = 6.0$ Hz, 2H, CH$_2$-CN), 2.73 (t, $J = 5.9$ Hz, 2H, CH$_2$-CCNH), 1.84-1.79 (m, 4H, (CH$_2$)$_2$-CH$_2$CN); 

$^{13}$C NMR (DMSO), $\delta$ (ppm): 165.97, 150.1, 148.42, 147.85, 138.70, 129.13, 128.07, 127.89, 123.24, 122.99, 119.86, 115.75, 108.51, 106.14, 101.35, 48.14, 33.35, 24.88, 22.65, 22.31; m/z (ESI MS): 416.2 (M+H)$^+$, 417.2 (M+2H)$^{2+}$, 418.2 (M+3H)$^{3+}$. Elem. analysis calc: (C$_{26}$H$_{27}$N$_3$O.1H$_2$O.0.5Et$_2$O): C 70.74, H 6.52, N 9.44%; found: C 70.75, H 6.37, N 9.33%.

5-(Benzo[d][1,3]dioxol-5-yl)-N-(2-(1,2,3,4-tetrahydroacridin-9-ylamino)ethyl)penta-2,4-dienamide (8a): Compound 4a and 5-(1,3-benzodioxol-5-yl)-2,4-pentadienoic acid afforded the title compound as a yellow solid, after recrystallization with n-hexan; yield: 62%; M.p. 130-132 °C. 

$^1$H NMR (CDCl$_3$), $\delta$ (ppm): 7.95 (d, $J = 8.1$ Hz, 1H, CH=CCNH), 7.87 (d, $J = 8.3$ Hz, 1H, CH=CN), 7.55-7.51 (m, 1H, CH-CHCN), 7.41-7.31 (m, 2H, CH-CHCCNH, CH=CHCONH), 6.97 (bs, 1H, CH=COCH$_3$), 6.89 (dd, $J = 8.1$ Hz, 1.5 Hz, 1H, CH=CHCOCH$_3$), 6.81-6.77 (m, 2H, CH=CHCHCHCONH,
(3.4-Dimethoxyphenyl)-N-(2-(1,2,3,4-tetrahydroacridin-9-ylamino)ethyl)acrylamide (9a): Compound 4a and 3,4-(dimethoxy)cinnamic acid afforded the title compound as a yellow solid after recrystallization from n-hexan; yield: 46% M.p. 84-86 °C. 1H NMR (DMSO), δ (ppm): 8.20 (t, J = 5.7 Hz, 1H, CH-CHCN), 8.12 (d, J = 8.1 Hz, 1H, CH-CCNH), 7.54-7.50 (m, 1H, CH-CN), 7.37 (d, J = 15.6 Hz, 1H, CH=CHCO), 7.15-7.10 (m, 2H, H arom), 6.98 (d, J = Hz, 1H, CH-CHCOCH₃), 6.47 (d, J = 15.7 Hz, 1H, CH-CONH), 5.56 (s, 1H, NH), 3.78 (s, 6H, OCH₃), 3.56-3.52 (m, 2H, CH₂-NHC), 3.43-3.39 (m, 2H, CH₂-NHCO), 2.90-2.87 (m, 2H, CH₂-CN), 2.75-2.72 (m, 2H, CH₂-CCNH), 1.82-1.79 (m, 4H, (CH₂)₂CH₂-CN); ¹³C NMR (DMSO), δ (ppm): 165.99 (C=O), 157.86 (C=N), 150.10 (C-OCH₃), 148.80 (C-OCH₃), 148.84 (C-NH), 138.92 (CH=CHCO), 128.22 (C-CH_), 127.81, 127.53, 123.20, 122.95, 121.30, 119.50, 119.51, 115.75, 111.66, 109.93, 55.47, 55.35, 48.18, 33.45, 24.89, 22.68, 22.35; m/z (ESI-MS): 432.2 (M+H)+, 433.2 (M+2H)²⁺, 434.3 (M+3H)³⁺. Elem. Analysis calc: (C₂₅H₂₅N₃O₃·0.7H₂O): C 70.31, H 6.90, N 9.46%; found: C 70.34, H 7.08, N 9.19%.

General procedure for the synthesis of the tacrine–hybrid compounds (5b-9b): The synthesis of the compounds 5b-9b followed the same protocol used to obtain the compounds 5a-9a, but using N-(1,2,3,4-tetrahydroacridin-9-yl)propane-1,3-diamine (4b), instead of N-(1,2,3,4-tetrahydroacridin-9-yl)etane-1,2-diamine (4a).

N-(3-(1,2,3,4-tetrahydroacridin-9-ylamino)propyl)cinnamamide (5b): Compound 4b and cinnamic acid afforded the title compound as a solid light brown after recrystallization with n-hexan and diethylether; yield: 32%; M.p. 130-132 °C. 1H NMR (CDCl₃), δ (ppm): 8.04 (d, J = 8.5 Hz, 1H, CH=CCNH), 7.90 (d, J = 8.43 Hz, 1H, CH=CN), 7.66 (d, J = 15.6 Hz, 1H, CH=CHCO), 7.56-7.49 (m, 3H, H Arom), 7.37-7.33 (m, 4H, H Arom), 6.39 (J = 15.6 Hz, 1H, CH-CO), 4.87 (s, 1H, NH), 3.56-3.51 (m, 4H, NH-CH₂CH₂CH₃-NH), 3.06 (bs, 2H, CH₂-C=N), 2.78 (bs, 2H, CH₃-C=CNH), 1.91-1.83
(m, 6H, CH₂(CH₂)₂CH₂, NHCH₂CH₂CH₂NH); 13C NMR (CDCl₃), δ (ppm): 166.66, 158.49, 150.53, 147.19, 141.41, 134.66, 129.78, 128.82, 128.52, 128.34, 127.78, 123.85, 122.58, 120.31, 116.54, 45.40, 36.95, 33.92, 31.54, 25.14, 23.07, 22.75; m/z (ESI MS): 386.3 (M+H)⁺, 387.3 (M+2H)²⁺, 388.3 (M+3H)³⁺. Elem. analysis calc: (C₂₅H₂₇N₃O·0.4H₂O): C 76.52, H 7.13, N 10.71%; found: C 76.54, H 7.34, N 10.71%.

5-Phenyl-N-(3-(1,2,3,4-tetrahydroacridin-9-ylamino)propyl)pent-2,4-dienamide (6b): Compound 4b and 5-phenyl-2,4-pentadienoic acid afforded the title compound as a white solid after recrystallization with n-hexan and diethylether; yield: 30%; M.p. 138-141 °C. ¹H NMR (CDCl₃), δ (ppm): 8.04 (d, J = 8.4 Hz, 1H, CH=CCNH), 7.90 (d, J = 8.4 Hz, 1H, CH=CN), 7.53 (t, J = 7.57 Hz, 1H, CH=CHCN), 7.46-7.40 (m, 3H, H arom), 7.36-7.28 (m, 4H, H arom), 6.85-6.82 (m, 2H, H arom), 5.99 (d, J = 14.9 Hz, 1H, CH-CO), 4.95 (s, 1H, NH), 3.52-3.49 (m, 4H, NH-CH₂-CH₂-CH₂-NH), 3.05 (bs, 2H, CH₂-C=N), 2.76 (bs, 2H, CH₂-CCNH), 1.90-1.78 (m, 6H, (CH₂)₂CH₂CN), 1.86-1.81 (m, 2H, CH₂CH₂NHCO); ¹³C NMR (CDCl₃), δ (ppm): 167.04, 158.29, 150.67, 146.99, 141.27, 139.46, 136.16, 128.75, 128.38, 128.28, 126.98, 126.17, 123.82, 123.49, 122.64, 120.21, 116.32, 45.28, 36.78, 33.79, 31.51, 25.12, 23.07, 22.70; m/z (ESI-MS): 412.2 (M+H)⁺, 413.1 (M+2H)²⁺. Elem. analysis calc: (C₂₇H₂₉N₃O·1.2H₂O): C 74.88, H 7.31, N 9.70%; found: C 74.90, H 7.10, N 9.51%.

3-(Benzo[d][1,3]dioxol-5-yl)-N-(3-(1,2,3,4-tetrahydroacridin-9-ylamino)propyl)acrylamide (7b): Compound 4b and 3,4-(methylenedioxy)cinnamic acid afforded the title compound as a beige solid, after recrystallization with n-hexan and diethylether; yield: 51%; M.p. 166-168 °C. ¹H NMR (400 Hz, CDCl₃), δ (ppm): 8.04 (d, J = 8.3 Hz, 1H, CH-CCNH), 7.91 (d, J = 8.3 Hz, 1H, CH-CN), 7.53 (dd, J = 7.0 Hz, 1.5 Hz, 1H, CH=CHCONH), 7.53 (d, J = 15.4 Hz, 1H, CH=CHCCNH), 6.98-6.96 (m, 2H, CH=CH-COCH₂, CH=CHCOCH₂), 6.79 (d, J = 8.4 Hz, 1H, CH=COCH₂), 6.22 (d, J = 15.5 Hz, 1H, CH=CONH), 6.08 (s, 1H, NH), 5.98 (s, 2H, OCH₂O), 3.55-3.45 (m, 4H, NHCH₂CH₂CH₂NH), 3.05 (bs, 2H, CH₂-CN), 2.78 (bs, 2H, CH₂-CCNH), 1.90 (bs, 4H, (CH₃)₂CH₂CN), 1.86-1.81 (m, 2H, CH₂CH₂NHCO); ¹³C NMR (400 Hz, CDCl₃), δ (ppm): δ 167.0, 158.8, 150.8, 149.3, 148.4, 147.2, 141.4, 129.2, 128.6, 128.5, 124.1, 124.0, 122.8, 120.4, 118.4, 116.6, 108.7, 106.5, 101.6, 45.5, 37.1, 33.8, 31.7, 25.3, 23.1, 22.9; m/z (ESI-MS): 430.2 (M+H)⁺, 431.1 (M+2H)²⁺. Elem. analysis calc: (C₂₆H₂₇N₃O₃·0.2H₂O): C 72.01, H 6.38, N 9.69%; found: C 72.04, H 6.55, N 9.57%. 
5-(Benzo[d][1,3]dioxol-5-yl)-N-(3-(1,2,3,4-tetrahydroacridin-9-ylamino)propyl)penta-2,4-dienamide (8b): Compound 4b and 5-(1,3-benzodioxol-5-yl)-2,4-pentadienoic acid afforded the title compound as a yellow solid after recrystallization with diethylether; yield: 43%; M.p. 171-173 °C. ¹H NMR (400 Hz, CDCl₃), δ (ppm): 8.04 (d, J = 8.0 Hz, 1H, CH=CCNH), 7.91 (d, J = 8.0 Hz, 1H, CH=CN), 7.56-7.52 (m, 1H, CH-CHCN), 7.43-7.33 (m, 2H, CH-CHCCNH, CH=CHCONH), 6.96 (d, J = 1.6 Hz, 1H, CH-COCH₂), 6.88 (dd, J = 8.1 Hz, 1.6 Hz, 1H, CH-CHCOCH₂), 6.80-6.76 (m, 2H, CH-(CH)₂CONH, CH=COCH₂), 6.69-6.62 (m, 1H, CH-(CH)₂CONH), 6.05 (1s, 1H, NH), 5.97 (s, 2H, OCH₂O), 5.93 (d, J = 14.9 Hz, 1H, CH-CO), 3.54-3.45 (m, 4H, NHCH₂-CH₂-CH₂NH), 3.06 (bs, 2H, CH₂-CN), 2.77 (bs, 2H, CH₂-CCNH), 1.90 (bs, 4H, (CH₂)₂-CH₂CN), 1.83-1.79 (m, 2H, CH₂CH₂CH₂NHCO); ¹³C NMR (400 MHz, CDCl₃), δ(ppm): 167.13, 158.45, 150.94, 148.50, 148.39, 147.11, 141.68, 139.44, 130.91, 128.62, 128.43, 124.65, 124.04, 122.86, 122.83, 122.78, 120.36, 116.49, 108.67, 105.89, 101.49, 45.45, 36.96, 33.89, 31.72, 25.29, 23.22, 22.86; m/z (ESI-MS): 456.2 (M+H)+, 457.1 (M+2H)²⁺. Elem. analysis calc: (C₂₈H₂₉N₃O₃.1H₂O): C 70.93, H 6.60, N 8.86%; found: C 70.94, H 6.54, N 8.76%.

3-(3,4-Dimethoxyphenyl)-N-(3-(1,2,3,4-tetrahydroacridin-9-ylamino)propyl)acrilamide (9b): Compound 4b and 3,4-(dimethoxy)cinnamic acid afforded the product as a beige solid after recrystallization with diethylether and n-hexan; yield: 49%; M.p. 160-162 °C. ¹H NMR (400 Hz, MeOD), δ (ppm): δ 8.13 (d, J = 8.5 Hz, 1H, CH-CCNH), 7.76 (d, J = 9.1 Hz, 1H, CH-CN), 7.55-7.52 (m, 1H, CH-CHCN), 7.47 (d, J = 15.7 Hz, 1H, CH=CHCONH), 7.38-7.34 (m, 1H, CH-CHCCNH), 7.13-7.09 (m, 2H, CHCHCOCH₃, CHCOCH₃), 6.94 (d, J = 8.2 Hz, 1H,CH-COCH₃), 6.44 (d, J = 15.7 Hz, 1H, CH-CONH), 3.85 (s, 3H, OCH₃), 3.84 (s, 3H, OCH₃), 3.56 (t, J = 6.8 Hz, 2H, CH₃(CH₂)₂NHCO), 3.39 (t, J = 6.8 Hz, 2H, CH₃NHCO), 2.96 (bs, 2H, CH₂-CN), 2.78 (bs, 2H, CH₂-CCNH), 1.90 (bs, 4H, (CH₂)₂CH₂CN), 1.88-1.83 (m, 2H, CH₂CH₂CH₂-NHCO); ¹³C NMR (400 MHz, MeOD), δ (ppm): 169.25, 159.03, 153.05, 152.29, 150.57, 147.60, 141.83, 129.75, 129.30, 128.01, 124.91, 124.21, 123.22, 121.44, 119.46, 117.22, 112.72, 111.36, 56.46, 56.42, 46.60, 37.88, 34.21, 32.13, 26.36, 24.12, 23.69; m/z (ESI-MS): 446.2 (M+H)+, 447.1 (M+2H)³⁺. Elem. Analysis calc: (C₂₈H₂₉N₃O₅.1H₂O): C 72.04, H 7.04, N 9.36%; found: C 72.24, H 7.24, N 9.37%.

General procedure for the synthesis of the tacrine–hybrid compounds (5c-9c): For the synthesis of the compounds 5c-9c was followed a protocol identical to that used to
obtain the products 5a-9a, but using $N$-(1,2,3,4-tetrahydroacridin-9-yl)butane-1,4-diamine (4c), instead of $N$-(1,2,3,4-tetrahydroacridin-9-yl)etane-1,2-diamine (4a).

$N$-(4-(1,2,3,4-tetrahydroacridin-9-ylamino)butyl)cinnamamide (5c): Compound 4c and cinnamic acid afforded the title compound as a light brown solid after recrystallization with n-hexan; yield: 39%; $^1$H NMR (400 Hz, CDCl$_3$), $\delta$ (ppm): 7.92-7.91 (m, 2H, CH=C-CN, CH-CN), 7.62 (d, $J$ = 15.6 Hz, 1H, CH=CHCO), 7.53 (t, $J$ = 7.4 Hz, 1H, CH-CHCN), 7.48-7.45 (m, 2H, CH-CHCCNH, C=C-CH), 7.36-7.32 (m, 4H, H arom), 6.38 (d, $J$ = 15.6 Hz, 1H, CH-CO), 5.94 (s, 1H, NH), 3.54-3.51 (m, 2H, CH$_2$(CH$_2$)$_3$NHCO), 3.45-3.40 (m, 2H, (CH$_2$)$_3$CH$_2$NHCO), 3.05 (bs, 2H, CH$_2$-CN), 2.69 (bs, 2H, CH$_2$-CCNH), 1.89 (bs, 4H, CH$_2$-CH$_2$CN, CH$_2$-CH2CCNH), 1.76-1.65 (m, 4H, CH$_2$(CH$_2$)$_2$CH$_2$NHCO); $^{13}$C NMR (400 MHz, CDCl$_3$), $\delta$ (ppm): 166.18, 158.27, 150.92, 147.02, 141.23, 134.94, 129.85, 128.97, 128.74, 128.39, 127.93, 124.04, 122.91, 120.72, 120.17, 116.22, 49.03, 39.46, 33.79, 29.02, 27.36, 24.99, 23.13, 22.79; m/z (ESI-MS): 400.2 (M+H)$^+$, 401.3 (M+2H)$^{2+}$, 402.2 (M+3H)$^{3+}$. M.p. 155-157 °C. Elem. analysis calc: (C$_{26}$H$_{29}$N$_3$O).0.5H$_2$O): C 76.56, H 7.40, N 10.30%; found: C 76.58, H 7.45, N 10.21%.

5-Phenyl-$N$-(4-(1,2,3,4-tetrahydroacridin-9-ylamino)butyl)penta-2,4-dienamide (6c): Compound 4c and 5-phenyl-2,4-pentadienoic acid afforded the title compound as a light brown solid after recrystallization with diethylether; yield: 43%; M.p. 83-85 °C. $^1$H NMR (400 Hz, MeOD), $\delta$ (ppm): 8.10 (d, $J$ = 8.0 Hz, 1H, CH-CCNH), 7.76 (d, $J$ = 8.1 Hz, 1H, CH-CN), 7.57-7.52 (m, 1H, CH-CHCN), 7.50-7.48 (m, 2H, CH-CHCCNH, CH-CHCO), 7.39-7.26 (m, 5H, H arom), 6.96 (d, $J$ = 15.4 Hz, 1H, CH=CHCHCHCO), 7.92-7.90 (m, 1H, CH=CCNH), 7.92-7.90 (m, 1H, CH-CHCHCO), 6.07 (d, $J$ = 15.0 Hz, 1H, CH-CO), 3.57 (t, $J$ = 6.9 Hz, 2H, CH$_2$(CH$_3$)NHCO), 3.27 (t, $J$ = 6.9 Hz, 2H, (CH$_2$)$_3$CH$_2$NHCO), 2.97 (bs, 2H, CH$_2$-CN), 2.75 (bs, 2H, CH$_2$-CCNH), 1.90 (bs, 4H, (CH$_2$)$_2$CH$_2$CN), 1.68-1.57 (m, 4H, (CH$_2$)$_2$CH$_2$NHCO); $^{13}$C NMR (400 MHz, MeOD), $\delta$ (ppm): 167.42, 159.23, 153.13, 147.93, 141.94, 140.41, 137.83, 129.83, 129.77, 128.10, 128.0, 127.64, 125.05, 124.85, 124.34, 121.44, 117.09, 40.18, 34.22, 29.66, 27.93, 26.24, 24.13, 23.71; m/z (ESI-MS): 426,3 (M+H)$^+$, 427.2 (M+2H)$^{2+}$, 428.2 (M+3H)$^{3+}$. M.p. 155-157 °C. Elem. analysis calc: (C$_{28}$H$_{31}$N$_3$O.0.6H$_2$O): C 77.07, H 7.44, N 9.63%; found: C 77.07, H 7.57, N 9.68%.

3-(Benzo[d][1,3]dioxol-5-yl)-$N$-(4-(1,2,3,4-tetrahydroacridin-9-ylamino)butyl)acrylamide (7c): Compound 4c and 3,4-(methylenedioxy)cinnamic acid afforded the title compound as a solid light brown, after recrystallization with diethylether; yield: 33%; M.p. 163-165 °C. $^1$H NMR (400 Hz, CDCl$_3$), $\delta$ (ppm): 7.94-
7.89 (m, 2H, CH-CCNH, CH-CN), 7.55-7.50 (m, 2H CH=CHCONH, CH-CN), 7.32 (t, J = 7.5 Hz, 1H, CH-CHCCNH), 6.95-6.93 (m, 2H, CH-COCH$_3$, CH-CHCOCH$_3$), 6.77 (d, J = 8.5 Hz, 1H, CH-COCH$_3$), 6.18 (d, J = 15.5 Hz, 1H, CH-CONH), 5.98 (s, 2H, OCH$_3$), 5.88 (s, 1H, NH), 3.99 (s, 1H, NH), 3.49-3.47 (bs, 2H, CH$_2$(CH$_2$)$_2$NHCO), 3.43-3.38 (m, 2H, CH$_2$NHCO), 3.04 (bs, 2H, CH$_2$-CN), 2.70 (bs, 2H, CH$_2$-CCNH), 1.89 (bs, 4H, (CH$_2$)$_2$CH$_2$CN), 1.75-1.63 (m, 4H, CH$_2$(CH$_2$)$_2$NHCO); $^{13}$C NMR (400 MHz, CDCl$_3$), $\delta$ (ppm): 166.31, 158.67, 150.66, 149.21, 148.37, 147.29, 140.93, 129.32, 128.85, 128.51, 123.99, 123.95, 122.84, 120.46, 118.71, 116.48, 108.67, 106.43, 101.58, 49.09, 39.47, 34.16, 29.19, 27.44, 25.02, 23.18, 22.91; m/z (ESI-MS): 444.2 (M+H)$^+$, 445.2 (M+2H)$^{2+}$, 446.2 (M+3H)$^{3+}$. Elem. analysis calc: (C$_{27}$H$_{29}$N$_3$O$_3$·0.2H$_2$O): C 72.55, H 6.63, N 9.40%; found: C 72.55, H 6.74, N 9.42%.

3-(3,4-Dimethoxyphenyl)-N-(4-(1,2,3,4-tetrahydroacridin-9-ylamino)butyl)acrylamide (9c): Compound 4c and 3,4-(dimethoxy)cinnamic acid afforded the title compound as a light brown solid, after recrystallization with diethylether; yield: 60%; M.p. 142-144 °C. $^1$H NMR (400 Hz, CDCl$_3$), $\delta$ (ppm): 7.94-7.89 (m, 2H, CH-CCNH, CH-CN), 7.57-7.52 (m, 2H, CH=CHCONH, CH-CN), 7.33 (t, J = 7.1 Hz, 1H, CH-CHCCNH), 7.05 (dd, J = 8.3 Hz, 1.8 Hz, 1H, CH-CHCOCH$_3$), 6.99-6.97 (m, 1H, CH$_2$-CONH), 6.82 (d, J = 8.3 Hz, 1H, CH$_2$-CONH), 6.23 (d, J = 15.5 Hz, 1H, CH-CONH), 5.86 (s, 1H, NH), 3.98 (s, 1H, NH), 3.89 (s, 3H, OCH$_3$), 3.87 (s, 3H, OCH$_3$), 3.52-3.47 (m, 2H, CH$_2$-NHCO), 3.44-3.39 (m, 2H, CH$_2$-NHCO), 3.04 (bs, 2H, CH$_2$-CCNH), 2.70 (sa, 2H, CH$_2$-CCNH), 1.89 (bs, 4H, (CH$_2$)$_2$CH$_2$CN), 1.73-1.63 (m, 4H, (CH$_2$)$_2$CH$_2$NHCO); $^{13}$C NMR (400 MHz, CDCl$_3$), $\delta$ (ppm): 166.66, 158.79, 150.73, 150.60, 149.26, 147.57, 141.04, 128.90, 128.47, 127.89, 123.96, 122.83, 122.10, 120.51, 118.60, 116.56, 111.23, 109.79, 56.10, 56.01, 49.08, 39.44, 34.20, 29.18, 27.45, 25.03, 23.18, 22.92; m/z (ESI-MS): 444.2 (M+H)$^+$, 445.2 (M+2H)$^{2+}$, 446.2 (M+3H)$^{3+}$. Elem. analysis calc.: (C$_{28}$H$_{33}$N$_3$O$_3$·0.05H$_2$O): C 73.05, H 7.25, N 9.12%; found: C 73.03, H 7.51, N 9.20%.

3-(3,4-Dihydroxyphenyl)-N-(2-(1,2,3,4-tetrahydroacridin-9-ylamino)ethyl)acrylamide (10a): A mixture of 7a (0.1130 g, 0.272 mmol) and n-Bu$_4$NI (3 equiv) in dry CH$_2$Cl$_2$ (15 mL) was stirred under N$_2$ atmosphere and cooled to -78 °C. To this solution was dropwise added an excess of a solution 1M BCl$_3$ in CH$_2$Cl$_2$ (ca 8 equiv). After ca 5 min the solution was allowed to warm to room temperature and stirred for 2 h. The reaction was quenched with ice water and it was left to stir for 30 min. A
precipitated was formed and the product was recrystallized from CH$_3$CN/Et$_2$O to afford the pure title as a yellow solid; yield: 83%; M.p. 196-198 °C. $^1$H NMR (400 Hz, MeOD) δ (ppm): 8.48 (d, $J = 8.7$ Hz, 1H, CH-CCNH), 7.86 (t, $J = 7.3$ Hz, 1H, CH-CHCN), 7.73 (d, 1H, $J = 7.6$ Hz, CH-CN), 7.60 (t, $J = 7.9$ Hz, 1H, CH-CHCCNH), 7.37 (d, $J = 15.6$ Hz, 1H, CH-CHCO), 6.83 (s, 1H, CH-COH), 6.88 (dd, $J = 8.2$, 2.0 Hz, 1H, CH-COH), 6.75 (d, $J = 8.2$ Hz, 1H, CH-CHCOH), 6.30 (d, $J = 15.6$ Hz, 1H, CH-CO), 4.16 (t, $J = 5.5$ Hz, 2H, CH$_2$-NHC) 3.70 (t, $J = 5.4$ Hz, 2H, CH$_2$-NHCO), 2.97 (t, $J = 5.9$ Hz, 2H, CH$_2$-CN), 2.75 (t, $J = 5.7$ Hz, 2H, CH$_2$-CCNH), 1.95 (m, 4H, CH$_3$CH$_2$-CH$_2$CN). $^{13}$C NMR (400 Hz, MeOD) δ (ppm): 170.77, 158.56, 149.10, 146.82, 143.09, 139.73, 134.08, 127.93, 126.65, 126.43, 122.33, 120.01, 117.42, 117.17, 116.47, 116.23, 115.03, 113.29, 50.80, 40.85, 29.30, 25.07, 22.99, 21.83; m/z (ESI-MS) 404.6 (M+H)$^+$. Elem. analysis calc: (C$_{24}$H$_{25}$N$_3$O$_3$·0.3Et$_2$O): C 71.08, H 6.25, N 10.41%; found: C 71.12, H 6.33, N 9.60%.

5-(3,4-dihydroxyphenyl)-N-(2-(1,2,3,4-tetrahydroacridin-9-ylamino)ethyl)penta-2,4-dienamide (11a): Using the procedure mentioned for 10a, but with compound 8a, the titled compound was obtained as a yellow solid; yield: 61%; M.p. 192-194 °C. $^1$H NMR (400 Hz, MeOD) δ (ppm): 8.50 (d, $J = 8.7$ Hz, 1H, CH-CCNH), 7.86 (t, $J = 7.7$ Hz, 1H, CH-CHCN), 7.77 (d, $J = 8.4$ Hz, 1H, CH-CN), 7.31 (dd, $J = 15.7$, 10.1 Hz, 1H, CH-CHCO), 6.97 (s, 1H, CH-COH), 6.87 (d, $J = 8.2$ Hz, 1H, CH-CO), 6.87-6.73 (m, 3H, CH-CHCCHCOH, CH-CCHCOH, CH-CHCOH), 6.02 (d, $J = 15.7$ Hz, 1H, CH-CO), 4.15 (t, $J = 5.5$ Hz, 2H, CH$_2$-NHC) 3.69 (t, $J = 5.5$ Hz, 2H, CH$_2$-NHCO), 3.00 (bs, 2H, CH$_2$-CN), 2.76 (bs, 2H, CH$_2$-CCNH), 1.96 (bs, 4H, CH$_2$CH$_2$-CH$_2$CN). $^{13}$C NMR (400 Hz, MeOD) δ (ppm): 170.5, 158.4, 148.1, 146.7, 143.4, 141.7, 139.7, 134.1, 129.8, 126.5, 124.5, 122.4, 121.3, 120.1, 116.6, 114.5, 113.2, 50.8, 40.77, 29.3, 24.9, 23.0, 21.8; m/z (ESI-MS) 430.4 (M+H)$^+$. Elem. analysis calc: (C$_{26}$H$_{27}$N$_3$O$_3$·0.6H$_2$O): C 70.92, H 6.46, N 9.54%; found: C 70.73, H 6.31, N 9.71%.

3-(3,4-Dihydroxyphenyl)-N-(3-(1,2,3,4-tetrahydroacridin-9-ylamino)propyl)acrylamide (10b): Using the procedure mentioned for 10a, but with compound 7b, the titled compound obtained as a pale green solid; yield: 86%; M.p. 152-154 °C. $^1$H NMR (400 Hz, MeOD) δ (ppm): 8.38 (d, $J = 8.6$ Hz, 1H, CH-CCNH), 7.81 (t, $J = 7.6$ Hz, 1H, CH-CHCN), 7.72 (d, $J = 8.4$ Hz, 1H, CH-CN), 7.56 (t, $J = 7.7$ Hz, 1H, CH-CHCCNH), 7.3 (d, $J = 15.6$ Hz, 1H, CH=CHCO), 6.97 (s, 1H, CH=COH), 6.87 (d, $J = 8.2$ Hz, 1H, CH=COH).
1H, CH-CO), 4.02 (t, J = 6.3 Hz, 2H, CH$_2$-NHC), 3.44 (t, J = 6.1 Hz, 2H, CH$_2$-NHCO), 2.97 (bs, 2H, CH$_2$-CN), 2.75 (bs, 2H, CH$_2$-CCNH), 2.05 (t, J = 6.2 Hz, 2H, CH$_2$-CH$_2$-NHC), 1.95 (bs, 4H, CH$_2$- CH$_2$-CH$_2$-CN). $^{13}$C NMR (400 Hz, MeOD) δ (ppm): 169.77, 158.17, 151.69, 148.94, 146.79, 142.48, 139.74, 134.03, 128.07, 126.36, 122.20, 120.05, 117.88, 117.29, 117.08, 116.66, 116.45, 114.98, 113.04, 37.36, 31.61, 29.29, 24.99, 22.98, 21.79; m/z (ESI-MS) 418.4 (M+H)$^+$. Elem. analysis calc.: (C$_{25}$H$_{27}$N$_3$O$_3$): C 71.92, H 6.52, N 10.06%; found: C 71.85, H 6.49, N 9.86%.

5-(3,4-Dihydroxyphenyl)-N-(3-(1,2,3,4-tetrahydroacridin-9-ylamino)propyl)penta-2,4-dienamide (11b): Using the procedure above for 10a, but with compound 8b, the pure title compound was obtained as a pale green solid; yield: 68%; M.p. 155-157 °C. $^1$H NMR (400 Hz, MeOD) δ (ppm): 8.35 (d, J = 8.7 Hz, CH-CCNH), 7.82 (t, J = 7.7 Hz, 1H, CH-CHCN), 7.73 (d, J = 8.1 Hz, 1H, CH-CN), 7.56 (t, J = 7.5 Hz, 1H, CH-CHCCNH), 7.21 (dd, J = 15.3, 9.2 Hz, 1H, CH=CHC), 6.91 (s, 1H, CH=COH), 6.81 (d, J = 8.1 Hz, 1H, CH=COH), 6.72 - 6.67 (m, 3H, CH-CH-CCH-CHCOH, ), 6.00 (d, J = 15.0 Hz, 1H, CH=CO), 3.99 (t, J = 6.5 Hz, 2H, CH$_2$-NHC), 3.43 (t, J = 6.2 Hz, 2H, CH$_2$-NHCO), 2.99 (bs, 2H, CH$_2$-CN), 2.73 (bs, 2H, CH$_2$-CCNH), 2.04 (t, J = 6.3 Hz, 2H, CH$_2$-CH$_2$NHCO), 1.95 (bs, 4H, CH$_2$- CH$_2$-CH$_2$-CN). $^{13}$C NMR (400 Hz, MeOD) δ (ppm): 169.72, 158.13, 158.65, 151.65, 147.94, 146.64, 142.93, 141.39, 139.74, 135.26, 134.03, 129.85, 126.39, 124.50, 122.66, 121.66, 120.08, 117.07, 116.44, 114.35, 113.04, 46.17, 37.32, 31.63, 29.30, 24.80, 21.79; m/z (ESI-MS) 444.4 (M+H)$^+$, 445.4 (M+H)$^{2+}$, 446.6 (M+3H)$^{3+}$. Elem. analysis calc.: (C$_{27}$H$_{29}$N$_3$O$_3$·H$_2$O): C 72.50, H 6.63, N 9.39%; found: C 72.50, H 6.45, N 9.31%.

**Molecular Modeling**

The strategy followed for the design of new inhibitors of AChE consisted in the pre-selection of molecules whose biological activity is known and the choice of suitable spacers to connect these two entities. The two main molecular entities chosen were tacrine (for providing inhibitory activity towards AChE) and derivatives of cinnamic acid or the correspondent 5-phenyl-2,4-pentadienoic acid (for providing antioxidant activity (Scheme 1). A virtual screening was then performed through a molecular docking study. It was necessary to retrieve the X-ray structure of AChE from RCSB Protein Data Bank with PDB code 1ODC, corresponding to the Torpedo californica variant (TcAChE).$^2$ The solvent and the co-crystalization molecules were removed and the hydrogen atoms were added with software Maestro v.9.3.$^3$ The new ligands were constructed with Maestro v.9.3
and submitted to Ghemical v.2.0 software, for geometry optimization and random conformational search of 1000 cycles and 2500 steps of optimization using Tripos 5.2 force field. The conformation with the lowest energy was saved and it was docked into the previously saved AChE structure, using GOLD program v.5.1.

The region of interest was defined in order to contain the residues within 10 Å from the position of the original ligand. The GOLD default parameters were used, and the ligands submitted to 100 genetic algorithm runs using the ASP scoring function. This scoring function has previously revealed, among the ones supplied by Gold (CHEMPLP, GoldScore, ChemScore e ASP), to give the best prediction with AChE inhibitors.

**Pharmacokinetic Properties**

To evaluate the potential of the new compounds as new-AD drugs some indicators of their pharmacokinetic profiles were calculated using QikProp v.2.5 program. The new ligands were built and minimized as previously mentioned for the docking studies. The structures were submitted to the calculations and parameters such as lipo-hydrophilic character (c log P), blood brain partition coefficient (log BB), the ability to be absorbed through the intestinal tract (Caco-2 cell permeability) and CNS activity were calculated. These predictions are for orally delivered drugs and assume nonactive transport.

**AChE inhibition**

The determination of the enzymatic activity was measured using an adaptation of Ellman’s method. The buffer solution used in this enzymatic assay was 2-[4-(2-hydroxyethyl)-1-piperazine]ethanesulfonic acid (HEPES) at 50 mM and pH 8. The Ellman reagent 5,5’-dithiobis-(2-nitrobenzoic acid) (DTNB, 3 mM) was prepared in HEPES buffer containing NaCl (50 mM) and MgCl₂ (20 mM). Acetylcholinesterase stock solution was prepared by dissolving 500 U (extracted from Electrophorus electricus and purchased from Sigma-Aldrich Type VI-S, lyophilized powder 500 units/mg protein) in TRIS buffer (50 mM, pH 8) (10 mL). The enzyme was later diluted from 5 U/mL with HEPES buffer to give the final AChE concentration conditions in the assay. An aqueous solution of the enzyme substract, acetylthiocholine iodide (AChI) 16 mM, was also prepared. Stock solutions of the tested compounds were prepared in methanol (1 mg/mL), due to their hydrophobic nature, and five different concentrations were used in order to obtain AChE inhibition ranging between 20 and 80%.
The final assay solution consisted in the addition of: 374 µL of HEPES (50 mM, pH 8), 476 µL of DTNB; a variable volume (10, 20, 30, 40 or 50 µL) of inhibitor solution, 25 µL of the AChE solution and the necessary amount of methanol to attain 0.925 mL of sample mixture in a 1 mL cuvette. The samples were left to incubate for 15 min. Subsequently, 75 µL of substrate solution was added. The initial rate of the enzymatic reaction was monitored by reading the solution absorbance at 405 nm, recorded on a Perkin-Elmer Lambda 35 UV-Vis spectrophotometer, during the first 5 min of the reaction time. Assays were also run with a blank solution containing all the components except the AChE solution (that was replaced by buffer HEPES), to account for the non-enzymatic reactions. The velocities of the reaction were calculated, as well as the enzyme activity. A control reaction was carried out using the sample solvent (methanol, 50 µL) in the absence of any tested compound and it was considered as 100% activity. Each concentration was analyzed in quintuplicate. The percent inhibition of the enzyme activity due to the presence of increasing test compound concentration was calculated by the following equation (1),

$$\%I = 100 - \left( \frac{v_i}{v_0} \times 100 \right)$$  \hspace{1cm} (1)

in which $v_i$ is the initial reaction rate in the presence of inhibitor, and $v_0$ is the initial rate of the control reaction. The inhibition curves were obtained by plotting the percentage of enzymatic inhibition vs. inhibitor concentration and a calibration curve was achieved from which the linear regression parameters were obtained. Data presented are the average of three independent experiments ± SD.

**Antioxidant Activity**

The antioxidant activity was determined by DPPH method previously described. The method consists in the addition, to a 2.5 mL of DPPH solution (0.002 M), of four samples of compound solution with different volumes. The volume required for the final volume of 3.5 mL was attained with methanol. The mixtures were left to incubate for 30 min at room temperature. The absorbance was measured at 517 nm against the corresponding blank. The control solution corresponds to the sample with only DPPH and methanol. The antioxidant activity was calculated using equation (2), in which
\[
\%AA = \left( \frac{A_{DPPH} - A_{sample}}{A_{DPPH}} \right) \times 100 \tag{2}
\]

\%AA is the antioxidant activity, \(A_{DPPH}\) is the absorbance of the DPPH solution (control solution) against the blank and \(A_{sample}\) is the absorbance of the sample against the blank. The compound concentrations provided 50% of antioxidant activity (EC\(_{50}\)) by plotting the anti-oxidant activity against the compound concentration.

**Inhibition of self-mediated Ab\(_{42}\) aggregation**

Aβ-peptide (1–42) (Aβ\(_{42}\)) was purchased from Aldrich as a lyophilized powder and stored at -20 °C. The samples were treated with 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP) to avoid self-aggregation and reserved. HFIP pre-treated Aβ\(_{42}\) samples were re-solubilized with a CH\(_3\)CN/Na\(_2\)CO\(_3\)/(300 μM)/NaOH (250 μM) (48.3:48.3:4.3, v/v/v) solvent mixture in order to have a stable stock solution. This Aβ\(_{42}\) alkaline solution (500 μM) was diluted in phosphate buffer (0.215 M, pH 8.0) to obtain a 80 μM solution. Compounds under study were firstly dissolved in methanol (1 mg/mL) due to their lipophilic nature, and then further diluted in phosphate buffer to a final concentration of 480 μM. To study the inhibition of Aβ\(_{42}\) aggregation, a reported method was followed, based on the fluorescence emission of thioflavin T (ThT).\(^{11,12}\) Firstly, Aβ\(_{42}\) solution samples (30 μL) and the compound solution (10 μL) were diluted with the phosphate buffer to a final concentration of 40 μM (Aβ) and 80 μM (compounds), and then were incubated for 24 h at 37 °C, without stirring. As for the control, a sample of the peptide was incubated under identical conditions but without the inhibitor. After incubation, the samples were added to a 96-well plate with 180 μL of 5 μM ThT in 50 mM glycine–NaOH (pH 8.5). Blank samples were prepared for each concentration in a similar way, devoid of peptide. After 5 min incubation with the dye (ThT), the fluorescence of all samples and control solutions was measured using a Cary Eclipse Fluorescence spectrophotometer at the following wavelengths: excitation (446 nm) and emission (490 nm). These experimental conditions were based on a preliminary time scan study of the ThT fluorescence spectrum (\(\lambda_{exc} = 446 \text{ nm}\)) which showed that the intensity of the fluorescence signal at maximum emission wavelength (490 nm) increased under a sigmoidal type behavior, reaching a plateau maximum after 5 min. Furthermore, within this working wavelength window, the compounds under study do not present any absorption (as shown Supplementary Figure...
2), thus with no interference on the ThT fluorescence signal. The percent inhibition of the self-induced aggregation due to the presence of the test compound was calculated on basis of Eq. (3), in which \( IF_i \) and \( IF_0 \) correspond to the fluorescence intensities, in the presence and absence of the test compound, respectively, minus the fluorescence intensities due to the respective blanks.

\[
\%I = 100 - \left( \frac{IF_i}{IF_0} \times 100 \right)
\]

(3)

**Cell treatments and viability**

The compounds from 5a to 11b were dissolved in DMSO. Neuroblastoma cells (SH-SY5Y) were incubated with these compounds, at final concentrations of 1.5, 2.5 and 5\( \mu \)M, for 25h at 37\(^\circ\)C. DMSO final concentration within the culture media was lower than 0.01% (v/v), which does not affects cell proliferation. Cell viability was evaluated by the quantitative colorimetric assay with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) according to the Mosmann method.\(^\text{13}\) In short, after incubation, the medium was replaced by MTT solution (0.5 mg mL\(^{-1}\) in sodium medium) and cells were incubated for 2 h, at 37 \(^\circ\)C. The resulting precipitates were solubilized with equal volume of acidic isopropanol (0.04 M HCl-isopropanol). The absorbance at 570 nm was measured by a Spectramax Plus 384 spectrophotometer (Molecular Devices). Units are expressed as percentage relatively to control (untreated cells).

The highest non-toxic concentration tested for each compound was selected to determine its protective effect against \( \text{A}_\beta_{42} \) peptides. \( \text{H}_2\text{O}_2 \) is able to cross the cell membrane\(^\text{14}\) and generate exogenous free radicals\(^\text{14,15}\), that lead to mitochondrial membrane depolarization, increasing the ROS production, and ultimately to cell death.\(^\text{16}\) Indeed, \( \text{H}_2\text{O}_2 \) induces intracellular ROS in SH-SY5Y cells,\(^\text{14,17}\) and for that reason compounds may act as direct antioxidants.

Therefore, cells were pre-incubated with the compounds during 1 h; after this time \( \text{A}_\beta_{42} \) (1 \( \mu \)M) and \( \text{H}_2\text{O}_2 \) (50 \( \mu \)M) were added for more 24 h at 37 \(^\circ\)C. For all the conditions tested, control experiments were performed.
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$^a$ Predicted values using program QikProp v.2.5 $^8$. $^b$ Calculated octanol/water partition coefficient. $^c$ Brain/blood partition coefficient.
Supplementary Figure 1. Docking results for the tacrine-cinnamate hybrids with TCACHe: superimposition of **8b** (purple) and **11b** (orange). H-bonds are represented as solid black lines.
Supplementary Figure 2. Absorption UV-Vis spectra registered for compound 8b at 2.05 ×10^{-5} M in metanol (blue) and in a 50% metanol-water mixture (pink).
**Supplementary Figure 3.** Dose-response effect of the hybrid compounds on cell viability. SH-SY5Y cells were incubated with each compound at final concentration per well of 1.5, 2.5 and 5 µM, for 25 h at 37 ºC. Cell viability was evaluated by MTT reduction assay. Results are expressed as percentage relatively to the control, with a mean ± SEM derived from 3-9 different experiments. *p < 0.05, **p < 0.01***p < 0.001, significantly different when compared to the control.
Novel tacrine-cinnamate and tacrine–cinnamylidenacetate as multitargeted compounds in view of AD drugs: anti-AChE and anti-oxidant activity as well as cell neuroprotection.

**Abbreviations**

Aβ, beta-amyloid; Aβ_{42}, amyloid-β peptide fragment 1-42; ACh, acetylcholine; AChE, acetylcholinesterase; AChI, acetylthiocholine iodide; AD, Alzheimer’s disease; BBB, blood–brain barrier; CAS, catalytic anionic site; CNS, central nervous system; DHE, dihydroethidium; DMSO, dimethylsulfoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; DTNB, 5,5’-dithiobis(2-nitrobenzoic acid); HEPES, 2-[4-(2-hydroxyethyl)-1-piperazine]ethanesulfonic acid; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PAS, peripheral anionic site; SH-SY5Y, human dopaminergic neuroblastoma cell line; T3P, 2,4,6-Tripropyl-1,3,5,2,4,6-trioxatriphosphorinane-2,4,6-trioxide; ThT, thioflavin T; TRIS, tris(hydroxymethyl)aminomethane.

**References**


2. [PDB, entry 1ODC](http://www.rcsb.org/pdb/explore/explore.do?structureId=1ODC).


