Discovery of Novel Dual-active 3-(4-(dimethylamino)phenyl)-7-aminoalcoxy-coumarin as Potent and Selective Acetylcholinesterase Inhibitor and Antioxidant.

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Supporting Information

Contents

General Procedures .................................................................2-12
Biological assessment results .....................................................13-17
References ................................................................................28
Copies of \textsuperscript{1}H, \textsuperscript{13}C NMR and Mass Spectra ..............................................29-51
Materials
All chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) on 0.25 mm Merck (Darmstadt, Germany) silica gel plates (60F-254) and visualized under UV lamp (254 and 365 nm). All melting points (mp) were measured using a Melting Point AAKER model PFM-II and were uncorrected. $^1$H NMR and $^{13}$C NMR spectra were measured on a BRUKER Ultrashield Plus spectrometer (Billerica, MA) at 25 °C and referenced to TMS. Chemical shifts are reported in ppm (δ) using the residual solvent line as internal standard. Splitting patterns are designed as s, singlet; d, doublet; t, triplet; m, multiplet; brs, broad singlet. The HRMS analysis was performed using a QExactive™ Hybrid Quadrupole Orbitrap Mass Spectrometer (Thermo Fisher Scientific, Waltham, USA) using electrospray ionization (ESI). Standard working solutions of compounds 4a-d, 5a-c and 6a-c (1000 ng·mL$^{-1}$) were prepared with water/methanol 7:3 and fortified with 0.1% formic acid and 5 mM NH$_4$COOH (ammonium formate), and the solutions were used by direct infusion.

General procedures of synthesis and spectral data
7-bromoalkoxy-coumarins (2a-d)

**A** In a 25 mL borosilicate reaction tube were added 2 mL of acetone, 10.0 – 15.0 mmol (4-6 eq.) of dibromoalkane (7a–d), as shown in the Table 1, and 5.0 mmol (2 eq.) of potassium carbonate (K$_2$CO$_3$). Thereafter, a solution of 2.5 mmol of 7-hydroxycoumarin (1) in 8 mL of acetone (CH$_3$COCH$_3$), previously prepared in a 25 mL beaker, was added dropwise to the reaction tube under stirring. Following, the reaction tube was sealed and the reaction kept at 60°C for 6 – 12h, being monitored by TLC. At the end of the reaction (complete consumption of 1) the solvent was removed in a rotary evaporator, the product diluted in 30 mL water and then extracted with 3 × 15 mL ethyl acetate. The combined organic phases were dried over sodium sulfate (Na$_2$SO$_4$), filtered and the solvent removed in a rotary evaporator. The products were collected as oils which were precipitated in presence of 50 mL hexanes and irradiation in an ultrasonic
bath. After filtration, the solids were dried at room temperature yielding the 7-bromoalkoxy-coumarin derivatives 2a-d.

Table 1. Amount of dibromoalkanes (7a-d) used for the reactions

| Compound | mmol |
|----------|------|
| 7a       | 15.0 |
| 7b       | 12.5 |
| 7c       | 10.0 |
| 7d       | 10.0 |

7-(2-bromoethoxy)-2H-chromen-2-one (2a)
Light yellow solid, 75% yield, mp: 132°C. \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.65 (d, \(J = 9.3\) Hz, 1H); 7.40 (d, \(J = 8.5\) Hz, 1H); 6.88 (dd, \(J = 8.4, 2.1\) Hz, 1H); 6.82 (d, \(J = 1.8\) Hz, 1H); 6.28 (d, \(J = 9.5\) Hz, 1H); 4.36 (t, \(J = 6.1\) Hz, 2H); 3.68 (t, \(J = 6.0\) Hz, 2H). \(^1\)C NMR (500 MHz, CDCl\(_3\)): \(\delta\) 161.14; 161.04; 155.73; 143.29; 128.93; 113.53; 113.02; 112.83; 101.63; 68.15; 28.41.

7-(3-bromopropoxy)-2H-chromen-2-one (2b)
Light yellow solid, 78% yield, mp: 101°C. \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.64 (d, \(J = 9.5\) Hz, 1H); 7.38 (d, \(J = 8.5\) Hz, 1H); 6.82-6.86 (m, 2H); 6.26 (d, \(J = 9.3\) Hz, 1H); 4.17 (t, \(J = 5.9\) Hz, 2H); 3.61 (t, \(J = 6.3\) Hz, 2H); 2.36 (quint, \(J = 6.1\) Hz, 2H). \(^1\)C NMR (500 MHz, CDCl\(_3\)): \(\delta\) 161.79; 161.11; 155.77; 143.33; 128.79; 118.52; 113.19; 112.69; 101.47; 65.80; 31.89; 29.54.

7-(4-bromobutoxy)-2H-chromen-2-one (2c)
Light yellow solid, 70% yield, mp: 65°C. \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.64 (d, \(J = 9.5\) Hz, 1H); 7.37 (d, \(J = 8.8\) Hz, 1H); 6.83 (dd, \(J = 8.5, 2.3\) Hz, 1H); 6.80 (d, \(J = 2.3\) Hz, 1H); 6.25 (d, \(J = 9.3\) Hz, 1H); 4.06 (t, \(J = 5.9\) Hz, 2H); 3.50 (t, \(J = 6.4\) Hz, 2H); 2.05-2.12 (m, 2H); 1.96-2.02 (m, 2H). \(^1\)C NMR (500 MHz, CDCl\(_3\)): \(\delta\) 162.01; 161.17; 155.82; 143.38; 128.74; 113.07; 112.82; 112.52; 101.28; 67.48; 33.19; 29.25; 27.59.

7-(5-bromopent oxy)-2H-chromen-2-one (2d)
Light orange oil, yield 68%. \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.64 (d, \(J = 9.5\) Hz, 1H); 7.37 (d, \(J = 8.5\) Hz, 1H); 6.83 (dd, \(J = 8.7, 2.1\) Hz, 1H); 6.80 (brs, 1H); 6.25 (d, \(J = 9.5\) Hz, 1H); 6.15 (t, \(J = 5.9\) Hz, 2H); 5.94 (dd, \(J = 5.9, 2.3\) Hz, 1H); 5.93 (dd, \(J = 8.5, 2.1\) Hz, 1H); 4.06 (t, \(J = 5.9\) Hz, 2H); 3.48 (t, \(J = 6.4\) Hz, 2H); 2.98-3.06 (m, 2H); 2.05-2.12 (m, 2H); 1.96-2.02 (m, 2H); 1.25-1.36 (m, 2H). \(^1\)C NMR (500 MHz, CDCl\(_3\)): \(\delta\) 162.01; 161.17; 155.82; 143.38; 128.74; 113.07; 112.82; 112.52; 101.28; 67.48; 33.19; 29.25; 27.59.
Hz, 1H); 4.03 (t, J = 6.1 Hz, 2H); 3.45 (t, J = 6.7 Hz, 2H); 1.92-1.99 (m, 2H); 1.82-1.89 (m, 2H); 1.61-1.69 (m, 2H). $^1$C NMR (500 MHz, CDCl$_3$): $\delta$ 162.16; 161.21; 155.84; 143.41; 128.71; 112.97; 112.88; 112.43; 101.26; 68.16; 33.45; 32.32; 28.12; 24.68.

3-bromo-7-bromoalkoxy-coumarins (3a-d)

B – In a 25 mL borosilicate reaction tube were added 1.7 mmol of the appropriate 7-bromoalkoxy-coumarin derivative (2a–d), 2.1 mmol (1.3 eq.) of bromine (Br$_2$), 5.0 mmol (3 eq.) of sodium acetate and 8 mL of glacial acetic acid. The reaction was kept under stirring at room temperature for 2h. After complete consumption of 2a–d, monitored by TLC, the reaction was added to a 50 mL beaker containing crushed ice. The precipitated was then vacuum filtered and dried at room temperature. Products were purified using an Isolera equipment (Biotage, model ISO-4SV) with a 10 g silica gel cartridge eluted with a mixture of hexanes: dichloromethane in a concentration gradient ranging from 50 – 90% of the most polar solvent.

3-bromo-7-(2-bromoethoxy)-2H-chromen-2-one (3a)

White solid, 95% yield, mp: 183°C. $^1$H NMR (500 MHz, DMSO-d$_6$): $\delta$ 8.55 (s, 1H); 7.63 (d, J = 8.8 Hz, 1H); 7.08 (d, J = 2.3 Hz, 1H); 7.02 (dd, J = 8.8, 2.5 Hz, 1H); 4.44 (t, J = 6.0 Hz, 2H); 3.84 (t, J = 6.1 Hz, 2H). $^1$C NMR (500 MHz, DMSO-d$_6$): $\delta$ 161.24; 156.81; 154.59; 145.28; 129.11; 113.39; 113.36; 106.91; 101.34; 68.50; 31.07.

3-bromo-7-(3-bromopropoxy)-2H-chromen-2-one (3b)

White solid, 97% yield, mp: 120°C. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.02 (s, 1H); 7.36 (d, J = 8.8 Hz, 1H); 6.88 (dd, J = 8.7, 2.4 Hz, 1H); 6.83 (d, J = 2.3 Hz, 1H); 4.18 (t, J = 5.8 Hz, 2H); 3.61 (t, J = 6.3 Hz, 2H); 2.36 (quint, J = 6.1 Hz, 2H). $^1$C NMR (500 MHz, CDCl$_3$): $\delta$ 162.01; 157.39; 154.95; 144.36; 128.13; 113.34; 113.19; 107.85; 101.38; 65.95; 31.84; 29.47.
3-bromo-7-(4-bromobutoxy)-2H-chromen-2-one (3c)
White solid, 98% yield, mp: 120°C. 1H NMR (500 MHz, CDCl3): δ 8.02 (s, 1H); 7.35 (d, J = 8.8 Hz, 1H); 6.86 (dd, J = 8.7, 2.4 Hz, 1H); 6.80 (d, J = 2.3 Hz, 1H); 4.06 (t, J = 5.9 Hz, 2H); 3.50 (t, J = 6.4 Hz, 2H); 2.05-2.12 (m, 2H); 1.96-2.03 (m, 2H). 13C NMR (500 MHz, CDCl3): δ 162.22; 157.45; 155.00; 144.41; 128.08; 113.47; 113.02; 107.70 101.17; 67.61; 33.15; 29.21; 27.56.

3-bromo-7-(5-bromopentoxy)-2H-chromen-2-one (3d)
White solid, 99% yield, mp: 123°C. 1H NMR (500 MHz, CDCl3): δ 8.02 (s, 1H); 7.35 (d, J = 8.8 Hz, 1H); 6.86 (dd, J = 8.7, 2.4 Hz, 1H); 6.80 (d, J = 2.3 Hz, 1H); 4.03 (t, J = 6.3 Hz, 2H); 3.45 (t, J = 6.8 Hz, 2H); 1.92-1.99 (m, 2H); 1.83-1.90 (m, 2H); 1.61-1.69 (m, 2H). 13C NMR (500 MHz, CDCl3): δ 162.38; 157.48; 155.03; 144.44; 128.06; 113.53; 112.95; 107.60 101.15; 68.32; 33.45; 32.30; 28.09; 24.68.

3-bromo-7-aminoalkoxy-coumarins (4a–d)

C – In a 25 mL borosilicate reaction tube were added 1.3 mmol of the appropriate 3-bromo-7-bromoalkoxycoumarin derivative (3a–d), 3.9 mmol (3 eq.) of piperidine (8) and 8 mL of acetonitrile. The tube was then sealed and the reaction kept under stirring at 60°C for 3 – 8 h. After complete consumption of the 3a–d, monitored by TLC, acetonitrile was removed in a rotary evaporator and the products were purified using an Isolera equipment (Biotage, model ISO-4SV) with a 10 g silica gel cartridge eluted with a mixture of dichloromethane: methanol in a concentration gradient ranging from 0 – 25% of the more polar solvent.

3-bromo-7-[2-(piperidin-1-yl)ethoxy]-2H-chromen-2-one (4a)
Light orange solid, 95% yield, mp: 215°C. 1H NMR (500 MHz, DMSO-d6): δ 8.57 (s, 1H); 7.64 (d, J = 8.5 Hz, 1H); 7.10 (brs, 1H); 7.02 (dd, J = 8.7, 1.7 Hz, 1H); 4.34 (brs, 2H); 2.87-3.16 (m, 6H); 1.64 (brs, 4H); 1.46 (brs, 2H). 13C NMR (500 MHz, DMSO-d6): δ 161.31; 156.78; 154.56; 145.27; 129.03; 113.42; 113.26; 106.81; 101.34; 53.45; 43.69; 22.18; 21.61. HRMS (ESI) m/z calculated for C16H19BrNO3 [M + H]+ 552.0548 was found to be 552.05390.
3-bromo-7-[3-(piperidin-1-yl)propoxy]-2H-chromen-2-one (4b)

Light orange solid, 97% yield, mp: 242°C. 1H NMR (500 MHz, CDCl3): δ 8.01 (s, 1H); 7.34 (d, J = 8.5 Hz, 1H); 6.88 (dd, J = 8.8, 2.2 Hz, 1H); 6.83 (d, J = 2.2 Hz, 1H); 4.21 (t, J = 5.7 Hz, 2H); 3.17 (t, J = 5.4 Hz, 2H); 2.87 (t, J = 5.7 Hz, 2H); 1.92-1.94 (m, 2H); 1.63-1.70 (m, 6H); 1.47-1.48 (m, 2H). 13C NMR (500 MHz, DMSO-d6): δ 161.56; 156.79; 154.59; 145.30; 129.06; 113.32; 113.17; 106.74; 101.16; 65.84; 53.22; 52.18; 23.20; 22.55; 21.27. HRMS (ESI) m/z calculated for C17H21BrNO3 [M + H]+ 366.0705 was found to be 366.0718.

3-bromo-7-[4-(piperidin-1-yl)butoxy]-2H-chromen-2-one (4c)

White solid, 98% yield, mp: 225°C. 1H NMR (500 MHz, CDCl3/ CD3OD): δ 7.97 (s, 1H); 7.29 (m, 1H); 6.78 (d, J = 10.1 Hz, 1H); 6.70 (brs, 1H); 3.97 (t, J = 5.8 Hz, 2H); 3.47-3.48 (m, 2H); 2.99-3.02 (m, 2H); 2.70 (brs, 2H); 1.93-1.99 (m, 4H); 1.78-1.81 (m, 4H). 13C NMR (500 MHz, CDCl3/ CD3OD): δ 161.95; 157.75; 154.64; 144.69; 128.18; 113.21; 113.03; 107.16; 101.12; 67.38; 56.64; 52.99; 25.94; 22.43; 21.51; 20.51. HRMS (ESI) m/z calculated for C18H23BrNO3 [M + H]+ 380.0861 was found to be 380.08518.

3-bromo-7-[5-(piperidin-1-yl)pentoxy]-2H-chromen-2-one (4d)

White solid, 99% yield, mp: 176°C. 1H NMR (500 MHz, DMSO-d6): δ 9.11 (brs, 1H); 8.56 (s, 1H); 7.62 (d, J = 8.8 Hz, 1H); 7.04 (d, J = 1.9 Hz, 1H); 6.98 (d, J = 8.5, 2.2 Hz, 1H); 4.10 (t, J = 6.3 Hz, 2H); 3.32 (brs, 2H); 3.02-3.05 (m, 2H); 2.85 (brs, 2H); 1.69-1.80 (m, 10H); 1.41-1.47 (m, 2H). 13C NMR (500 MHz, DMSO-d6): δ 162.02; 156.83; 154.66; 145.35; 129.02; 113.32; 112.93; 106.47; 101.02; 68.06; 55.64; 52.00; 27.83; 22.86; 22.62; 22.52; 21.35. HRMS (ESI) m/z calculated for C19H25BrNO3 [M + H]+ 394.1018 was found to be 394.10079.

3-phenyl-7-aminoalkoxy-coumarins (5a-5c and 6a-6c)

D – In a 10 mL borosilicate reaction tube were added 0.14 mmol of the appropriate 3-bromine-7-aminoalkoxy-coumarin derivative (4a; 5b; 6d), 0.20 mmol (1.4 eq.) of properly
substituted phenylboronic acid (9-10) and 0.41 mmol (3 eq.) of potassium carbonate in 4 ml of a solvent mixture (water: ethanol: toluene (2: 1: 1)). The reaction was then degassed with nitrogen gas for 10 minutes. Following, 0.01 mmol (7 mol%) of Pd(PPh3)_4 (catalyst) was added under nitrogen flux, the reaction tube sealed and the reaction kept under stirring and heating at 65°C for 3 – 5 h. After complete consumption of 4a; b; d, monitored by TLC, the solvent was removed in a rotary evaporator. The products were purified using an Isolera equipment (Biotage, model ISO-4SV) with a 10 g silica gel cartridge eluted with a mixture of dichloromethane: methanol in a concentration gradient ranging from 0 – 25% of the more polar solvent.

3-phenyl-7-[2-(piperidin-1-yl)ethoxy]-2H-chromen-2-one (5a)
Ligthy yellow solid, 75% yield, mp: 90°C. 1H NMR (500 MHz, DMSO-d6): δ 8.20 (s, 1H); 7.67-7.71 (m, 3H); 7.45 (t, J = 7.4 Hz, 2H); 7.39 (t, J = 7.4 Hz, 1H); 7.04 (brs, 1H); 6.98 (dd, J = 8.5 Hz, 1.9 Hz, 1H); 4.18 (t, J = 5.7 Hz, 2H); 2.68 (t, J = 5.7 Hz, 2H); 2.43 (brs, 4H); 1.49-1.51 (m, 4H); 1.37-1.38 (m, 2H). 13C NMR (500 MHz, DMSO-d6): δ 161.65; 159.99; 154.81; 140.87; 129.69; 128.32; 128.20; 128.18; 123.14; 113.08; 113.02; 100.76; 66.38; 57.15; 54.37; 25.55; 23.91. HRMS (ESI) m/z calculated for C_{22}H_{24}NO_3 [M + H]^+ 350.1756 was found to be 350.17459.

3-phenyl-7-[3-(piperidin-1-yl)propoxy]-2H-chromen-2-one (5b)
Ligthy yellow solid, 75% yield, mp: 114°C. 1H NMR (500 MHz, CDCl3): δ 7.76 (s, 1H); 7.69-7.70 (m, 2H); 7.39-7.46 (m, 4H); 6.86-6.87 (m, 2H); 4.09 (brs, 2H); 2.47-2.55 (m, 6H); 2.05-2.06 (m, 2H); 1.64 (brs, 4H); 1.47 (brs, 2H). 13C NMR (500 MHz, CDCl3): δ 162.04; 160.92; 155.27; 140.05; 135.03; 128.78; 128.39; 128.37; 124.67; 113.23; 113.07; 100.99; 67.04; 55.63; 54.53; 26.37; 25.73; 24.25. HRMS (ESI) m/z calculated for C_{23}H_{26}NO_3 [M + H]^+ 364.1913 was found to be 364.19085.

3-phenyl-7-[5-(piperidin-1-yl)pent oxy]-2H-chromen-2-one (5c)
Ligthy yellow solid, 80% yield, mp: 198°C. 1H NMR (500 MHz, CDCl3/ CD3OD): δ 7.44 (s, 1H); 7.28 (m, 1H); 7.00-7.10 (m, 5H); 6.47-6.51 (m, 2H); 3.68 (t, J = 5.5 Hz, 2H); 2.41-2.52 (m, 6H); 1.43-1.48 (m, 8H); 1.15-1.22 (m, 4H). 13C NMR (500 MHz, CDCl3/ CD3OD): δ 161.86; 161.36; 154.89; 140.44; 134.63; 128.81; 128.13; 128.10; 124.27; 113.10; 112.96; 100.63; 67.87; 57.59; 53.35; 28.27; 24.03; 23.30; 22.31. HRMS (ESI) m/z calculated for C_{25}H_{30}NO_3 [M + H]^+ 392.2226 was found to be 392.22138.
3-[4-(dimethylamino)phenyl]-7-[2-(piperidin-1-yl)ethoxy]-2H-chromen-2-one (6a)

Green solid, 70% yield, mp: 140°C. ¹H NMR (500 MHz, CDCl₃): δ 7.67 (s, 1H); 7.63 (d, J = 8.8 Hz, 2H); 7.40 (d, J = 8.5 Hz, 1H); 6.84-6.87 (m, 2H); 6.77 (d, J = 8.8 Hz, 2H); 4.20 (t, J = 5.8 Hz, 2H); 3.01 (s, 6H); 2.86 (t, J = 5.7 Hz, 2H); 2.59 (brs, 4H); 1.66 (quin, J = 5.5 Hz, 4H); 1.48 (brs, 2H). ¹³C NMR (500 MHz, CDCl₃): δ 161.58; 161.17; 154.86; 150.78; 137.33; 129.41; 128.57; 125.15; 122.90; 114.17; 113.07; 112.27; 101.32; 66.47; 57.75; 55.22; 40.63; 25.84; 24.17. HRMS (ESI) m/z calculated for C₂₄H₂₉N₂O₃ [M + H]^+ 393.2178 was found to be 393.2174.

3-[4-(dimethylamino)phenyl]-7-[3-(piperidin-1-yl)propoxy]-2H-chromen-2-one (6b)

Yellow solid, 75% yield, mp: 153°C. ¹H NMR (500 MHz, CDCl₃): δ 7.67 (s, 1H); 7.63 (d, J = 8.8 Hz, 2H); 7.39 (d, J = 9.1 Hz, 1H); 6.83-6.85 (m, 2H); 6.78 (d, J = 8.8 Hz, 2H); 4.08 (t, J = 6.1 Hz, 2H); 3.01 (s, 6H); 2.49-2.58 (m, 6H); 2.04-2.1 (m, 2H); 1.65-1.67 (m, 4H); 1.48 (brs, 2H). ¹³C NMR (500 MHz, CDCl₃): δ 161.35; 161.26; 154.58; 150.52; 137.13; 129.15; 128.29; 124.78; 122.70; 113.76; 112.76; 112.03; 100.95; 66.90; 55.72; 54.55; 40.39 26.31; 25.61; 24.17. HRMS (ESI) m/z calculated for C₂₅H₃₁N₂O₃ [M + H]^+ 407.2335 was found to be 407.23246.

3-[4-(dimethylamino)phenyl]-7-[5-(piperidin-1-yl)pentoxy]-2H-chromen-2-one (6c)

Yellow solid, 77% yield, mp: 206°C. ¹H NMR (500 MHz, CDCl₃): δ 7.67 (s, 1H); 7.63 (d, J = 8.5 Hz, 2H); 7.40 (d, J = 8.5 Hz, 1H); 6.81-6.84 (m, 2H); 6.77 (d, J = 8.8 Hz, 2H); 4.02 (t, J = 6.3 Hz, 2H); 3.00 (s, 6H); 2.61-2.72 (m, 6H); 1.79-1.89 (m, 8H); 1.50-1.56 (m, 4H). ¹³C NMR (500 MHz, CDCl₃): δ 161.34; 161.23; 154.65; 150.51; 137.14; 129.14; 128.34; 124.75; 122.66; 113.74; 112.71; 112.01; 100.88; 68.09; 58.38; 53.96; 40.38; 28.68; 25.07; 24.30; 23.85; 23.32. HRMS (ESI) m/z calculated for C₂₇H₃₃N₂O₃ [M + H]^+ 435.2648 was found to be 435.26382.

**Biological assessment**

**Anticholinesterase activity assays**

The anticholinesterase activity was determined according to adapted Ellman's method¹. All of the solution were prepared in 0.02 M tris-HCl buffer (pH = 7.5), stock solutions of test compounds prepared in DMSO (50 mM), and the experiment conducted in triplicate. To a flat-bottom 96-well transparent plate were added 150μL of treatment solutions with inhibitors 4a-d, 5a-c, 6a-c and donepezil at eight different concentrations...
serially diluted. Negative (without treatment) and vehicle (DMSO, final concentration 0.2% v/v for AChE and 0.8% v/v for BuChE) controls were kept for reference. Following, there were added 60 μL of 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent) at 1.1 mM and 30 μL of electric eel acetylcholinesterase (EeAChE) or equine serum butyrylcholinesterase (EqBuChE) at 0.20 U/mL in the presence of 1 mg/mL bovine serum albumin (BSA). Absorbance was then recorded using an iMark plate reader (Bio-Rad) equipped with a λ = 415 nm light filter and this measure used as a blank reference. After a 10-minute incubation at 30°C, 24 μL of 2.75 mM acetyltiocholine iodide (ACTI) or S-butyrylthiocholine iodide (BCTI) were added and the absorbance recorded after a 10-minute incubation at 30°C at λ = 415 nm for 3 times within 30 seconds. Enzyme activity was calculated as percentage of untreated control discounting the blank reference. The final inhibitor concentration ranged from 100 – 0.00001 μM for AChE (dilution factor = 10) and from 400 – 0.1 μM for BuChE (dilution factor = 4). IC50 values were calculated on Graphpad Prism 7.0 using the non-linear regression model for dose-response inhibition.

**Enzymatic kinetic study**

The enzymatic kinetic of cholinesterase inhibition was determined according to adapted Ellman's method1. All of the solution were prepared in 0.02 M tris-HCl buffer (pH = 7.5), stock solutions of test compounds prepared in DMSO (50 mM), and the experiment conducted in triplicate. To a flat-bottom 96-well transparent plate were added 150μL of treatment solutions with inhibitors 4d and 6a at two different concentrations (Table 2) distributed in eight sets of triplicates each. Eight sets of untreated triplicates were used as negative control. Following, there were added 60 μL of 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent) at 1.1 mM and 30 μL of electric eel acetylcholinesterase (EeAChE) or equine serum butyrylcholinesterase (EqBuChE) at 0.20 U/mL in presence of 1 mg/mL bovine serum albumin (BSA). Absorbance was then recorded using an iMark plate reader (Bio-Rad) equipped with a λ = 415 nm light filter and this measure used as a blank reference. After 10-minute incubation at room temperature, 24 μL of acetyltiocholine iodide (ACTI) or S-butyrylthiocholine iodide (BCTI) at eight concentrations serially dilluted (factor = 1.3) from 2.75 – 0.44 mM (final concentration: 0.25 – 0.04 mM) were added to all wells and the absorbance recorded after incubation for 0, 5, 10, 15 and 20 minutes at room temperature at λ = 415 nm. The Lineweaver–Burk reciprocal plots were obtained by
plotting a 1/velocity versus 1/[substrate] graph for two different inhibitor concentrations and untreated control. The linear regression of each data-set shows a convergent behavior, in ways the region to where the curves converge determine the type of inhibition observed. $K_i$, $K_i'$ (competitive and noncompetitive inhibition constants respectively), $K_m$ (Michaelis-Menten constant) and $V_{max}$ values were calculated with Graphpad Prism 7.0 using the non-linear regression models for enzyme kinetics - inhibition and enzyme kinetics - substrate vs. velocity.

**Table 2.** Inhibitor concentrations for the kinetic study on AChE and BuChE

| Compound | Inhibitor concentrations (µM) | 4d       | 6a       |
|----------|--------------------------------|----------|----------|
| AChE     |                                | 0.1 and 0.2 | 0.01 and 0.03 |
| BuChE    |                                | 2 and 5   | 3 and 6  |

**Molecular modeling**

In order to get insight at the molecular level of possible reasons for the observed enzyme inhibition data, we implemented a molecular docking study with an electric eel (*Electrophorus electricus*) acetylcholinesterase (EeAChE) and a horse (*Equus caballus*) butyrylcholinesterase (EqBuChE). Cholinesterases have two important binding sites: the active site, where the enzymes hydrolyze their substrates; and the peripheral site, both located at the extremities of a gorge in the enzyme structure. Both sites were explored in the docking study, to determine which one is the most suitable for the connection of these compounds.

There are three EeAChE structures available in the Protein Data Bank (PDB): 1EEA, 1C2B and 1C2O, all of them without any co-crystallized ligand, and we chose 1C2O for the docking studies because it has the best crystallographic resolution (4.2 Å). For EqBuChE, there is no available crystallographic structure, so it was necessary the construction of a 3D model from a sequence available in the UniProtKB/Swiss-Prot protein sequence database (entry Q9N1N9). It was used for construction of a homology model with the automated mode of the protein structure homology-modeling server, Swiss-Model, using as template the human BuChE in complex with $N$-((1-(2,3-dihydro-1H-inden-2-yl)piperidin-3-yl)methyl)-$N$-(2-methoxyethyl)-2-naphth-amide (PDB code 4TPK). The resolution of the template was 2.52 Å, the identity between
sequences was 90.40%, with a coverage 0.95. The global and per-residue model quality has been assessed using the QMEAN scoring function. As a previous test for the ability of the docking program GOLD 5.6 (CCDC Software Ltd., Cambridge, UK) to find reliable solutions for the docking to cholinesterases, we implemented redocking experiments. Since all the three acetylcholinesterases to be tested do not have ligands in their crystallographic structures, all of the scoring functions available in the program were tested through redocking procedures with the PDB structure 2CMF (Torpedo californica AChE co-crystallized with alkylene-linked bis-tacrine dimer). Hydrogen atoms were added to proteins structures based on ionization and tautomeric states defined by GOLD. To allow the best orientation of hydrogen bonds involving the serine, threonine, tyrosine and lysine side chains, they were set free to rotate during the docking procedure. In the course of the searching procedure, 100,000 genetic operations (crossover, migration, mutation) were used for each docking run. Radius of binding sites for the enzymes was tested at 15 Å and 20 Å around atoms from adequate amino acids selected based on literature information for each binding site. The same score function, corresponding amino acids and radius were adopted for the docking studies with the EqBuChE model. Spartan’14 program [Wavefunction, Inc.] was utilized to construct and optimize the molecules in study, with the PM6 method. Several poses were obtained for each compound in all proteins, and the best-ranked pose for each one was chosen for analysis of the interactions with the amino acid residues. In the GOLD docking program, the docking functions yield the “fitness scores”, which are dimensionless values. In each case, the score values are a guide of how good the docking pose is, with a higher score indicating a better docking result.

**Ferric reducing ability of plasma (FRAP) antioxidant assay**
FRAP solution was freshly prepared by mixing 25 mL acetate buffer (0.30 M) at pH 3.6, 2.5 mL ferric chloride (20 mM) and 2.5 mL 2,4,6-tris(2-pyridyl)-s-triazine (10 mM). In a test tube containing 4.5 mL of FRAP reagent were added 0.5 mL of 500 µM solutions of 4a-d, 5a-c and 6a-c (in methanol). After a 10-minute incubation at 37°C the absorbance was recorded in a spectrophotometer at λ = 593 nm using methanol as blank reference. A standard curve was obtained by plotting the absorbance read after treatment with 10, 25, 30, 50, 75 and 100 µM quercetin and results, expressed as mmol of quercetin (Q) equivalent to mol of inhibitor (mmol Q/mol), obtained in triplicates.
**Murine neuroblastoma cell (N2a) culture and cell viability assay.**

N2a cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 0.1% gentamicin in a 5% CO₂ atmosphere. N2a cells were transferred to a 96-well plate (~10,000 cells/cm²) and incubated for 24 h, before treatment with the compounds at 10 or 50 µM. After being treated, cells were grown for 48 hours. Cell viability was evaluated by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay. MTT was added to a final concentration of 0.5 mg/mL into the wells (samples and controls), and the plates were incubated for 4 h at 37 °C. After, all the media was removed and produced formazan crystals were solubilized with DMSO and quantified by reading the OD at 570 nm in a 96-well plate reader (SpectraMax® Paradigm®, Molecular Devices). Means and standard deviations were calculated from triplicates.
Biological assessment results

*Enzymatic kinetic study*

**Table 3.** Kinetics parameters of 4d and 6a on AChE and BuChE

| Concentration (μM) | Vₘₐₓ ± SD (μM/min) | Kₘ (μM) ± SD (μM/min) | Kᵢ (nM) ± SD (nM) | Kᵢ’ (nM) ± SD (nM) |
|-------------------|---------------------|------------------------|-----------------|---------------------|
| 4d on AChE       |                     |                        |                 |                     |
| 0                 | 3.064 ± 0.063       | 23.06 ± 2.744          | 21.273 ± 0.816  | 157.350 ± 4.674     |
| 0.1               | 2.209 ± 0.060       | 48.083 ± 2.189         |                 |                     |
| 0.2               | 1.752 ± 0.198       | 81.645 ± 7.743         |                 |                     |
| 4d on BuChE      |                     |                        |                 |                     |
| 0                 | 6.306 ± 0.138       | 78.160 ± 0.141         | 2129 ± 148      | 6621 ± 322          |
| 0.1               | 5.791 ± 0.185       | 97.903 ± 0.309         |                 |                     |
| 0.2               | 4.669 ± 0.229       | 114.500 ± 1.061        |                 |                     |
| 6a on AChE       |                     |                        |                 |                     |
| 0                 | 3.168 ± 0.188       | 25.290 ± 0.651         | 1.610 ± 0.066   | 10.499 ± 0.438      |
| 0.01              | 1.762 ± 0.128       | 56.185 ± 3.910         |                 |                     |
| 0.03              | 1.254 ± 0.043       | 76.095 ± 1.025         |                 |                     |
| 6a on BuChE      |                     |                        |                 |                     |
| 0                 | 5.811 ± 0.201       | 80.190 ± 0.625         | 2585 ± 236      | 7376 ± 558          |
| 0.01              | 4.837 ± 0.399       | 109.483 ± 5.504        |                 |                     |
| 0.03              | 3.224 ± 0.284       | 112.827 ± 1.961        |                 |                     |

*a* Standard deviation (SD) of triplicate independent experiments  

*b* Kᵢ is the competitive inhibition constant  

*c* Kᵢ’ is the non-competitive inhibition constant
Figure 1. Graphs of AChE inhibition percentage vs. inhibitor concentration
**Figure 2.** Graphs of BuChE inhibition percentage vs. inhibitor concentration

**Figure 3.** Lineweaver-Burk plots of EeAChE inhibition kinetics of compound 4d. Inset: concentrations used for 4d are depicted with [I] graphic symbol.
4d 6a

Figure 4. Lineweaver-Burk plots of EqBuChE inhibition kinetics of compounds 4d and 6a. Inset: concentrations used for 4d and 6a are depicted with [I] graphic symbol.

Molecular modeling

Table 4. Fitness scores (Goldscore)* obtained by molecular docking in cholinesterases

| Ligand | EeAChE | EqBuChE |
|-------|--------|---------|
| 4a    | 64.9   | 57.6    |
| 6a    | 78.1   | 63.0    |
| 6c    | 71.4   | 67.9    |

*GOLD 5.6 program

Figure 5. Superposition of the interaction poses of compounds 6a (A, carbon atoms in yellow) and 4a (B, carbon atoms in purple) with EeAChE obtained by molecular docking (Goldscore function). Color code: oxygen atoms, red; nitrogen atoms, blue;
hydrogen atoms, white; carbon atoms, green. Figure generated with PyMol 0.99 (DeLano Scientific LLC).

**Cytotoxicity Evaluation**

**Table 5**: Neuroblastoma cell viability after compound treatment at 10 and 50 µM.

| Compound | Concentration (µM) | Mean ± SD     |
|----------|--------------------|--------------|
| 4a       | 10                 | 89.16 ± 11.28|
|          | 50                 | 103.2 ± 13.68|
| 4b       | 10                 | 93.34 ± 8.19 |
|          | 50                 | 87.60 ± 4.39 |
| 4c       | 10                 | 94.43 ± 4.28 |
|          | 50                 | 88.88 ± 19.01|
| 4d       | 10                 | 80.10 ± 7.27 |
|          | 50                 | 62.11 ± 10.09|
| 5a       | 10                 | 98.37 ± 8.92 |
|          | 50                 | 105.5 ± 14.93|
| 5b       | 10                 | 83.55 ± 6.58 |
|          | 50                 | 57.06 ± 8.72 **|
| 5c       | 10                 | 117.4 ± 4.86 |
|          | 50                 | 13.47 ± 1.19 ***|
| 6a       | 10                 | 92.15 ± 43.03|
|          | 50                 | 115.9 ± 4.28 |
| 6b       | 10                 | 84.11 ± 11.1 |
|          | 50                 | 16.79 ± 8.41 ***|
| 6c       | 10                 | 59.04 ± 8.21 **|
|          | 50                 | 10.09 ± 3.09 ***|

MTT reduction was evaluated as described in the Experimental Procedures. Data are expressed as the mean ± standard deviation of the percentage of MTT reduction relative to the value for control cells (cells without treatment, 100% viability). **P < 0.01; ****P < 0.0001.
**ADMET Evaluation**

**Compound 4a**

![Chemical Structure](image)

### Physicochemical Properties

| Property                  | Value                  |
|---------------------------|------------------------|
| Formula                   | C16H18BrNO3            |
| Molecular weight          | 352.22 g/mol           |
| Fraction Csp3             | 0.44                   |
| Num. rotatable bonds      | 4                      |
| Num. H-bond acceptors     | 4                      |
| Num. H-bond donors        | 0                      |
| Molar Refractivity        | 90.21                  |
| TPSA                      | 42.68 Å²               |

### Lipophilicity

| Property                  | Value                  |
|---------------------------|------------------------|
| Log $P_{ow}$ (iLOGP)      | 3.40                   |
| Log $P_{ow}$ (XLOGP3)     | 3.66                   |
| Log $P_{ow}$ (WLOGP)      | 3.04                   |
| Log $P_{ow}$ (MLOGP)      | 2.73                   |
| Log $P_{ow}$ (SILICOS-IT) | 3.91                   |
| Consensus Log $P_{ow}$    | 3.35                   |

### Water Solubility

| Property                  | Value                  |
|---------------------------|------------------------|
| Log $S$ (ESOL)            | -4.42                  |
| Solubility                | 1.35e-02 mg/ml ; 3.82e-05 mol/l |
| Class                     | Moderately soluble     |

### Pharmacokinetics

| Property                  | Value                  |
|---------------------------|------------------------|
| GI absorption             | High                   |
| BBB permeant              | Yes                    |
| P-gp substrate            | No                     |
| CYP1A2 inhibitor          | Yes                    |
| CYP2C19 inhibitor         | Yes                    |
| CYP2C9 inhibitor          | Yes                    |
| CYP2D6 inhibitor          | Yes                    |
| CYP3A4 inhibitor          | No                     |

### Druglikeness

| Property                  | Value                  |
|---------------------------|------------------------|
| Lipinski                  | Yes; 0 violation       |
| Ghose                     | Yes                    |
| Veber                     | Yes                    |
| Egan                      | Yes                    |
| Muegge                    | Yes                    |
| Bioavailability Score     | 0.55                   |

18
### Compound 4b

![Chemical Structure](image)

#### Physicochemical Properties

| Property                  | Value         |
|---------------------------|---------------|
| Formula                   | C17H20BrNO3   |
| Molecular weight          | 366.25 g/mol  |
| Fraction Csp3             | 0.47          |
| Num. rotatable bonds      | 5             |
| Num. H-bond acceptors     | 4             |
| Num. H-bond donors        | 0             |
| Molar Refractivity        | 95.02         |
| TPSA                      | 42.68 Å²      |

#### Lipophilicity

| Property                  | Value         |
|---------------------------|---------------|
| Log $P_{ow}$ (iLOGP)      | 3.62          |
| Log $P_{ow}$ (XLOGP3)     | 4.02          |
| Log $P_{ow}$ (WLOGP)      | 3.43          |
| Log $P_{ow}$ (MLOGP)      | 2.97          |
| Log $P_{ow}$ (SILICOS-IT) | 4.29          |
| Consensus Log $P_{ow}$    | 3.67          |

#### Water Solubility

| Property        | Value         |
|-----------------|---------------|
| Log $S$ (ESOL)  | -4.65         |
| Solubility      | 8.20e-03 mg/ml ; 2.24e-05 mol/l |
| Class           | Moderately soluble |

#### Pharmacokinetics

| Property                  | Value         |
|---------------------------|---------------|
| GI absorption             | High          |
| BBB permeant              | Yes           |
| P-gp substrate            | No            |
| CYP1A2 inhibitor          | Yes           |
| CYP2C19 inhibitor         | Yes           |
| CYP2C9 inhibitor          | Yes           |
| CYP2D6 inhibitor          | Yes           |
| CYP3A4 inhibitor          | No            |

#### Druglikeness

| Property   | Value         |
|------------|---------------|
| Lipinski   | Yes; 0 violation |
| Ghose      | Yes           |
| Veber      | Yes           |
| Egan       | Yes           |
| Muegge     | Yes           |
| Bioavailability Score | 0.55    |
## Compound 4c

![Chemical Structure](image)

### Physicochemical Properties

| Property                        | Value                        |
|---------------------------------|------------------------------|
| Formula                         | C18H22BrNO3                  |
| Molecular weight                | 380.28 g/mol                 |
| Fraction Csp3                   | 0.50                         |
| Num. rotatable bonds            | 6                            |
| Num. H-bond acceptors           | 4                            |
| Num. H-bond donors              | 0                            |
| Molar Refractivity              | 99.83                        |
| TPSA                            | 42.68 Å²                     |

### Lipophilicity

| Property                        | Value |
|---------------------------------|-------|
| Log \( P_{ow} \) (iLOGP)        | 3.90  |
| Log \( P_{ow} \) (XLOGP3)       | 4.38  |
| Log \( P_{ow} \) (WLOGP)        | 3.82  |
| Log \( P_{ow} \) (MLOGP)        | 3.20  |
| Log \( P_{ow} \) (SILICOS-IT)   | 4.66  |
| Consensus Log \( P_{ow} \)      | 3.99  |

### Water Solubility

| Property                        | Value                        |
|---------------------------------|------------------------------|
| Log \( S \) (ESOL)              | -4.88                        |
| Solubility                      | 4.98e-03 mg/ml ; 1.31e-05 mol/l |
| Class                           | Moderately soluble           |

### Pharmacokinetics

| Property                        | Value                        |
|---------------------------------|------------------------------|
| GI absorption                   | High                         |
| BBB permeant                    | Yes                          |
| P-gp substrate                  | No                           |
| CYP1A2 inhibitor                | Yes                          |
| CYP2C19 inhibitor               | Yes                          |
| CYP2C9 inhibitor                | Yes                          |
| CYP2D6 inhibitor                | Yes                          |
| CYP3A4 inhibitor                | No                           |

### Druglikeness

| Property                        | Value                        |
|---------------------------------|------------------------------|
| Lipinski                        | Yes; 0 violation             |
| Ghose                           | Yes                          |
| Veber                           | Yes                          |
| Egan                            | Yes                          |
| Muegge                          | Yes                          |
| Bioavailability Score           | 0.55                         |
**Compound 4d**

| Physicochemical Properties               |       |
|------------------------------------------|-------|
| Formula                                  | C19H24BrNO3 |
| Molecular weight                         | 394.30 g/mol |
| Fraction Csp3                            | 0.53  |
| Num. rotatable bonds                     | 7     |
| Num. H-bond acceptors                    | 4     |
| Num. H-bond donors                       | 0     |
| Molar Refractivity                       | 104.64 |
| TPSA                                     | 42.68 Å² |

| Lipophilicity                            |       |
|------------------------------------------|-------|
| Log $P_{ow}$ (iLOGP)                     | 4.15  |
| Log $P_{ow}$ (XLOGP3)                    | 4.74  |
| Log $P_{ow}$ (WLOGP)                     | 4.21  |
| Log $P_{ow}$ (MLOGP)                     | 3.42  |
| Log $P_{ow}$ (SILICOS-IT)                | 5.05  |
| Consensus Log $P_{ow}$                   | 4.31  |

| Water Solubility                         |       |
|------------------------------------------|-------|
| Log $S$ (ESOL)                           | -5.12 |
| Solubility                               | 3.01e-03 mg/ml ; 7.63e-06 mol/l |
| Class                                    | Moderately soluble |

| Pharmacokinetics                         |       |
|------------------------------------------|-------|
| GI absorption                            | High  |
| BBB permeant                             | Yes   |
| P-gp substrate                           | No    |
| CYP1A2 inhibitor                         | Yes   |
| CYP2C19 inhibitor                        | Yes   |
| CYP2C9 inhibitor                         | Yes   |
| CYP2D6 inhibitor                         | Yes   |
| CYP3A4 inhibitor                         | Yes   |

| Druglikeness                             |       |
|------------------------------------------|-------|
| Lipinski                                 | Yes; 0 violation |
| Ghose                                    | Yes   |
| Veber                                    | Yes   |
| Egan                                     | Yes   |
| Muegge                                   | Yes   |
| Bioavailability Score                    | 0.55  |
## Compound 5a

![Chemical Structure](image)

### Physicochemical Properties

| Property                        | Value                      |
|---------------------------------|----------------------------|
| Formula                         | C22H23NO3                  |
| Molecular weight                | 349.42 g/mol               |
| Fraction Csp3                   | 0.32                       |
| Num. rotatable bonds            | 5                          |
| Num. H-bond acceptors           | 4                          |
| Num. H-bond donors              | 0                          |
| Molar Refractivity              | 107.95                     |
| TPSA                            | 42.68 Å²                   |

### Lipophilicity

| Property                        | Value       |
|---------------------------------|-------------|
| Log $P_{ow}$ (iLOGP)            | 3.87        |
| Log $P_{ow}$ (XLOGP3)           | 4.40        |
| Log $P_{ow}$ (WLOGP)            | 3.94        |
| Log $P_{ow}$ (MLOGP)            | 3.26        |
| Log $P_{ow}$ (SILICOS-IT)       | 4.81        |
| Consensus Log $P_{ow}$          | 4.06        |

### Water Solubility

| Property                        | Value                      |
|---------------------------------|----------------------------|
| Log $S$ (ESOL)                  | -4.90                     |
| Solubility                      | 4.36e-03 mg/ml ; 1.25e-05 mol/l |
| Class                           | Moderately soluble         |

### Pharmacokinetics

| Property                        | Value       |
|---------------------------------|-------------|
| GI absorption                   | High        |
| BBB permeant                    | Yes         |
| P-gp substrate                  | No          |
| CYP1A2 inhibitor                | Yes         |
| CYP2C19 inhibitor               | Yes         |
| CYP2C9 inhibitor                | Yes         |
| CYP2D6 inhibitor                | Yes         |
| CYP3A4 inhibitor                | Yes         |

### Druglikeness

| Property                        | Value       |
|---------------------------------|-------------|
| Lipinski                        | Yes; 0 violation |
| Ghose                           | Yes         |
| Veber                           | Yes         |
| Egan                            | Yes         |
| Muegge                          | Yes         |
| Bioavailability Score           | 0.55        |
### Compound 5b

![Chemical Structure](image)

#### Physicochemical Properties

| Property                          | Value                        |
|----------------------------------|------------------------------|
| Formula                          | C23H25NO3                    |
| Molecular weight                 | 363.45 g/mol                 |
| Fraction Csp3                    | 0.35                         |
| Num. rotatable bonds             | 6                            |
| Num. H-bond acceptors            | 4                            |
| Num. H-bond donors               | 0                            |
| Molar Refractivity               | 112.76                       |
| TPSA                             | 42.68 Å²                     |

#### Lipophilicity

| Property                  | Value |
|---------------------------|-------|
| Log $P_{ow}$ (iLOGP)      | 4.13  |
| Log $P_{ow}$ (XLOGP3)     | 4.76  |
| Log $P_{ow}$ (WLOGP)      | 4.33  |
| Log $P_{ow}$ (MLOGP)      | 3.47  |
| Log $P_{ow}$ (SILICOS-IT) | 5.20  |
| Consensus Log $P_{ow}$    | 4.38  |

#### Water Solubility

| Property      | Value                           |
|----------------|---------------------------------|
| Log $S$ (ESOL) | -5.13                          |
| Solubility     | 2.67e-03 mg/ml; 7.33e-06 mol/l  |
| Class          | Moderately soluble              |

#### Pharmacokinetics

| Property                  | Value |
|---------------------------|-------|
| GI absorption             | High  |
| BBB permeant              | Yes   |
| P-gp substrate            | Yes   |
| CYP1A2 inhibitor          | Yes   |
| CYP2C19 inhibitor         | Yes   |
| CYP2C9 inhibitor          | Yes   |
| CYP2D6 inhibitor          | Yes   |
| CYP3A4 inhibitor          | Yes   |

#### Druglikeness

| Property | Value |
|----------|-------|
| Lipinski | Yes; 0 violation |
| Ghose    | Yes   |
| Veber    | Yes   |
| Egan     | Yes   |
| Muegge   | Yes   |
| Bioavailability Score | 0.55 |

![Graph](image)
**Compound 5c**

![Chemical Structure](image)

| Physicochemical Properties |  |
|----------------------------|---|
| **Formula**                | C25H29NO3 |
| **Molecular weight**       | 391.50 g/mol |
| **Fraction Csp3**          | 0.40 |
| **Num. rotatable bonds**   | 8 |
| **Num. H-bond acceptors**  | 4 |
| **Num. H-bond donors**     | 0 |
| **Molar Refractivity**     | 122.37 |
| **TPSA**                   | 42.68 Å² |

| Lipophilicity |  |
|---------------|---|
| **Log P<sub>ow</sub> (iLOGP)** | 4.53 |
| **Log P<sub>ow</sub> (XLOGP3)** | 5.47 |
| **Log P<sub>ow</sub> (WLOGP)** | 5.11 |
| **Log P<sub>ow</sub> (MLOGP)** | 3.89 |
| **Log P<sub>ow</sub> (SILICOS-IT)** | 5.98 |
| **Consensus Log P<sub>ow</sub>** | 5.00 |

| Water Solubility |  |
|------------------|---|
| **Log S (ESOL)** | -5.59 |
| **Solubility**   | 9.98e-04 mg/ml; 2.55e-06 mol/l |
| **Class**        | Moderately soluble |

| Pharmacokinetics |  |
|------------------|---|
| **GI absorption** | High |
| **BBB permeant**  | Yes |
| **P-gp substrate** | Yes |
| **CYP1A2 inhibitor** | Yes |
| **CYP2C19 inhibitor** | Yes |
| **CYP2C9 inhibitor** | Yes |
| **CYP2D6 inhibitor** | Yes |
| **CYP3A4 inhibitor** | Yes |

| Druglikeness |  |
|--------------|---|
| **Lipinski** | Yes; 0 violation |
| **Ghose**    | Yes |
| **Veber**    | Yes |
| **Egan**     | Yes |
| **Muegge**   | No; 1 violation: XLOGP3>5 |
| **Bioavailability Score** | 0.55 |
## Compound 6a

![Chemical structure of Compound 6a](image)

### Physicochemical Properties

| Property                        | Value                        |
|---------------------------------|------------------------------|
| Formula                         | C24H28N2O3                   |
| Molecular weight                | 392.49 g/mol                 |
| Fraction Csp3                   | 0.38                         |
| Num. rotatable bonds            | 6                            |
| Num. H-bond acceptors           | 4                            |
| Num. H-bond donors              | 0                            |
| Molar Refractivity              | 122.16                       |
| TPSA                            | 45.92 Å²                     |
| Lipophilicity                   |                              |
| Log $P_{ow}$ (iLOGP)            | 4.12                         |
| Log $P_{ow}$ (XLOGP3)           | 4.52                         |
| Log $P_{ow}$ (WLOGP)            | 4.01                         |
| Log $P_{ow}$ (MLOGP)            | 3.12                         |
| Log $P_{ow}$ (SILICOS-IT)       | 4.49                         |
| Consensus Log $P_{ow}$          | 4.05                         |
| Water Solubility                |                              |
| Log $S$ (ESOL)                  | -5.13                        |
| Solubility                      | 2.89e-03 mg/ml ; 7.36e-06 mol/l |
| Class                           | Moderately soluble           |
| Pharmacokinetics                |                              |
| GI absorption                   | High                         |
| BBB permeant                    | Yes                          |
| P-gp substrate                  | No                           |
| CYP1A2 inhibitor                | Yes                          |
| CYP2C19 inhibitor               | Yes                          |
| CYP2C9 inhibitor                | Yes                          |
| CYP2D6 inhibitor                | Yes                          |
| CYP3A4 inhibitor                | Yes                          |
| Druglikeness                    |                              |
| Lipinski                        | Yes; 0 violation             |
| Ghose                           | Yes                          |
| Veber                           | Yes                          |
| Egan                            | Yes                          |
| Muegge                          | Yes                          |
| Bioavailability Score           | 0.55                         |

25
**Compound 6b**

| **Physicochemical Properties** |  |
|-------------------------------|-------------------------------|
| **Formula**                   | C25H30N2O3                   |
| **Molecular weight**          | 406.52 g/mol                 |
| **Fraction Csp3**             | 0.40                          |
| **Num. rotatable bonds**      | 7                             |
| **Num. H-bond acceptors**     | 4                             |
| **Num. H-bond donors**        | 0                             |
| **Molar Refractivity**        | 126.97                        |
| **TPSA**                      | 45.92 Å²                      |
| **Lipophilicity**             |                               |
| Log $P_{\text{ow}}$ (iLOGP)   | 4.40                          |
| Log $P_{\text{ow}}$ (XLOGP3)  | 4.88                          |
| Log $P_{\text{ow}}$ (WLOGP)   | 4.40                          |
| Log $P_{\text{ow}}$ (MLOGP)   | 3.33                          |
| Log $P_{\text{ow}}$ (SILICOS-IT) | 4.88                     |
| Consensus Log $P_{\text{ow}}$ | 4.38                          |
| **Water Solubility**          |                               |
| Log $S$ (ESOL)                | -5.37                         |
| Solubility                    | 1.74e-03 mg/ml ; 4.29e-06 mol/l |
| Class                         | Moderately soluble            |
| **Pharmacokinetics**          |                               |
| GI absorption                 | High                          |
| BBB permeant                  | Yes                           |
| P-gp substrate                | Yes                           |
| CYP1A2 inhibitor              | No                            |
| CYP2C19 inhibitor             | Yes                           |
| CYP2C9 inhibitor              | Yes                           |
| CYP2D6 inhibitor              | Yes                           |
| CYP3A4 inhibitor              | Yes                           |
| **Druglikeness**              |                               |
| Lipinski                      | Yes; 0 violation              |
| Ghose                         | Yes                           |
| Veber                         | Yes                           |
| Egan                          | Yes                           |
| Muegge                        | Yes                           |
| Bioavailability Score         | 0.55                          |

![Graph showing physicochemical properties](image-url)
### Compound 6c

![Chemical Structure](image)

#### Physicochemical Properties

| Property                        | Value               |
|---------------------------------|---------------------|
| Formula                         | C27H34N2O3          |
| Molecular weight                | 434.57 g/mol        |
| Fraction Csp3                   | 0.44                |
| Num. rotatable bonds            | 9                   |
| Num. H-bond acceptors           | 4                   |
| Num. H-bond donors              | 0                   |
| Molar Refractivity              | 136.58              |
| TPSA                            | 45.92 Å²            |

#### Lipophilicity

| Property (Log P<sub>ow</sub>) | Value |
|------------------------------|-------|
| iLOGP                        | 4.84  |
| XLOGP3                       | 5.59  |
| WLOGP                        | 5.18  |
| MLOGP                        | 3.73  |
| SILICOS-IT                   | 5.68  |
| Consensus Log P<sub>ow</sub> | 5.01  |

#### Water Solubility

| Property             | Value                                      |
|----------------------|--------------------------------------------|
| Log S (ESOL)         | -5.83                                      |
| Solubility           | 6.40e-04 mg/ml; 1.47e-06 mol/l             |
| Class                | Moderately soluble                         |

#### Pharmacokinetics

| Property                        | Value               |
|---------------------------------|---------------------|
| GI absorption                   | High                |
| BBB permeant                    | Yes                 |
| P-gp substrate                  | Yes                 |
| CYP1A2 inhibitor                | No                  |
| CYP2C19 inhibitor               | No                  |
| CYP2C9 inhibitor                | No                  |
| CYP2D6 inhibitor                | Yes                 |
| CYP3A4 inhibitor                | Yes                 |

#### Druglikeness

| Property   | Value                                      |
|------------|--------------------------------------------|
| Lipinski   | Yes; 0 violation                           |
| Ghose      | No; 1 violation: MR>130                     |
| Veber      | Yes                                        |
| Egan       | Yes                                        |
| Muegge     | No; 1 violation: XLOGP3>5                   |
| Bioavailability Score | 0.55                                      |
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$^1$H NMR spectra of 2a

$^{13}$C NMR spectra of 2a
$^1$H NMR spectra of 2b

$^{13}$C NMR spectra of 2b
$^1$H NMR spectra of 2c

$^{13}$C NMR spectra of 2c
$^1$H NMR spectra of 2d

$^{13}$C NMR espectra of 2d
$^1$H NMR spectra of 3a

$^{13}$C NMR spectra of 3a
$^{1}$H NMR spectra of 3b

$^{13}$C NMR spectra of 3b
$^1$H NMR spectra of 3c

$^{13}$C NMR spectra of 3c
$^1$H NMR spectra of 3d

$^{13}$C NMR spectra of 3d
$^{1}$H NMR spectra of 4a

$^{13}$C NMR spectra of 4a
HRMS spectra of 4a

\[ \text{HRMS spectra of 4a} \]

\[ \text{HRMS spectra of 4a} \]

\[ \text{H NMR spectra of 4b} \]

\[ \text{H NMR spectra of 4b} \]

\[ \text{H NMR spectra of 4b} \]
\(^{13}\)C NMR spectra of 4b

HRMS spectra of 4b
$^1$H NMR spectra of 4c

$^{13}$C NMR spectra of 4c
HRMS spectra of 4c

\[ \text{HRMS spectra of 4c} \]

\[ \text{\(^1\)H NMR spectra of 4d} \]
$^{13}$C NMR spectra of 4d

HRMS spectra of 4d
$^1$H NMR spectra of 5a

$^{13}$C NMR spectra of 5a
HRMS spectra of 5a

H NMR spectra of 5b
$^{13}$C NMR spectra of 5b

HRMS spectra of 5b
$^1$H NMR spectra of 5c

$^{13}$C NMR spectra of 5c
HRMS spectra of 5c

\[ \text{HRMS spectra of } 5c \]

\[ \text{H NMR spectra of } 6a \]
$^{13}$C NMR spectra of 6a

HRMS spectra of 6a
$^1$H NMR spectra of 6b

$^{13}$C NMR spectra of 6b
HRMS spectra of 6b

\[ \text{HRMS spectra of 6b} \]

\[ \text{\textsuperscript{1}H NMR spectra of 6c} \]

\[ \text{\textsuperscript{1}H NMR spectra of 6c} \]
$^{13}$C NMR spectra of 6c

HRMS spectra of 6c