Supporting Information

Synthesis of novel 1,2,3-triazole-dihydro[3,2-*c*]chromenones as acetylcholinesterase inhibitors

Mina Saeedi, Shirin Ansari, Mohammad Mahdavi, Reyhaneh Sabourian, Tahmineh Akbarzadeh, Alireza Foroumadi, and Abbas Shafiee

Synthesis of 4-(prop-2-yn-1-yloxy)benzaldehyde 3: A solution of 4-hydroxybenzaldehyde **1** (1 mmol) and propargyl bromide **2** (1 mmol) in DMF (8 mL) was heated at 80 °C for 12 h. After completion of reaction, the precipitated product was filtered off, washed with water, dried at 50 °C, and used for further reaction.

Synthesis of 2-amino-5-oxo-4-(4-(prop-2-yn-1-yloxy)phenyl)-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile 6: A solution of 4-(prop-2-yn-1-yloxy)benzaldehyde **3** (1 mmol), 4-hydroxycoumarine **4** (1 mmol), malonitrile **5** (1 mmol), and DBU (1 mmol) was heated at room temperature for 7 h. After completion of reaction, the precipitated product was filtered off, washed with cold ethanol, dried at 50 °C, and used for further reaction.

Synthesis of azide derivatives 9: A mixture of benzyl bromide or chloride derivative **7** (1 mmol) and sodium azide **8** (1 mmol) was stirred at 80 °C for 3-5 h. After completion of reaction, the precipitated product was filtered off, washed with water, dried at 50 °C, and used for the next reaction.

Synthesis of 1,2,3-triazole-dihydro[3,2-*c*]chromenone derivatives 10: General Procedure: A solution of 2-amino-5-oxo-4-(4-(prop-2-yn-1-yloxy)phenyl)-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile **6** (1 mmol), azide derivative **9** (1 mmol), and CuI (5 mol%) in DMF (8 mL) was stirred at 80 °C for 4-5 h. After completion of reaction, the mixture was cooled down to room temperature. Then, water (25 mL) was added to the mixture and the organic phase was extracted using ethyl acetate (3 × 25). It was dried over Na₂SO₄ and the solvent was evaporated under vacuum. Crude product was recrystallized from ethanol to give pure product **10**.

2-Amino-4-(4-((1-(4-methylbenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-oxo-4*H*,5*H*-pyrano[3,2-*c*]chromene-3-carbonitrile (10a).

Yield: 80%, white crystals, mp 230-232 °C. IR (KBr): 3351, 3320, 3079, 2955, 2197, 1715, 1673, 1606, 1507 cm^{-1} . ^1H NMR (500 MHz: DMSO- d_6): 2.26 (s, 3H, CH₃), 4.40 (s, 1H, H₄), 5.11 (s, 2H, CH₂), 5.71 (s, 2H, CH₂), 6.95 (d, J = 8.8 Hz, 2H, H_{3'}, H_{5'}), 7.11-7.22 (m, 6H, H_{2'}, H_{6'}, H_{2''}, H_{3''}, H_{5''}, H_{6''}), 7.39 (s, 2H, NH₂), 7.45 (d, J = 8.0 Hz, 1H, H₇), 7.48 (t, J = 8.0 Hz, 1H, H₉), 7.70 (td, J = 8.0, 1.5 Hz, 1H, H₈), 7.89 (d, J = 8.0 Hz, 1H, H₁₀), 8.25 (s, 1H, triazole). ^{13}C NMR (125 MHz: DMSO- d_6): 20.7 (Me), 36.2 (C₄), 52.7 (benzyl CH₂), 58.2 (C₃), 61.1 (OCH₂), 104.3 (4*H*-pyran), 113.0 (C_{3'} and 5'), 114.6 (coumarin), 116.6 (coumarin), 119.4 (CN), 122.5 (triazole), 124.6 (Ar), 124.7 (Ar), 128.1 (Ar), 128.8 (Ar), 129.4 (Ar), 132.9 (Ar), 133.0 (Ar), 135.8 (Ar), 137.6 (Ar), 143.0 (triazole), 152.1 (C_{4'}), 153.2 (coumarin, C=O), 157.2 (C₂), 158.0 (4*H*-pyran, C=O), 159.6 (C=O (C₅)). Anal. calcd. for C₃₀H₂₃N₅O₄: C, 69.62; H, 4.48; N, 13.53. Found: C, 69.80; H, 4.32; N, 13.71.

2-Amino-4-(4-((1-(2-methylbenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-oxo-4*H*,5*H*-pyrano[3,2-*c*]chromene-3-carbonitrile (10b)

Yield: 75%, white crystals, mp 207-209 °C. IR (KBr): 3351, 3320, 2909, 2197, 1714, 1675, 1606 cm^{-1} . ^1H NMR (500 MHz: DMSO- d_6): 2.29 (s, 3H, CH₃), 4.40 (s, 1H, H₄), 5.10 (s, 2H, CH₂), 5.54 (s, 2H, CH₂), 5.88 (s, 1H, H₄), 6.60 (d, J = 8.0 Hz, 1H, H_{4''}), 6.66 (t, J = 8.0 Hz, 1H, H_{5''}), 7.20 (t, J = 7.7 Hz, 1H, H₉), 7.19-7.37 (m, 9H, H_{2'}, H_{3'}, H_{5'}, H_{6'}, H_{6''}, H₇, H₈, NH₂), 7.66 (dd, J = 7.7 Hz, 1H, H₁₀), 8.05 (s, 1H, triazole). Anal. calcd. for C₃₀H₂₃N₅O₄: C, 69.62; H, 4.48; N, 13.53. Found: C, 69.51; H, 4.50; N, 13.68.

2-Amino-4-(4-((1-(2-chlorobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-oxo-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile (10c)

Yield: 82%, white crystals, mp 142-144 °C. IR (KBr): 3318, 3184, 2195, 1719, 1673, 1605 cm^{-1} . ^1H NMR (500 MHz: DMSO- d_6): 4.40 (s, 1H, H₄), 5.11 (s, 2H, CH₂), 5.70 (s, 2H, CH₂), 6.96 (d, J = 8.4 Hz, 2H, H_{3'}, H_{5'}), 7.17-7.21 (m, 3H, H_{2'}, H_{6'}, H_{6''}), 7.33-7.38 (m, 4H, H_{4''},

H5", NH₂), 7.44-7.50 (m, 3H, H7, H9, H3"), 7.70 (t, *J* = 7.8 Hz, 1H, H8), 7.89 (d, *J* = 7.8 Hz, 1H, H10), 8.24 (s, 1H, triazole). ¹³C NMR (125 MHz: DMSO-*d*₆): 36.1 (C4), 50.5 (benzyl CH₂), 58.0 (C3), 60.9 (OCH₂), 104.1 (4*H*-pyran), 113.0 (C3' and 5'), 114.5 (coumarin), 116.5 (coumarin), 119.2 (CN), 122.4 (triazole), 124.6 (Ar), 125.0 (Ar), 127.6 (Ar), 128.7 (Ar), 129.5 (Ar), 130.1 (Ar), 130.4 (Ar), 132.5 (Ar), 132.8 (Ar), 133.1 (Ar), 135.7 (Ar), 142.8 (triazole), 152.0 (C4'), 153.0 (coumarin, C-O), 157.0 (C2), 157.8 (4*H*-pyran, C-O), 159.4 (C=O (C5)). Anal. calcd. for C₂₉H₂₀ClN₅O₄: C, 64.75; H, 3.75; N, 13.02. Found: C, 64.87; H, 3.59; N, 12.87.

2-Amino-4-(4-((1-(2,3-dichlorobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-oxo-4*H*,5*H*-pyrano[3,2-*c*]chromene-3-carbonitrile (10d)

Yield: 77%, white crystals, mp 136-137 °C. IR (KBr): 3321, 3184, 2909, 2195, 1719, 1672, 1605 cm⁻¹. ¹H NMR (500 MHz: DMSO-*d*₆): 4.36 (s, 1H, H4), 5.08 (s, 2H, CH₂), 5.72 (s, 2H, CH₂), 6.95 (d, *J* = 8.5 Hz, 2H, H3', H5'), 7.09 (d, *J* = 7.2 Hz, 1H, H6") 7.14 (d, *J* = 8.5 Hz, 2H, H2', H6'), 7.32-7.48 (m, 3H, H5", NH₂), 7.42 (d, *J* = 7.5 Hz, 1H, H7), 7.46 (t, *J* = 7.5 Hz, 1H, H9), 7.61 (d, *J* = 7.2 Hz, 1H, H4"), 7.67 (t, *J* = 7.5 Hz, 1H, H8), 7.85 (d, *J* = 7.5 Hz, 1H, H10), 8.24 (s, 1H, triazole). ¹³C NMR (125 MHz: DMSO-*d*₆): 36.1 (C4), 48.6 (benzyl CH₂), 58.1 (C3), 60.9 (OCH₂), 104.2 (4*H*-pyran), 113.0 (C3' and 5'), 114.6 (coumarin), 116.5 (coumarin), 119.2 (CN), 122.4 (triazole), 124.6 (Ar), 124.9 (Ar), 128.7 (Ar), 128.9 (Ar), 129.0 (Ar), 130.3 (Ar), 131.4 (Ar), 131.6 (Ar), 132.8 (Ar), 135.7 (Ar), 135.9 (Ar), 142.5 (triazole), 152.1 (C4'), 153.1 (coumarin, C-O), 157.1 (C2), 157.9 (4*H*-pyran, C-O), 159.5 (C=O (C5)). Anal. calcd. for C₂₉H₁₉Cl₂N₅O₄: C, 60.85; H, 3.35; N, 12.24. Found: C, 60.63; H, 3.47; N, 12.36.

2-Amino-4-(4-((1-(2,4-dichlorobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-oxo-4*H*,5*H*-pyrano[3,2-*c*]chromene-3-carbonitrile (10e)

Yield: 70%, white crystals, mp 231-233 °C. IR (KBr): 3400, 3308, 3132, 2206, 1714, 1675, 1608 cm⁻¹. ¹H NMR (500 MHz: DMSO-*d*₆): 4.40 (s, 1H, H4), 5.11 (s, 2H, CH₂), 5.71 (s, 2H, CH₂), 6.96 (d, *J* = 8.7 Hz, 2H, H3', H5'), 7.17-7.21 (m, 3H, H2', H6', H6''), 7.33-7.51 (m, 6H, H7, H9, H3'', H5'', NH₂), 7.71 (td, *J* = 7.9, 1.5 Hz, 1H, H8), 7.89 (dd, *J* = 7.9, 1.5 Hz, 1H, H10), 8.25 (s, 1H, triazole). ¹³C NMR (125 MHz: DMSO-*d*₆): 36.1 (C4), 50.6 (benzyl CH₂), 58.1 (C3), 61.0 (OCH₂), 104.2 (4*H*-pyran), 113.0 (C3' and 5'), 114.6 (coumarin), 116.6 (coumarin), 119.3 (CN), 122.4 (triazole), 122.5 (Ar), 124.7 (Ar), 125.1 (Ar), 127.7 (Ar), 128.8 (Ar), 129.6 (Ar), 130.4 (Ar), 132.6 (Ar), 132.9 (Ar), 133.2 (Ar), 135.8 (Ar), 142.9 (triazole), 152.1 (C4'), 153.1 (coumarin, C-O), 157.1 (C2), 157.9 (4*H*-pyran, C-O), 159.5 (C=O (C5)). Anal. calcd. for C₂₉H₁₉Cl₂N₅O₄: C, 60.85; H, 3.35; N, 12.24. Found: C, 60.73; H, 3.28; N, 12.11.

2-Amino-4-(4-((1-(2,6-dichlorobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-oxo-4*H*,5*H*-pyrano[3,2-*c*]chromene-3-carbonitrile (10f)

Yield: 70%, white crystals, mp 162-164 °C. IR (KBr): 3430, 3329, 3180, 2196, 1723, 1673, 1605 cm⁻¹. ¹H NMR (500 MHz: DMSO-*d*₆): 4.40 (s, 1H, H4), 5.08 (s, 2H, CH₂), 5.80 (s, 2H, CH₂), 6.95 (d, *J* = 8.5 Hz, 2H, H3', H5'), (d, *J* = 8.5 Hz, 2H, H2', H6'), 7.37 (s, 2H, NH₂), 7.45-7.50 (m, 3H, H7, H9, H4''), 7.57 (d, *J* = 8.0 Hz, 2H, H3'', H5''), 7.71 (t, *J* = 7.8 Hz, 1H, H8), 7.89 (d, *J* = 7.8 Hz, 1H, H10), 8.22 (s, 1H, triazole). ¹³C NMR (125 MHz: DMSO-*d*₆): 36.2 (C4), 51.2 (benzyl CH₂), 58.1 (C3), 61.0 (OCH₂), 104.3 (4*H*-pyran), 113.0 (C3' and 5'), 114.7 (coumarin), 116.6 (coumarin), 119.4 (CN), 122.5 (triazole), 124.7 (Ar), 125.3 (Ar), 128.7 (Ar), 128.8 (Ar), 129.0 (Ar), 130.6 (Ar), 132.9 (Ar), 135.8 (Ar), 135.9 (Ar), 143.0 (triazole), 152.1 (C4'), 153.2 (coumarin, C-O), 157.1 (C2), 158.0 (4*H*-pyran, C-O), 159.6 (C=O (C5)). Anal. calcd. for C₂₉H₁₉Cl₂N₅O₄: C, 60.85; H, 3.35; N, 12.24. Found: C, 60.70; H, 3.46; N, 12.41.

2-Amino-4-(4-((1-(4-bromobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-oxo-4*H*,5*H*-pyrano[3,2-*c*]chromene-3-carbonitrile (10g)

Yield: 80%, white crystals, mp 222-224 °C. IR (KBr): 3425, 3349, 2197, 1713, 1673, 1605 cm⁻¹. ¹H NMR (500 MHz: DMSO-*d*₆): 4.40 (s, 1H, H4), 5.10 (s, 2H, CH₂), 5.59 (s, 2H, CH₂), 6.96 (d, *J* = 8.4 Hz, 2H, H3', H5'), 7.18 (d, *J* = 8.4 Hz, 2H, H2', H6'), 7.27 (d, *J* = 8.0 Hz, 2H, H2'', H6''), 7.39 (s, 2H, NH₂), 7.45 (d, *J* = 8.0 Hz, 1H, H7), 7.48 (t, *J* = 8.0 Hz, 1H, H9), 7.56 (d, *J* = 8.0 Hz, 2H, H3'', H5''), 7.70 (t, *J* = 8.0 Hz, 1H, H8), 7.89 (t, *J* = 8.0 Hz, 1H, H10), 8.28 (s, 1H, triazole). ¹³C NMR (125 MHz: DMSO-*d*₆): 36.1 (C4), 52.1 (benzyl CH₂), 58.1 (C3), 61.1 (OCH₂), 104.2 (4*H*-pyran), 113.0 (C3' and 5'), 114.6 (coumarin), 116.5 (coumarin), 119.3 (CN), 121.4 (triazole), 122.4 (Ar), 124.7 (Ar), 128.8 (Ar), 129.7 (Ar), 130.2 (Ar), 131.7 (Ar), 132.9 (Ar), 135.4 (Ar), 135.8 (Ar), 143.1 (triazole), 152.1 (C4'), 153.1 (coumarin, C-O), 157.1 (C2), 157.9 (4*H*-pyran, C-O), 159.5 (C=O (C5)). Anal. calcd. for C₂₉H₂₀BrN₅O₄: C, 59.81; H, 3.46; N, 12.02. Found: C, 59.68; H, 3.57; N, 11.87.

2-Amino-4-(4-((1-(4-bromobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-5-oxo-4*H*,5*H*-pyrano[3,2-*c*]chromene-3-carbonitrile (10h)

Yield: 75%, white crystals, mp 235-237 °C. IR (KBr): 3370, 3345, 2935, 2193, 1710, 1670, 1607 cm⁻¹. ¹H NMR (500 MHz: DMSO-*d*₆): 3.69 (s, 3H, OCH₃), 4.41 (s, 1H, H4), 5.06 (s, 2H, CH₂), 5.59 (s, 2H, CH₂), 6.73 (d, *J* = 8.2 Hz, 1H, H5'), 6.86 (d, *J* = 1.0 Hz, 1H, H2'), 7.04 (dd, *J* = 8.2, 1.0 Hz, 1H, H6'), 7.27 (d, *J* = 8.0 Hz, 2H, H2'', H6''), 7.38 (s, 2H, NH₂), 7.45-7.47 (m, 2H, H7, H9), 7.56 (d, *J* = 8.0 Hz, 2H, H3'', H5''), 7.71 (t, *J* = 8.0 Hz, 1H, H8), 7.89 (t, *J* = 8.0 Hz, 1H, H10), 8.26 (s, 1H, triazole). ¹³C NMR (125 MHz: DMSO-*d*₆): 36.5 (C4), 52.1 (benzyl CH₂), 55.5 (OMe), 58.0 (benzyl CH₂), 61.7 (OCH₂), 104.1 (4*H*-pyran), 111.8 (C5'), 113.1 (C2'), 113.6 (coumarin), 116.6 (coumarin), 119.5 (CN), 119.6 (Ar), 121.5 (triazole), 122.5 (Ar), 124.7 (Ar), 124.9 (Ar), 130.3 (Ar), 131.7 (Ar), 132.9 (Ar), 135.4 (Ar),

136.5 (Ar), 143.1 (triazole), 146.6 (C4'), 148.8 (C3'), 152.1 (coumarin, C-O), 153.3 (C2), 158.0 (4*H*-pyran, C-O), 159.6 (C=O (C5)). Anal. calcd. for C₃₀H₂₂ BrN₅O₅: C, 58.84; H, 3.62; N, 11.44. Found: C, 58.69; H, 3.74; N, 11.29.

Product 10a

Product 10b

Product 10c

Product 10d

Product 10e

Product 10f

Product 10g

Product 10h

***In vitro* AChE assay**

Acetylcholinesterase (AChE, E.C. 3.1.1.7, Type V-S, lyophilized powder, from electric eel, 1000 unit), acetylthiocholine iodide (ATCI), and 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) were obtained from Sigma-Aldrich. Potassium dihydrogen phosphate, dipotassium hydrogen phosphate, potassium hydroxide, and sodium hydrogen carbonate were purchased from Fluka. The solutions of the title compounds were prepared in a mixture of DMSO (5 mL) and methanol (5 mL) and diluted in 0.1 M $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ buffer (pH 8.0) to give the final assay concentrations. All experiments were performed at 25 °C. Six different concentrations were examined for each product in triplicate.

To measure *in vitro* AChE activity, modified Ellman method was performed^[27,33,36] using a 96-well plate reader (BioTek ELx808). Each well contained 50 μL potassium phosphate buffer ($\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$, 0.1 M, pH 8), 25 μL sample dissolved in 50% methanol and 50% DMSO and 25 μL enzyme (final concentration 0.22 U/mL in buffer). After preincubation for 15 min at room temperature, 125 μL DTNB (3 mM in buffer) was added. Characterization of the hydrolysis of ATCI catalyzed by AChE was performed spectrometrically at 405 nm followed by the addition of substrate (ATCI 3 mM in water). The change in absorbance was measured at 405 nm after 40 min. A control experiment was conducted under the same conditions without inhibitor and the blank contained buffer, water, DTNB, and substrate.