Supporting Information

The role of lipophilicity in transmembrane anion transport

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General Procedures and Methods

Commercial reagents were used without any further purification. NMR spectra were recorded in Varian Mercury-300 MHz and Varian Unity Inova-400 MHz spectrometers. Chemical shifts are reported in ppm with using residual solvent peak as reference, coupling constants are reported in Hz. High resolution mass spectra (HRMS) were recorded on a Micromass Autospec S-2 spectrometer using EI at 70eV. 4-Methoxy-2,2'-bipyrrole-5-carboxaldehyde was prepared as described.¹

Synthesis of Tambjamine derivatives

Compounds 1-16 were synthesised using modifications of the previously reported method.² In a typical procedure, 4-Methoxy-2,2'-bipyrrole-5-carboxaldehyde (190 mg, 1 mmol,)¹ was mixed with the corresponding amine (1.3 mmol, 1.3 equivalents) in 10 mL of chloroform. 40 uL of acetic acid were added and the mixture stirred at 60 °C until TLC showed disappearance of the staring material. The reaction mixture was diluted with 40 mL of dichloromethane and washed with HCl 1M (3×25 mL). The organic fraction was dried over Na₂SO₄ and the solvent evaporated to yield 1-16 as yellow solids/oils in excellent yields.

(1) (Z)-1-(3-methoxy-5-(1H-pyrrol-2-yl)-2H-pyrrol-2-ylidene)-N-methylmethanamine hydrochloride

Yield (85%).

¹H NMR (300 MHz, CDCl₃): δ = 13.59 (br s, 1H), 10.57 (br s, 1H), 9.24 (br s, 1H), 7.28 (d, *J* = 14.7 Hz, 1H), 7.06 (m, 1H), 6.74 (m, 1H), 6.28 (m, 1H), 5.93 (m, 1H), 3.92 (s, 3H), 3.26 (d, *J* = 4.7 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃): δ = 164.02, 142.56, 141.75 (CH), 124.28 (CH), 122.85, 113.43 (CH), 111.11 (CH), 111.00, 91.36 (CH), 58.72 (CH₃), 37.02 (CH₃). HRMS (EI) m/z [M]+ calcd for [C₁₁H₁₃N₃O] 203.1053; found: 203.1058

(2) (Z)-N-((3-methoxy-5-(1H-pyrrol-2-yl)-2H-pyrrol-2-ylidene)methyl)ethanamine, Tambjamine D

Yield (86%). UV-Vis (CHCl₃): λ_{max} 415nm ($\epsilon = 41356 \text{ M}^{-1}\text{cm}^{-1}$)

¹H-NMR (CDCl₃, 300MHz): δ = 13.63 (s, br, 1H) , 10.59 (s, br, 1H), 9.46 (d, br, 1H), 7.36 (d, *J* = 15.0 Hz, 1H), 7.06 (m, 1H), 6.73 (m, 1H), 6.28 (m, 1H), 5.94 (m, 1H), 3.93 (s, 3H), 3.56 (m, 2H), 1.42 7.36 (t, *J* = 7.3 Hz, 3H). ¹³C-NMR (CDCl₃, 75 MHz): δ = 163.95, 142.26, 140.22(CH), 123.92 (CH), 122.87, 113.19, 110.92 (CH), 110.90, 91.44 (CH), 58.69 (CH₃), 45.56 (CH₂), 15.60 (CH₃). HRMS (EI) m/z calcd for [C₁₂H₁₅N₃O] 217.12096; found: 217.1211

(3) (Z)-N-((3-methoxy-5-(1H-pyrrol-2-yl)-2H-pyrrol-2-ylidene)methyl)propan-1-amine hydrochloride

Yield (90%).

¹H NMR (300 MHz, CDCl₃): δ = 13.43 (br s, 1H), 10.58 (br s, 1H), 9.38 (d, *J* = 13.9 Hz, 1H), 7.28 (d, *J* = 14.9 Hz, 1H), 6.97 (m, 1H), 6.72 (m, 1H), 6.21 (m, 1H), 5.93 (m, 1H), 3.83 (s, 3H), 3.38 (m, 2H), 1.72 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H).

¹³C NMR (75 MHz, CDCl₃): δ = 163.98, 142.33, 140.65 (CH), 124.05 (CH), 122.88, 113.27 (CH), 110.93 (CH), 110.89, 91.40 (CH), 58.70 (CH₃), 52.76 (CH₂), 23.83 (CH₂), 11.31 (CH₃). HRMS (EI) m/z [M]+ calcd for [C₁₃H₁₇N₃O] 231.1366; found:231.1370

¹ K. Dairi, S. Tripathy, G. Attardo, J.-F. Lavallee, *Tetrahedron Lett.* 2006, 47, 2605.

² D. M. Pinkerton, M. G. Banwell, A. C. Willis, Org. Lett. 2007, 9, 5127.

(4) (Z)-N-((3-methoxy-5-(1H-pyrrol-2-yl)-2H-pyrrol-2-ylidene)methyl)butan-1-amine hydrochloride

Yield (91%).

¹H NMR (300 MHz, CDCl₃): δ = 13.37 (br s, 1H), 10.58 (br s, 1H), 9.38 (d, *J* = 14.4 Hz, 1H), 7.26 (d, *J* = 14.9 Hz, 1H), 6.95 (m, 1H), 6.71 (m, 1H), 6.20 (m, 1H), 5.94 (m, 1H), 3.81 (s, 3H), 3.40 (m, 2H), 1.65 (m, 2H), 1.36 (m, 2H), 0.88 (t, *J* = 7.3 Hz, 3H).

¹³C NMR (75 MHz, CDCl₃): δ = 163.94, 142.28, 140.60 (CH), 123.99 (CH), 122.87, 113.23 (CH), 110.92 (CH), 110.86, 91.42 (CH), 58.69 (CH₃), 50.86 (CH₂), 32.42 (CH₂), 19.90 (CH₂), 13.79 (CH₃). HRMS (EI) m/z [M]+ calcd for [C₁₄H₁₉N₃O] 245.15226; found: 245.1523

(5) (Z)-N-((3-methoxy-5-(1H-pyrrol-2-yl)-2H-pyrrol-2-ylidene)methyl)pentan-1-amine hydrochloride

Yield (86%).

¹H NMR (300 MHz, CDCl₃): δ = 13.44 (br s, 1H), 10.58 (br s, 1H), 9.38 (br s, 1H), 7.26 (d, *J* = 14.9 Hz, 1H), 6.95 (m, 1H), 6.71 (m, 1H), 6.19 (m, 1H), 5.93 (m, 1H), 3.82 (s, 3H), 3.38 (m, 2H), 1.68 (m, 2H), 1.30 (m, 4H), 0.84 (t, *J* = 4.8 Hz, 3H).

¹³C NMR (75 MHz, CDCl₃): δ = 163.94, 142.31, 140.56 (CH), 123.98 (CH), 122.89, 113.23 (CH), 110.91, 91.38 (CH), 58.67 (CH₃), 51.10 (CH₂), 30.12 (CH₂), 28.75 (CH₂), 22.35 (CH₂), 14.06 (CH₃). HRMS (EI) m/z [M]+ calcd for [C₁₅H₂₁N₃O] 259.1679; found: 259.1679

(6) (Z)-N-((3-methoxy-5-(1H-pyrrol-2-yl)-2H-pyrrol-2-ylidene)methyl)hexan-1-amine hydrochloride

Yield (92%).

¹H NMR (300 MHz, CDCl₃): $\delta = 13.50$ (br s, 1H), 10.58 (br s, 1H), 9.37 (br s, 1H), 7.29 (d, J = 14.9 Hz, 1H), 7.00 (m, 1H), 6.72 (m, 1H), 6.23 (m, 1H), 5.93 (m, 1H), 3.86 (s, 3H), 3.43 (m, 2H), 1.71 (m, 2H), 1.31 (m, 6H), 0.85 (t, J = 6.5 Hz, 3H).

¹³C NMR (75 MHz, CDCl₃): δ = 163.92, 142.33, 140.54 (CH), 124.14 (CH), 122.89, 113.28 (CH), 110.92, 110.86, 91.35 (CH), 58.69 (CH₃), 51.21 (CH₂), 31.46 (CH₂), 30.45 (CH₂), 26.37 (CH₂), 22.68 (CH₂), 14.23 (CH₃).

HRMS (EI) m/z [M]+ calcd for $[C_{16}H_{23}N_3O]$ 273.1836; found: 273.1846

(7) (Z)-N-((3-methoxy-5-(1H-pyrrol-2-yl)-2H-pyrrol-2-ylidene)methyl)heptan-1-amine hydrochloride

Yield (90%).

¹H NMR (300 MHz, CDCl₃): δ = 13.49 (br s, 1H), 10.58 (br s, 1H), 9.41 (d, *J* = 14.3 Hz, 1H), 7.27 (d, *J* = 14.9 Hz, 1H), 6.97 (m, 1H), 6.71 (m, 1H), 6.21 (m, 1H), 5.93 (m, 1H), 3.84 (s, 3H), 3.38 (m, 2H), 1.69 (m, 2H), 1.28 (m, 8H), 0.82 (t, *J* = 6.6 Hz, 3H).

¹³C NMR (75 MHz, CDCl₃): δ = 163.93, 142.32, 140.54 (CH), 124.03 (CH), 122.90, 113.24 (CH), 110.90, 91.35 (CH), 58.67 (CH₃), 51.15 (CH₂), 31.78 (CH₂), 30.46 (CH₂), 28.95 (CH₂), 26.64 (CH₂), 22.74 (CH₂), 14.22 (CH₃).

HRMS (EI) m/z [M]+ calcd for $[C_{17}H_{25}N_3O]$ 287.1992; found: 287.2001

(8) (Z)-N-((3-methoxy-5-(1H-pyrrol-2-yl)-2H-pyrrol-2-ylidene)methyl)octan-1-amine hydrochloride

Yield (92%).

¹H NMR (300 MHz, CDCl₃): δ = 13.42 (br s, 1H), 10.58 (br s, 1H), 9.38 (d, *J* = 14.3 Hz, 1H), 7.27 (d, *J* = 14.9 Hz, 1H), 6.97 (m, 1H), 6.71 (m, 1H), 6.21 (m, 1H), 5.93 (m, 1H), 3.83 (s, 3H), 3.40 (m, 2H), 1.68 (m, 2H), 1.27 (m, 10H), 0.82 (t, *J* = 6.4 Hz, 3H).

¹³C NMR (75 MHz, CDCl₃): δ = 163.91, 142.26, 140.56 (CH), 124.04 (CH), 122.88, 113.23 (CH), 110.91, 110.86, 91.38 (CH), 58.69 (CH₃), 51.18 (CH₂), 31.95 (CH₂), 29.30 (2 C) (CH₂), 29.28 (CH₂), 26.70 (CH₂), 22.82 (CH₂), 14.23 (CH₃).

HRMS (EI) m/z [M]+ calcd for $[C_{18}H_{27}N_3O]$ 301.2149; found: 301.2150

(9) (Z)-N-((3-methoxy-5-(1H-pyrrol-2-yl)-2H-pyrrol-2-ylidene)methyl)nonan-1-amine hydrochloride

Yield (93%).

¹H NMR (300 MHz, CDCl₃): $\delta = 13.41$ (br s, 1H), 10.60 (br s, 1H), 9.41 (d, J = 11.9 Hz, 1H), 7.26 (d, J = 14.7 Hz, 1H), 6.94 (m, 1H), 6.71 (m, 1H), 6.19 (m, 1H), 5.93 (m, 1H), 3.81 (s, 3H), 3.39 (m, 2H), 1.67 (m, 2H), 1.26 (m, 10H), 0.81 (t, J = 6.7 Hz, 3H).

¹³C NMR (75 MHz, CDCl₃): δ = 163.93, 142.30, 140.55 (CH), 124.95 (CH), 122.89, 113.21 (CH), 110.89, 91.39 (CH), 58.66 (CH₃), 51.12 (CH₂), 31.97 (CH₂), 30.43 (CH₂), 29.54 (CH₂), 29.37 (CH₂), 29.28 (CH₂), 26.66 (CH₂), 22.81 (CH₂), 14.27 (CH₃). MS (EI) m/z [M]+ calcd for [C₁₉H₂₉N₃O] 315.2305; found: 315.23

(10) (Z)-N-((3-methoxy-5-(1H-pyrrol-2-yl)-2H-pyrrol-2-ylidene)methyl)decan-1-amine hydrochloride

Yield (89%).

¹H NMR (300 MHz, CDCl₃): δ = 13.44 (br s, 1H), 10.59 (br s, 1H), 9.40 (d, *J* = 14.1 Hz, 1H), 7.27 (d, *J* = 14.8 Hz, 1H), 6.98 (m, 1H), 6.71 (m, 1H), 6.21 (m, 1H), 5.92 (m, 1H), 3.84 (s, 3H), 3.41 (m, 2H), 1.69 (m, 2H), 1.28 (m, 14H), 0.82 (t, *J* = 6.5 Hz, 3H).

¹³C NMR (75 MHz, CDCl₃): δ = 163.92, 142.30, 140.54 (CH), 124.06 (CH), 122.88, 113.25 (CH), 110.91, 91.36 (CH), 58.68 (CH₃), 51.17 (CH₂), 32.06 (CH₂), 30.47 (CH₂), 29.71 (CH₂), 29.62 (CH₂), 29.46 (CH₂), 29.31 (CH₂), 26.70 (CH₂), 22.87 (CH₂), 14.33 (CH₃). HRMS (EI) m/z [M]+ calcd for [C₂₀H₃₁N₃O] 329.24616; found: 329.2466

(11) (Z)-N-((3-methoxy-5-(1H-pyrrol-2-yl)-2H-pyrrol-2-ylidene)methyl)dodecan-1-amine hydrochloride, BE-18591

Yield (86%). UV-Vis (CHCl₃): λ_{max} 415 nm (ϵ = 48386 M⁻¹cm⁻¹)

¹H-NMR (CDCl₃, 300MHz): $\delta = 13.66$ (s, br, 1H) , 10.60 (s, br, 1H), 9.44 (m, 1H), 7.33 (d, J = 15.0 Hz, 1H), 7.06 (m, 1H), 6.73 (m, 1H), 6.27 (m, 1H), 5.94 (m, 1H), 3.92 (s, 3H), 3.47 (m, 2H), 1.75 (m, 2H), 1.24 (br, 15H), 0.87 (t, J = 6.6 Hz, 3H).

¹³C-NMR (CDCl₃, 75 MHz): δ = 163.53, 141.89, 140.16(CH), 123.60 (CH), 122.48, 112.82(CH), 110.52 (CH), 110.47, 91.00 (CH), 58.28 (CH₃), 50.76 (CH₂), 31.70 (CH₂), 30.08 (CH₂), 29.41 (CH₂), 29.36 (CH₂), 29.23 (CH₂), 19.14 (CH₂), 28.92 (CH₂), 26.30 (CH₂), 22.48 (CH₂), 13.94 (CH₃). MS (EI) m/z calcd for [C₂₂H₃₅N₃O] 357.2775; found: 357.27

(12) (Z)-N-((3-methoxy-5-(1H-pyrrol-2-yl)-2H-pyrrol-2-ylidene)methyl)-2-methylpropan-1-amine hydrochloride, Tambjamine C

Yield (88%). UV-Vis (CHCl₃): λ_{max} 415nm (ε = 60087 M⁻¹cm⁻¹) ¹H-NMR (CDCl₃, 300MHz): δ = 13.43 (s, br, 1H), 10.61 (s, br, 1H), 9.35 (m, 1H), 7.23 (d, *J* = 14.8 Hz, 1H), 6.95 (m, 1H), 6.72 (m, 1H), 6.20 (m, 1H), 5.95 (m, 1H), 3.82 (s, 3H), 3.22 (t, *J* = 6.4, 2H), 1.94 (hp, *J* = 6.7 Hz, 1H), 0.95 (d, *J* = 6.7 Hz, 6H). ¹³C-NMR (CDCl₃, 75 MHz): δ = 164.07, 142.40, 140.91(CH),

124.06 (CH), 122.85, 113.32(CH), 110.93 (CH), 110.81, 91.45 (CH), 58.75 (CH₂), 58.71 (CH₃), 29.73 (CH), 20.02 (CH₃). HRMS (EI) m/z calcd for [C₁₄H₁₉N₃O] 245.1523; found: 245,1528.

(13) (Z)-N-((3-methoxy-5-(1H-pyrrol-2-yl)-2H-pyrrol-2-ylidene)methyl)-3-methylbutan-1-amine hydrochloride, Tambjamine K

Yield (93%).

¹H NMR (300 MHz, CDCl₃): δ = 13.41 (br s, 1H), 10.57 (br s, 1H), 9.37 (d, *J* = 14.4 Hz, 1H), 7.27 (d, *J* = 14.9 Hz, 1H), 6.97 (m, 1H), 6.72 (m, 1H), 6.21 (m, 1H), 5.93 (m, 1H), 3.83 (s, 3H), 3.43 (m, 2H), 1.65 (m, 4H), 0.89 (d, *J* = 6.4 Hz, 6H).

¹³C NMR (75 MHz, CDCl₃): δ = 163.90, 142.28, 140.54 (CH), 124.05 (CH), 122.88, 113.25 (CH), 110.92 (2 peaks) (CH), 91.39 (CH), 58.70 (CH₃), 49.39 (CH₂), 39.11 (CH₂), 25.64 (CH), 22.50 (CH₃). HRMS (EI) m/z [M]+ calcd for [C₁₅H₂₁N₃O] 259.1679; found: 259.1681

(14) (Z)-N-((3-(benzyloxy)-5-(1H-pyrrol-2-yl)-2H-pyrrol-2-ylidene)methyl)propan-1-amine hydrochloride

Yield (83%).

¹H-NMR (CDCl₃, 300MHz): $\delta = 13.57$ (br s, 1H), 10.61 (s, br, 1H), 9.48 (d, J = 13.8 Hz, 1H), 7.40-7.32 (6H), 7.00 (m, 1H), 6.73 (m, 1H), 6.25 (m, 1H), 6.02 (m, 1H), 5.10 (s, 2H), 3.40 (m, 2H), 1.75 (m, 2H), 0.99 (d, J = 7.4 Hz, 3H).

¹³C-NMR (CDCl₃, 75 MHz): δ = 162.77, 142.34, 140.82 (CH), 135.18, 129.09 (CH), 129.04 (CH), 128.26 (CH), 124.24 (CH), 122.88, 113.35 (CH), 111.15, 110.95 (CH), 92.28 (CH), 73.62 (CH₂), 52.84 (CH₂), 23.88 (CH₂), 11.34 (CH₃).

HRMS (EI) m/z calcd for [C₁₉H₂₁N₃O] 307.1679; found: 307.1679

(**15**) (Z)-N-((3-(benzyloxy)-5-(1H-pyrrol-2-yl)-2H-pyrrol-2-ylidene)methyl)pentan-1-amine hydrochloride

Yield (81%).

¹H NMR (300 MHz, CDCl₃): $\delta = 13.59$ (br s, 1H), 10.61 (br s, 1H), 9.49 (d, J = 14.5 Hz, 1H), 7.39 (m, 5H), 7.35 (d, J = 15.0 Hz, 1H), 6.99 (m, 1H), 6.73 (m, 1H), 6.24 (m, 1H), 6.02 (m, 1H), 5.09 (s, 2H), 3.41 (m, 2H), 1.73 (m, 2H), 1.24 (m, 14H), 0.88 (t, J = 6.6 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃): $\delta = 162.73$, 142.31, 140.74 (CH), 135.22, 129.05 (CH), 129.01 (CH), 128.21 (CH), 124.17 (CH), 122.90, 113.30 (CH), 111.20, 110.93 (CH), 92.27 (CH), 73.62 (CH₂), 51.20 (CH₂), 30.17 (CH₂), 28.79 (CH₂), 22.39 (CH₂), 14.10 (CH₃).

HRMS (EI) m/z [M]+ calcd for [C₂₁H₂₅N₃O] 335.1992; found: 335.2008

(16) (Z) - N - ((3 - (benzy loxy) - 5 - (1H - pyrrol - 2 - yl) - 2H - pyrrol - 2 - ylidene) methyl) decan - 1 - amine hydrochloride

Yield (83%).

¹H NMR (300 MHz, CDCl₃): $\delta = 13.62$ (br s, 1H), 10.61 (br s, 1H), 9.49 (d, J = 14.5 Hz, 1H), 7.42 (m, 5H), 7.35 (d, J = 15.0 Hz, 1H), 7.03 (m, 1H), 6.72 (m, 1H), 6.26 (m, 1H), 6.01 (m, 1H), 5.11 8 s, 2H), 3.44 (m, 2H), 1.73 (m, 2H), 1.24 (m, 14H), 0.86 (t, J = 6.6 Hz, 3H).

¹³C NMR (75 MHz, CDCl₃): δ = 162.72, 142.33, 140.71 (CH), 135.20, 129.09 (CH), 129.04 (CH), 128.23 (CH), 124.30 (CH), 122.88, 113.32 (CH), 111.18, 110.92 (CH), 92.21 (CH), 73.62 (CH₂), 51.28 (CH₂), 32.08 (CH₂), 30.53 (CH₂), 29.73 (CH₂), 29.65 (CH₂), 29.48 (CH₂), 29.33 (CH₂), 26.74 (CH₂), 22.90 (CH₂), 14.35 (CH₃).

HRMS (EI) m/z [M]+ calcd for [C₂₆H₃₅N₃O] 405.2775; found: 405.2782

Anion binding titrations

In order to estimate an association constant in solution the perchlorate salts of compounds 2 and 7 were prepared by successive treatment a dichloromethane solutions of the corresponding hydrochloride salts with diluted NaOH followed by diluted HClO₄ (three times). These two compounds were titrated with tetrabutyl ammonium chloride in d_6 -DMSO. And the data processed using WinEQNMR 2 software.³

³ M. J. Hynes, J. Chem. Soc. Dalton Trans., 1993, 311.



Fig. S1 Stack plot of ¹H-NMR (d_6 -DMSO, 300MHz) of compound **2**.HClO₄ upon addition of increasing ammounts of TBACl.



Fig. S2 Stack plot of ¹H-NMR (d_6 -DMSO, 300MHz) of compound **2**.HClO₄ upon addition of increasing ammounts of TBACl (downfield region).



Fig. S3 Changes in the chemical shift corresponding to NH1 (Figure S10) are fitted to a 1:1 model using WinEQNMR2. K_a = 721 (±87) M⁻¹.



Fig. S4 Stack plot of ¹H-NMR (d_6 -DMSO, 300MHz) of compound **7**.HClO₄ upon addition of increasing ammounts of TBACl.



Fig. S5 Stack plot of ¹H-NMR (d_6 -DMSO, 300MHz) of compound **7**.HClO₄ upon addition of increasing ammounts of TBACl (downfield region).



Fig. S6 Changes in the chemical shift corresponding to NH1 (Figure S10) are fitted to a 1:1 model using WinEQNMR2. K_a = 795 (±61) M⁻¹.

Lipophilicity calculations

Lipophilicity is a parameter of prime importance in drug discovery. It is essential for predicting ADMET properties and druglikeness of a given molecule. Therefore, there is an enormous interest in developing computational methods of calculating logP. LogP (the octanol/water partition coefficient) is the parameter most commonly used for estimating the lipophilicity of a compound. LogP can be experimentally obtained by the traditional shake-flask method.⁴ Nevertheless this is time consuming and intensive research efforts in developing computational methods to estimate logP values of small molecules have been made.⁵ There are a number of methods to calculate the logP values of a substance. Essentially they can be classified as substructure based methods and properties based methods. Substructure based methods rely in calculations of logP values of fragments/atoms of the molecule and expressing the final logP value as the sum of the individual contributions of the different fragments. Properties based methods use descriptions of the whole molecule. VCCLab software allows the calculations of LogP using different methods such as ALOGPS, AC LogP, ALOGP, MLOGP, KowWIN, XLOPGP2 or XLOGP3, and we used consensus LogP as the average of these values.⁶ Four different forms of the anion transporters were considered for the LogP calculations, corresponding to neutral and protonated forms of these compounds (Fig. S7). The results are summarised in Table S1. There is a linear correlation between calculated logP for forms I-IV for all compounds, therefore plots of logP vs transport activity only differed in the location of the logP value corresponding to the best performance compounds and an increase of the uncertainty of logP values intrinsic to protonated forms (Fig. S8).



Fig. S7 Tautomeric forms I-IV used for lipophilicity calculations.

⁴ A. Leo, C. Hansch, D. Elkins, *Chem. Rev.*, 1971, **71**, 525.

⁵ R. Mannhold, G. I. Poda, C. Ostermann, I. V. Tetko, J. Pharm. Sci., 2009, 98, 861.

⁶ (a) I. V. Tetko, J. Gasteiger, R. Todeschini, A. Mauri, D. Livingstone, P. Ertl, V. A. Palyulin, E. V. Radchenko, N. S. Zefirov, A. S. Makarenko, V. Y. Tanchuk, V. V. Prokopenko, *Comput. Aid. Mol. Des.*, **2005**, 19, 453; (b) VCCLAB, Virtual Computational Chemistry Laboratory, http://www.vcclab.org, 2005.

Compound	LogP I	LogP II	LogP III	LogP IV
1	0.93 ± 0.46	1.50 ± 0.62	0.08 ± 1.57	0.65 ± 1.21
2	1.34 ±0.51	1.86 ±0.63	0.45 ± 1.53	1.00 ± 1.16
3	1.78 ± 0.52	2.30 ± 0.66	0.85 ± 1.48	1.40 ± 1.13
4	2.22 ± 0.57	2.73 ±0.71	1.25 ± 1.46	1.79 ± 1.13
5	2.69 ±0.63	3.19 ±0.77	1.67 ± 1.47	2.22 ± 1.17
6	3.16 ±0.71	3.64 ±0.83	$2.10\pm\!\!1.50$	2.63 ± 1.22
7	3.63±0.79	4.10 ± 0.91	2.52 ± 1.54	3.05 ± 1.30
8	4.26 ± 0.69	4.70 ±0.73	3.06 ± 1.61	3.59 ± 1.33
9	4.70±0.74	5.10 ± 0.80	3.47±1.66	4.01 ± 1.42
10	5.13±0.81	5.63±0.89	3.88±1.74	4.41±1.53
11	6.09 ± 0.92	6.52 ± 1.07	4.71 ±1.91	5.23 ± 1.78
12	2.11 ±0.56	2.62 ± 0.65	1.16 ± 1.45	1.71 ± 1.10
13	2.59 ± 0.64	3.07 ±0.71	1.58 ± 1.49	2.12 ± 1.16
14	3.44±0.69	3.81±0.78	$2.24{\pm}1.42$	2.78 ± 1.14
15	4.20 ± 0.78	4.68 ±0.92	3.04 ± 1.49	3.57 ±1.29
16	6.64±0.96	7.05 ± 1.08	5.21±1.93	5.73±1.84

Table S1 Calculated LogP values for compounds 1-16.



Fig. S8: Representation of logP values calculated for compounds 1-16 using the different tautomeric forms I-IV vs transport activity.

We also gathered experimental data for measuring the lipophilicity of compounds **1-16** and checking the reliability of calculated logP values. An easy, yet reliable method, for determine the relative lipophilicity of a class of molecules involve the use of HPLC and reverse phase column.⁷ The retention time of each

 ⁷ (a) L.-G. Danielsson, Y.-H. Zhang, trends in analytical chemistry, 1996, 15, 188-196; (b) D. Henry, J. H. Block, J. L. Anderson, G. R. Carlson, J. Med. Chem., 1976, 19, 619; (c) K. Valkó, J. Chromatogr. A, 2004, 1037, 299.

molecule on the reverse phase column is related to its lipophilicity. The products were dissolved in MeOH at concentration of 1 to 2 mM and injected (2uL) on a phenomenex kromasil c8 (100Å, 250x4.6mm, 5 micron) using an Agilent 1100 HPLC. They were eluted using a linear gradient of 20% to 95% of acetonitrile in water in 30 min (Both solvent were modified by 0.1% of formic acid). The peaks were detected at 254nm with reference on 550nm.

The retention time of each molecule was plotted against the calculated logP showing a linear correlation, proving that the calculated logP are reliable (Fig. S9).



Fig. S9 Representation of retention times vs calculated logP values for compounds 1-16, showing the linear relationships between both parameters.

Membrane transport assays

Preparation of Phospholipid Vesicles. A chloroform solution (20 mg/mL) of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (Sigma-Aldrich) was evaporated *in vacuo* using a rotary evaporator and the lipid film obtained was dried under high vacuum for at least 2 hours. The lipid film was rehydrated by addition of a sodium chloride solution (451 mM NaCl and 20 mM phosphate buffer, pH 7.2) followed by vortexing. The lipid suspension was then subjected to nine freeze-thaw cycles and twenty-nine extrusions through a 200 nm polycarbonate Nucleopore membrane using a LiposoFast Basic extruder (Avestin, Inc.). The resulting unilamellar vesicles were dialyzed against Na₂SO₄ solution (150 mM Na₂SO₄ and 20 mM phosphate buffer, pH 7.2) to remove unencapsulated chloride.

ISE Transport Assays. Unilamellar POPC vesicles (200 nm mean diameter) containing an encapsulated solution of 451 mM NaCl and 20 mM phosphate buffer, pH 7.2, were suspended in a solution 150 mM Na₂SO₄ and 20 mM phosphate buffer, pH 7.2, for a final lipid concentration of 0.5 mM and a total volume of 5 mL. A DMSO solution of the carrier molecule, typically 5 μ L to avoid influence of the solvent molecules in the assay, was added, followed by a solution of NaHCO₃ (500 mM in Na₂SO₄ buffered to pH 7.2 with 20 mM sodium phosphate salts) for a final bicarbonate concentration of 40 mM. The chloride release from vesicles was monitored using a sympHony chloride selective electrode. At the end of the experiment the vesicles were lysed with detergent (triton-X 10% dispersion in water, 60 μ L) to release all chloride ions; the resulting value was considered to represent 100% release and used as such.



Fig. S10 Chloride efflux upon addition of 1-5 (1 μ M, 0.2 % molar carrier to lipid) to vesicles composed of POPC. The vesicles contained NaCl (451 mM NaCl and 20 mM phosphate buffer, pH 7.2) and were immersed in Na₂SO₄ (150 mM Na₂SO₄ and 20 mM phosphate buffer, pH 7.2). At the end of the experiment the vesicles were lysed with detergent to release all chloride ions and the resulting value was considered to represent 100% release and used as such. Each trace represents the average value of three independent experiments.



Fig. S11 Fitting of the initial slope resulting from the chloride efflux promoted by 1-5 under the conditions described in Fig. S10 used for comparative purposes.



Fig. S12 Chloride efflux upon addition of **6-10** (1 μ M, 0.2 % molar carrier to lipid) to vesicles composed of POPC. The vesicles contained NaCl (451 mM NaCl and 20 mM phosphate buffer, pH 7.2) and were immersed in Na₂SO₄ (150 mM Na₂SO₄ and 20 mM phosphate buffer, pH 7.2). At t=0 a NaHCO₃ solution to (500 mM in Na₂SO₄ buffered to pH 7.2 with 20 mM sodium phosphate salts) was added for a final concentration of 40 mM and the chloride efflux was monitored for 5 minutes. The vesicles were lysed with detergent to release all chloride ions and the resulting value was considered to represent 100% release and used as such. Each trace represents the average value of three independent experiments.



Fig. S13 Fitting of the initial slope resulting from the chloride efflux promoted by **1-6** under the conditions described in Fig. S12 used for comparative purposes.



Fig. S14 Chloride efflux upon addition of **11-16** (0.1 μ M, 0.02 % molar carrier to lipid) to vesicles composed of POPC. The vesicles contained NaCl (476 mM NaCl and 10 mM phosphate buffer, pH 7.2) and were immersed in NaNO₃ (476 mM NaNO₃ and 10 mM phosphate buffer, pH 7.2). Once the electrode reading was stable the carrier was added and the chloride efflux was monitored for 5 minutes. At the end of the experiment the vesicles were lysed with detergent to release all chloride ions and the resulting value was considered to represent 100% release and used as such.



Fig. S15 Fitting of the initial slope resulting from the chloride efflux promoted by **11-16** under the conditions described in Fig. S14 used for comparative purposes.

¹³C NMR liposome Assays.

6 mL of a chloroform solution of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (20 mg/mL) (Sigma-Aldrich) was evaporated *in vacuo* using a rotary evaporator and the lipid film obtained was dried under high vacuum for at least 2 hours. The lipid film was rehydrated by addition of 2 mL of NaH¹³CO₃ 500 mM followed by careful vortexing. The lipid suspension was then subjected to nine freeze-thaw cycles and seven extrusions through a 200 nm polycarbonate Nucleopore membrane using a LiposoFast Basic extruder (Avestin, Inc.). The resulting suspension of vesicles was dialyzed against Na₂SO₄ solution (150 mM Na₂SO₄ and 20 mM phosphate buffer, pH 7.2 to exchange unencapsulated bicarbonate. 380 µL of this suspension were placed in a NMR sample and 40 µL of D₂O added. An initial ¹³C NMR was acquired (400 pulses). A NaCl was added next for a external chloride concentration of 50 mM followed by 5 µL of a 10 mM solution of the compound studied in DMSO. After 5 minutes a ¹³C NMR was acquired (400 pulses). Finally 5 µL of a 40 mM solution of MnCl₂ were added and a ¹³C NMR was acquired (400 pulses).



Fig. S16 ¹³C NMR spectra evidencing the facilitated bicarbonate/chloride exchange. a) POPC vesicles loaded with 500 mM NaH¹³CO₃ dispersed in 162 mM Na₂SO₄ buffered at pH 7.2 with 20 mM phosphate. b) After addition of NaCl (50 mM) and active tambjamine carrier **8** (0.16 % mol carrier to lipid concentration). c) After addition of MnCl₂ (0.5 mM) a paramagnetic reagent affecting only extravesicular $H^{13}CO_3^{-}$ anions the signal is broaden to the baseline.

Hill plot analyses. Transport assays were carried out using various concentrations of carriers using the conditions described above. The chloride efflux (%) 290 s was plotted as a function of the carrier concentration and the data fitted to the Hill equation: $y=V_{max} x^n / (k^n + x^n)$ where x is the carrier concentration, V_{max} is the maximum chloride efflux (100%) and y is the chloride efflux at 290 s (%). *n* is the Hill coefficient and *k* is the carrier concentration needed to reach 50% of chloride efflux *k* and *n* are the parameters to be fitted. The results are summarized in Table S2. *n* (Hill coefficient) values close to 1 supported the discrete carrier mechanism for the transport mediated by these compounds.



Table S2 Overview of hill analyses for compounds 7,9,14 and 15.



Fig. S17 Right: Chloride efflux upon addition of 7 (5-0.05 μ M, 1-0.01 % molar carrier to lipid) to vesicles composed of POPC. The vesicles contained NaCl (451 mM NaCl and 20 mM phosphate buffer, pH 7.2) and were immersed in Na₂SO₄ (150 mM Na₂SO₄ and 20 mM phosphate buffer, pH 7.2). At t=0 a NaHCO₃ solution to (500 mM in Na₂SO₄ buffered to pH 7.2 with 20 mM sodium phosphate salts) was added for a final concentration of 40 mM and the chloride efflux was monitored for 5 minutes. The vesicles were lysed with detergent to release all chloride ions and the resulting value was considered to represent 100% release and used as such; Left: Hill plot corresponding to chloride efflux under these conditions.



Fig. S18 Right: Chloride efflux upon addition of **9** (5-0.05 μ M, 1-0.01 % molar carrier to lipid) to vesicles composed of POPC. The vesicles contained NaCl (451 mM NaCl and 20 mM phosphate buffer, pH 7.2) and were immersed in Na₂SO₄ (150 mM Na₂SO₄ and 20 mM phosphate buffer, pH 7.2). At t=0 a NaHCO₃ solution to (500 mM in Na₂SO₄ buffered to pH 7.2 with 20 mM sodium phosphate salts) was added for a final concentration of 40 mM and the chloride efflux was monitored for 5 minutes. The vesicles were

lysed with detergent to release all chloride ions and the resulting value was considered to represent 100% release and used as such; Left: Hill plot corresponding to chloride efflux under these conditions.



Fig S19 Right: Chloride efflux upon addition of **14** (5-0.05 μ M, 1-0.01 % molar carrier to lipid) to vesicles composed of POPC. The vesicles contained NaCl (451 mM NaCl and 20 mM phosphate buffer, pH 7.2) and were immersed in Na₂SO₄ (150 mM Na₂SO₄ and 20 mM phosphate buffer, pH 7.2). At t=0 a NaHCO₃ solution to (500 mM in Na₂SO₄ buffered to pH 7.2 with 20 mM sodium phosphate salts) was added for a final concentration of 40 mM and the chloride efflux was monitored for 5 minutes. The vesicles were lysed with detergent to release all chloride ions and the resulting value was considered to represent 100% release and used as such; Left: Hill plot corresponding to chloride efflux under these conditions.



Fig S20 Right: Chloride efflux upon addition of **15** (5-0.05 μ M, 1-0.01 % molar carrier to lipid) to vesicles composed of POPC. The vesicles contained NaCl (451 mM NaCl and 20 mM phosphate buffer, pH 7.2) and were immersed in Na₂SO₄ (150 mM Na₂SO₄ and 20 mM phosphate buffer, pH 7.2). At t=0 a NaHCO₃ solution to (500 mM in Na₂SO₄ buffered to pH 7.2 with 20 mM sodium phosphate salts) was added for a final concentration of 40 mM and the chloride efflux was monitored for 5 minutes. The vesicles were lysed with detergent to release all chloride ions and the resulting value was considered to represent 100% release and used as such; Left: Hill plot corresponding to chloride efflux under these conditions.

Computational Details

The protonated tambjamine derivatives **1-11** were described with default parameters from the General AMBER Force Field (GAFF),⁸ apart the bond length between ylidene and bipyrrole fragments and the ylidene bond length, which were changed based on the crystal structure data. The ideal bond lengths and the corresponding force constants are given in Figure S21 with the atom types for bipyrrole-enamine moiety transport unit.



Fig S21 GAFF atom types for the bipyrrole-enamine moiety assigned by *antechamber*. The cc-ce bond length was changed to 1.3700 Å and the force constant to 440.30 kcalmol⁻¹Å⁻², while for ce-nh the corresponding bond stretching terms were changed to 1.3120 Å and 534.90 kcalmol⁻¹Å⁻², respectively.

The Molecular Dynamics (MD) studies were carried out with the AMBER software package⁹ using the following protocol:

Multi-conformational RESP charge fitting of the transporters.

Due to the conformational flexibility imposed by the R alkyl substituents and as well as the *syn* and *anti* configurations that the bipyrrole moiety can assume derived from its eventual fluxional behaviour, four distinct conformations of each transporter were used to calculate the atomic point charges by means of multi-conformational RESP charge fitting methodology. Therefore, the transporters **1-11** were initially geometry optimized at the HF/6-31G* level of theory using Gaussian09¹⁰ with a starting random structure. Subsequently, parameters from GAFF were assigned to the transporters and RESP atomic charges were fitted to the electrostatic potential obtained at the HF/6-31G* using 4 concentric layer of points per atom and 6 points per unit area (Gaussian IOP 6/33=2, 6/41=4, 6/42=6) in agreement with the methodology followed in the force field reference.⁸ Then, transporters **1-11** were submitted to a 3 ns MD run in the gas phase at 1000 K using sander,¹⁰ which allows a stochastic covering of the conformational space of the transporters, and saving a trajectory file composed of 30000 structures. All these structures were further minimized by molecular mechanics (MM), through 1000 steps of the steepest descent method, followed

⁸ (a) J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman and D. A. Case, *J Comput Chem*, 2004, **25**, 1157-1174. (b) J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman and D. A. Case, *Journal of Computational Chemistry*, 2005, **26**, 114-114.

⁹ D. A. Case, T. A. Darden, T.E. Cheatham III, C. L. Simmerling, J. Wang, R. E. Duke, R. Luo, R. C. Walker, W. Zhang, K. M. Merz, B. P. Roberts, B. Wang, S. Hayik, A. Roitberg, G. Seabra, I. Kolossvai, K. F. Wong, F. Paesani, J. Vanicek, J. Liu, X. Wu, S. R. Brozell, T. Steinbrecher, H. Gohlke, Q. Cai, X. Ye, J. Wang, M.-J. Hsieh, G. Cui, D. R. Roe, D. H. Mathews, M. G. Seetin, C. Sagui, V. Babin, T. Luchko, S. Gusarov, A. Kovalenko and P. A. Kollman, *AMBER 11*, (2010) University of California, San Francisco.

¹⁰ M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. J. A. Montgomery, J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, *Gaussian 09*, (2009) Gaussian, Inc., Pittsburgh PA.

by the conjugate gradient method until a convergence criterion of 0.0001 kcalmol⁻¹ was achieved. The MM minimized conformations of each transporter were then clustered by root-mean-square deviation (RMSD) similarity and 4 different conformations were chosen for each transporter.

These 4 conformations were again geometry optimized at the HF/6-31G* level of theory and the electrostatic potential was calculated for each of them, allowing the calculation of multi-conformational RESP atomic point charges, using identical weights for all conformations.

Simulations in Water and Surface Area calculations.

The lowest-energy conformation found in the previous step for tambjamine derivatives **1-11** was immersed in a cubic box composed of 2090 SPC/E model water¹¹ molecules using PACKMOL.¹² The solvent was initially relaxed, while keeping the solute fixed with a harmonic restraint of 500 kcalmol⁻¹ Å⁻², followed by a MM minimization off all system. Subsequently, the system was heated to 300 K during 50 ps using the Langevin thermostat with a collision frequency of 1 ps⁻¹ in an NVT ensemble. After 200 ps of equilibration in a NPT ensemble at 1 atm with isotropic pressure scaling using relaxation time of 2 ps, the data were collected during 25 ns for all transporters. The SHAKE algorithm was used to constrain all bonds involving hydrogen atoms, thus allowing the usage of 2 fs time step. A 10 Å cut-off was used for the non-bonded van der Waals interactions. Frames were saved every 1.0 ps leading to a trajectory file containing 25000 structures. All unconstrained MD simulations were carried out with the pmemd.cuda AMBER executable, able to accelerate explicit solvent Particle Mesh Ewald (PME)¹³ calculations through the use of GPUs.¹⁴

The polar surface area (PSA) was calculated over the 25000 frames, taking into account only the polar atoms (N and O) of the bipyrrole entity of tambjamine derivatives **1-11** and the N-H hydrogen atoms, using the Linear Combinations of Pairwise Overlaps (LCPO) algorithm¹⁵ as implemented in the *cpptraj* utility of Ambertools 1.5.¹⁰ The total surface area (TSA) was calculated in the same way, but taking into account all atoms of the transporters.

The results obtained are listed in Table S3 and plotted in Fig. S22, and demonstrate that the Total Surface Area increases with the increase of the R alkyl substituent, while the Polar Surface Area remains almost constant, regardless of the R substituent.

¹¹ H. J. C. Berendsen, J. R. Grigera and T. P. Straatsma, *J Phys Chem-Us*, 1987, **91**, 6269-6271.

¹² L. Martinez, R. Andrade, E. G. Birgin and J. M. Martinez, *J Comput Chem*, 2009, **30**, 2157-2164.

¹³ (a) T. Darden, D. York and L. Pedersen, J Chem Phys, 1993, 98, 10089-10092. (b) U. Essmann, L. Perera, M. L. Berkowitz, T. Darden, H. Lee and L. G. Pedersen, Journal of Chemical Physics, 1995, 103, 8577-8593.

¹⁴ (a) A. W. Goetz, R. Salomon-Ferrer, D. Poole, S. L. Grand and R. C. Walker, "Routine microsecond molecular dynamics simulations with AMBER – Part II: Particle Mesh Ewald" (*in preparation*), 2011. (b) see <u>http://ambermd.org/gpus/</u> (accessed in 2011/11/20)

¹⁵ J. Weiser, P. S. Shenkin and W. C. Still, Journal of Computational Chemistry, 1999, 20, 217-230.

Transporter	TSA (Ų)	PSA (Ų)
1	345.25 ± 4.57	$78.54{\pm}~1.45$
2	376.49 ± 3.95	$74.85{\pm}~1.08$
3	404.20 ± 6.86	$72.07{\pm}~1.49$
4	430.04 ± 9.85	$69.68{\pm}2.17$
5	463.80 ± 9.11	$70.64{\pm}~1.94$
6	$492.35{\pm}11.84$	$70.54{\pm}~2.04$
7	522.02 ± 12.51	$70.38{\pm}2.34$
8	$545.44{\pm}21.18$	$69.23{\pm}~3.47$
9	$576.76{\pm}22.10$	$69.70{\pm}~3.27$
10	597.16 ± 34.93	$68.64{\pm}~4.24$
11	641.31 ± 51.56	$67.60{\pm}~5.62$

Table S3. Computed TSA and PSA values for compounds 1-11 with the standard deviations (average \pm standard deviation).



Fig. S22 Calculated TSA (blue markers) and PSA (red markers) values for the different transporters.

Estimation of the relative binding free energies for the chloride-protonated tambjamine complexes.

The chloride anion in the protonated tambjamine associations 1-7 was described with van der Waals parameters developed to be used along the SPC/E water model¹⁶ and with a charge set to -1. The structures of the chloride protonated complexes were established in gas phase via a quenched molecular dynamics run of 1 ns following a protocol identical to described above for conformational analyses of free protonated tambjamine derivatives. The lowest energy binding arrangement of each complex or the corresponding free tambjamine was subsequently solvated with 1273 DMSO molecules¹⁷ affording a cubic box, which as further equilibrated under periodic conditions using a multistage protocol equivalent to that explained above for the simulations carried out in water solution with free protonated transporters **1-11**. After 200 ns of a NPT run performed with the PMEMD CUDA module within the AMBER11, the density of the cubic boxes were in agreement with the experimental density of the DMSO and then the system was considered equilibrated and suitable to be used in the subsequent constrained MD simulations.

¹⁶ I. S. Joung and T. E. Cheatham, *J Phys Chem B*, 2008, **112**, 9020-9041.

¹⁷ T. Fox and P. A. Kollman, J Phys Chem B, 1998, **102**, 8070-8079.

The relative binding free energies of the protonated tambjamines derivatives to the chloride anion ($\Delta\Delta G$ binding) were estimated by constrained MD simulations *via* thermodynamic integration using the thermodynamic cycle depicted in Fig. S23.



Where, the relative binding free energy of two tambjamine derivatives is given by:

$$\Delta \Delta G = \Delta G_3 - \Delta G_2 = \Delta G_4 - \Delta G_1 \tag{1}$$

The values of ΔG_3 and ΔG_2 were computationally assessed as follows: the R substituent of a tambjamine derivative (Tb₁) was alchemically mutated into the R substituent of another tambjamine (Tb₂), by coupling its Hamiltonian to a mutation variable (λ), which spanned from 0 to 1 along the mutation Tb₁ \rightarrow Tb₂. The corresponding free energy calculated by the thermodynamic integration is given by the integral:

$$\Delta G = G(\lambda = 1) - G(\lambda = 0) = \int_{\lambda=0}^{\lambda=1} \langle \frac{\partial v}{\partial \lambda} \rangle_{\lambda} d\lambda$$
⁽²⁾

where G and V represents the free and potential energy, respectively.

This transformation was performed independently for an isolated tambjamine or in the presence of the chloride ion, *i.e.*, for a tambjamine in its "free" and ion bounded states using a dual topology in three independent steps. In the first one, the R substituent of Tb_1 disappeared with the annihilation of their partial atomic charges; in second one, the van der Waals parameters of the R substituent of Tb_2 , keeping the atomic charges of both substituents switched off using soft-core potentials; and, finally, in third step the R substituent of Tb_2 appeared concomitantly with its charges.

The mutation was divided in nine windows with λ assuming the discrete values of 0.01592, 0.08198, 0.19331, 0.33787, 0.5, 0.66213, 0.80669, 0.91802 and 0.98408. Each window consisted of a constrained molecular dynamics simulation divided into an initial equilibration stage equivalent to the described above for the MD simulations performed in water solution, but using shorter simulation times for the initial NVT (50 ps) and NPT (150 ps) runs, followed by a data collection step of 300 ps for the charges designed simulation and 500 ps for the van der Waals specific simulations, carried out at 300 K using a NPT ensemble and 2 fs time step. The remaining simulation settings are the same used in the MD simulations carried out in the water solution.

The free energy given by the equation 2 was estimated through the Gaussian quadrature method, as defined in the AMBER11 manual,⁹ using selected λ values and the corresponding weights. Afterwards the relative free energies ($\Delta\Delta G = \Delta G_3$ - ΔG_2) were computed and their values are summarised in Table S4.

The tambjamines have comparable affinities for chloride, independently of the chain size length. Furthermore, a negative energy means that the mutation is favoured. The energy differences found are marginal, indicating that all transporters, independently of the alkyl substituent, have equivalent binding affinities for chloride in agreement with experimental binding data.

From	То	$\Delta \Delta G$
$\mathbf{R} = \text{ethyl} \ (2)$	R = methyl (1)	-0.09
$\mathbf{R} = \operatorname{propyl}\left(3\right)$	$\mathbf{R} = \text{ethyl} \ (2)$	0.11
$\mathbf{R} = \mathbf{butyl} \ (4)$	$\mathbf{R} = \operatorname{propyl}\left(3\right)$	-0.08
R = pentyl (5)	$\mathbf{R} = \mathbf{butyl} \ (4)$	0.45
$\mathbf{R} = \mathbf{hexyl} \ (6)$	R = pentyl (5)	0.24
R = heptyl (7)	$\mathbf{R} = \mathbf{hexyl} \ (6)$	-0.68

Table S4 Relative binding free energies (kcalmol⁻¹) for chloride tambjamine associations.

NMR spectra of compounds 1-16



Fig. S24 ¹H NMR spectrum of compound **1**.HCl (CDCl₃).



Fig. S25 ¹³C NMR spectrum of compound **1**.HCl (CDCl₃).



Fig. S26 ¹H NMR spectrum of compound **2**.HCl (CDCl₃).



Fig. S27 ¹³C NMR spectrum of compound **2**.HCl (CDCl₃).



Fig. S29 ¹³C NMR spectrum of compound **3**.HCl (CDCl₃).

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Fig. S30 ¹H NMR spectrum of compound **4**.HCl (CDCl₃).



Fig. S31 ¹³C NMR spectrum of compound **4**.HCl (CDCl₃).



Fig. S32 ¹H NMR spectrum of compound **5**.HCl (CDCl₃).



Fig. S33 ¹³C NMR spectrum of compound **5**.HCl (CDCl₃).

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Fig. S34 ¹H NMR spectrum of compound **6**.HCl (CDCl₃).



Fig. S35 ¹³C NMR spectrum of compound **6.**HCl (CDCl₃).



Fig. S36 ¹H NMR spectrum of compound 7.HCl (CDCl₃).



Fig. S37 ¹³C NMR spectrum of compound **7.**HCl (CDCl₃).



Fig. S38 ¹H NMR spectrum of compound 8.HCl (CDCl₃).



Fig. S39 ¹³C NMR spectrum of compound **8.**HCl (CDCl₃).



Fig. S40 ¹H NMR spectrum of compound **9**.HCl (CDCl₃).





Fig. S43 ¹³C NMR spectrum of compound **10** .HCl (CDCl₃).





Fig. S45 ¹³C NMR spectrum of compound **11.**HCl (CDCl₃).



Fig. S46 ¹H NMR spectrum of compound **12.**HCl (CDCl₃).



Fig. S47 ¹³C NMR spectrum of compound **12.**HCl (CDCl₃).



Fig. S48 ¹H NMR spectrum of compound **13.**HCl (CDCl₃).



Fig. S49 ¹³C NMR spectrum of compound **13.**HCl (CDCl₃).



Fig. S50 ¹H NMR spectrum of compound **14.**HCl (CDCl₃).



Fig. S51 ¹³C NMR spectrum of compound **14.**HCl (CDCl₃).



Fig. S52 ¹H NMR spectrum of compound **15.**HCl (CDCl₃).



Fig. S53 ¹³C NMR spectrum of compound **15.**HCl (CDCl₃).



Fig. S54 ¹H NMR spectrum of compound **16.**HCl (CDCl₃).



Fig. S55 ¹³C NMR spectrum of compound **16.**HCl (CDCl₃).