Towards “drug-like” indole-based transmembrane anion transporters

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Electronic Supporting Information

Calculated log P and molecular masses of the previous generation of transporters

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$^a$ clog P calculated using Fieldview Version 2.0.2 for Macintosh (Wildman-Crippen model).
**Synthesis**

**General comments**

All solvents and starting materials were purchased from commercial sources and used without further purification unless otherwise stated. Dry DCM was obtained by distillation over CaH$_2$, Aniline was distilled prior to use.

We have reported the synthesis of compounds 1, 3, 5, and 10 previously.

**Synthesis of 1-hexyl-(1H-indol-7-yl)thiourea, 2**

7-nitroindole (0.205g, 1.26mmol) was dissolved in methanol (30mL) with palladium on carbon (~0.05g) and the reaction vessel flooded with hydrogen. The mixture was stirred for three hours at room temperature. The palladium catalyst was removed by filtration and the filtrate reduced *in vacuo*. The crude product was dissolved in DCM (30mL) with thiophosgene (0.1mL, 1.30mmol). Saturated aqueous sodium bicarbonate solution (30mL) was added and the reaction mixture stirred for fifteen hours. The organic phase was extracted and hexylamine (0.3mL, 2.27mmol) added. The reaction mixture was stirred at reflux for fifteen hours. The crude reaction mixture was washed with water (200mL) and the organic phase reduced *in vacuo* to yield an orange oil. The crude mixture was purified by column chromatography (1:10 methanol/DCM), the product fractions were combined and reduced *in vacuo* and the resulting oil triturated in hexane (50mL). The precipitate was removed by filtration and dried under vacuum to yield a cream coloured solid (0.221g). 64% yield. **Mp:** 97-99°C; **IR (Golden Gate) ν$_{\text{max}}$/cm$^{-1}$:** 3380 (indole NH stretch), 3330, 3190 (thiourea NH stretches), 3020, 2920, 2860 (CH stretches); **LRMS (ES$^-$) m/z:** 274.2 [M-H]$^-$; **HRMS (ES$^+$) m/z:** act: 298.1356 [M+Na]$^+$, cal: 298.1354; **$^1$H NMR (300MHz, DMSO-$d_6$, δ = ppm):** 10.89 (s, 1H, indole NH), 9.22 (s, 1H, thiourea NH), 7.51-7.40 (m, 2H, Ar CH), 7.29 (t, J=2.55Hz, 1H, Ar CH), 7.02-6.93 (m, 2H, Ar CH, thiourea NH), 6.46 (dd, J=1.83, 2.91Hz, 1H, Ar CH), 3.55-3.40 (m, 2H, CH$_2$), 1.52 (m, 2H, CH$_2$), 1.26 (br. s, 6H, CH$_3$), 0.87 (t, J=6.60Hz, CH$_3$); **$^{13}$C NMR (100MHz, DMSO-$d_6$, δ = ppm):** 180.7 (CS), 131.2 (Ar C), 129.5 (Ar C), 125.6 (Ar CH), 122.9 (C), 119.0 (Ar CH), 118.0 (Ar CH), 118.0 (Ar CH), 101.6 (Ar CH), 44.3 (CH$_2$), 31.0 (CH$_2$), 28.5 (CH$_2$), 26.0 (CH$_2$), 22.0 (CH$_2$), 13.9 (CH$_3$)
Synthesis of 1-(2-butyl-5-(trifluoromethyl)-1H-indol-7-yl)-3-hexylurea, 3

2-butyl-7-nitro-5(trifluoromethyl)-1H-indole\(^6\) (0.106g, 0.37mmol) was dissolved in ethanol (25mL) with palladium on carbon (~0.05g) and the reaction vessel flooded with hydrogen. The mixture was stirred at reflux for five hours. The palladium catalyst was removed by filtration and the filtrate reduced in vacuo to yield a white solid. The solid was dissolved in DCM (50mL) with 1,1'-carbonyldiimidazole (0.234g, 1.44mmol). The reaction mixture was stirred under nitrogen at room temperature for five hours. Hexylamine (2.0mL, 15.14mmol) was added and the reaction mixture was stirred at reflux for eighteen hours. The reaction mixture was reduced in vacuo to yield a brown oil which was purified by column chromatography (1:50 methanol/hexane) to yield a white solid (0.085g). 60% yield. 

\[
\begin{align*}
\text{Mp: } & 102-104^\circ C; \\
\text{IR (Golden Gate) } & v_{\text{max}}/\text{cm}^{-1}: 3390 \text{ (indole NH), 3350 \text{ (urea NH stretch), 2960, 2930, 2870 (CH stretches), 1630 (CO stretch), 1110 (trifluoromethyl CF stretch); } \\
\text{LRMS (ES) } m/z: & 382.3 [M-H]^-; \\
\text{HRMS (ES) } m/z: & \text{act: 406.2072 [M+Na]^+}, \text{ cal: 406.2082; } \\
\text{1H NMR (300MHz, DMSO-\text{d}_6)} & \delta = \text{ppm}: 10.89 \text{ (s, 1H, indole NH), 8.38 \text{ (s, 1H, urea NH), 7.53 \text{ (s, 1H, Ar CH), 7.43 \text{ (s, 1H, Ar CH), 6.28 \text{ (br. s, 2H, Ar CH, urea NH), 3.13 \text{ (dt, J=7.08, 6.04Hz, 2H, CH}_2, 2.76 \text{ (app. t, J=7.56, 2H, CH}_2, 1.75-1.63 \text{ (m, 2H, CH}_2, 1.53-1.42 \text{ (m, 2H, CH}_2, 1.42-1.22 \text{ (m, 8H, CH}_2, 0.92(t, J=7.08Hz, 3H, CH}_3, 0.88 \text{ (t, J=7.04Hz, 3H, CH}_3); } \\
\text{13C NMR (100MHz, DMSO-\text{d}_6)} & \delta = \text{ppm}: 155.3 \text{ (C0), 141.8 \text{ (Ar C), 128.9 \text{ (Ar C), 128.6 (Ar C), 125.6 (q, J = 269.8Hz, CF}_3), 125.1 \text{ (Ar C), 119.9 (q, J = 30.2Hz, Ar C), 110.6 \text{ (Ar CH), 106.6 \text{(ArCH), 100.0 \text{(ArCH), 39.4 \text{(CH}_2), 31.1 \text{(CH}_2, 30.8 \text{(CH}_2, 29.7 \text{(CH}_2, 27.2 \text{(CH}_2, 26.1 \text{(CH}_2, 22.1 \text{(CH}_2, 21.8 \text{(CH}_2, 14.0 \text{(CH}_3, 13.7 \text{(CH}_3); } \\
\text{19F NMR (282MHz, DMSO-\text{d}_6, } & \delta = \text{ppm}: -58.67 \text{ (CF}_3).}
\end{align*}
\]

Synthesis of 1-(2-butyl-5-(trifluoromethyl)-1H-indol-7-yl)-3-hexylthiourea, 4

2-butyl-7-nitro-5(trifluoromethyl)-1H-indole\(^6\) (0.100g, 0.35mmol) was dissolved in methanol (20mL) with palladium on carbon (~0.05g) and the reaction vessel flooded with hydrogen. The mixture was stirred at reflux for three hours. The palladium catalyst was removed by filtration and the filtrate reduced in vacuo to yield a white solid. The solid was dissolved in DCM (50mL) with thiophosgene (0.3mL, 3.91mmol). Saturated aqueous
sodium bicarbonate solution (100mL) was added to the reaction mixture which was then stirred at room temperature for five hours. The organic portion was separated and reduced in vacuo to yield an orange oil. The oil was redissolved in DCM (50mL) with hexylamine (2.0mL, 15.14mmol) and stirred at reflux for eighteen hours. The reaction mixture was reduced in vacuo to yield a yellow oil which was purified by column chromatography (DCM). The product fractions were combined and reduced in vacuo to yield a colourless oil. The oil was triturated in water (50mL) to yield product as a cream solid (0.065g). 47% yield. Mp: 78-80°C; IR (Golden Gate) ν max/cm⁻¹: 3390 (indole NH stretch), 3290, 2850 (thiourea NH stretches), 2950, 2930, 2850 (CH stretches), 1100 (trifluoromethyl CF stretch); LRMS (ES⁻) m/z: 398.2 [M-H]-; HRMS (ES⁺) m/z: act: 400.2019 [M+H]+, cal: 400.2034; ¹H NMR (300MHz, DMSO-d₆, δ = ppm): 11.19 (s, 1H, indole NH), 9.25 (s, 1H, thiourea NH), 7.72 (br. s, 1H, thiourea NH), 7.65 (s, 1H, Ar CH), 7.20 (s, 1H, Ar CH), 6.33 (s, 1H, Ar CH), 3.45 (br. m, 2H, CH₂), 2.74 (t, J=7.53Hz, 2H, CH₂), 1.67 (m, 2H, CH₂), 1.53 (br. s, 2H, CH₂), 1.40-1.20 (m, 8H, CH₂), 0.91 (t, J=7.14Hz, 3H, CH₃), 0.87 (t, J=7.98Hz, 3H, CH₃); ¹³C NMR (100MHz, DMSO-d₆, δ = ppm): 180.8 (CS), 143.2 (Ar C), 132.7 (Ar C), 129.6 (Ar C), 125.4 (q, J = 269.2Hz, CF₃), 123.0 (Ar C), 119.6 (q, J = 30.8Hz, Ar C), 114.1 (Ar CH), 113.5 (Ar CH), 99.9 (Ar CH), 44.4 (CH₂), 31.1 (CH₂), 30.9 (CH₂), 28.4 (CH₂), 27.1 (CH₂), 26.1 (CH₂), 22.1 (CH₂), 21.8 (CH₂), 13.9 (CH₃), 13.7 (CH₃); ¹⁹F NMR (282MHz, DMSO-d₆, δ = ppm): -58.45 (CF₃).

Synthesis of 1-(1H-indol-7-yl)-3-phenylthiourea, 6

7-nitroindole (0.457g, 2.82mmol) was dissolved in methanol (25mL) with palladium on carbon (~0.05g) and the reaction vessel flooded with hydrogen. The mixture was stirred for ninety minutes at room temperature. The palladium catalyst was removed by filtration and the filtrate reduced in vacuo to yield a white solid (0.412g). A portion of the solid (0.201g, 1.52mmol) was dissolved in pyridine (5mL) with phenylisothiocyanate (0.4mL, 3.34mmol) and stirred under nitrogen for eighteen hours at room temperature. Pyridine was removed in vacuo to yield a cream coloured solid. The crude product was purified by column chromatography (1:50 methanol/DCM). Product fractions were combined and reduced in vacuo to yield the product as a white solid (0.088g). 20% yield. Mp: 230-232°C; IR (Golden Gate) ν max/cm⁻¹: 3320 (indole NH stretch), 3120 (thiourea NH stretch), 2950, 2930, 2850 (CH stretches).
stretch), 2970 (thiourea NH stretch); **LRMS (ES) m/z:** 266.1 [M-H]; **HRMS (ES⁺) m/z:** act: 290.0727 [M+Na]+, cal: 290.0728; **1H NMR (400MHz, DMSO-d₆, δ = ppm):** 10.97 (s, 1H, indole NH), 9.72 (s, 1H, thiourea NH), 9.54 (s, 1H, thiourea NH), 7.56 (d, J=8.01Hz, 2H, Ar CH), 7.44 (d, J=7.32Hz, 1H, Ar CH), 7.38-7.27 (m, 3H, Ar CH), 7.12 (t, J=7.32Hz, 1H, Ar CH), 7.08-6.93 (m, 2H, Ar CH), 6.47 (s, 1H, Ar CH);

**13C NMR (75MHz, DMSO-d₆, δ = ppm):** 180.3 (CS), 139.7 (Ar C), 131.6 (Ar C), 129.4 (Ar C), 128.3 (Ar CH), 125.5 (Ar CH), 124.3 (Ar CH), 123.8 (Ar CH), 123.6 (Ar CH), 118.9 (Ar CH), 118.8 (Ar CH), 118.2 (Ar CH), 101.5 (Ar CH).

Synthesis of 1-(1H-indol-7-yl)-3-(4-(trifluoromethyl)phenyl)urea, 7

7-nitroindole (0.199g, 1.23mmol) was dissolved in methanol (20mL) with palladium on carbon (∼0.05g) and the reaction vessel flooded with hydrogen. The mixture was stirred for two hours at room temperature. The palladium catalyst was removed by filtration and the filtrate reduced in vacuo to yield a white solid. The solid was dissolved in DCM (50mL) with 4-(trifluoromethyl)phenylisocyanate (0.3mL, 2.10mmol) and stirred under nitrogen for ninety minutes. The reaction mixture was filtered and the resulting solid washed with DCM (200mL) to yield product as a grey solid (0.201g), 51% yield. **Mp:** 225-226°C; **IR (Golden Gate) v_max/cm⁻¹:** 3390 (indole NH stretch), 3310, 3290 (urea NH stretches), 1640 (urea CO stretch), 1100 (trifluoromethyl CF stretch); **LRMS (ES) m/z:** 318.1 [M-H]; **HRMS (ES⁺) m/z:** act: 342.0823 [M+Na]+, cal: 342.0830; **1H NMR (300MHz, DMSO-d₆, δ = ppm):** 10.73 (s, 1H, indole NH), 9.23 (s, 1H, urea NH), 8.59 (s, 1H, urea NH), 7.71 (d, J=8.67Hz, 2H, Ar CH), 7.64 (d, J=8.67Hz, 2H, Ar CH), 7.36-7.31 (m, 2H, Ar CH), 7.10 (d, J=7.17Hz, 1H, Ar CH), 6.95 (t, J=7.89Hz, 1H, Ar CH), 6.45 (dd, J=2.64, 1.89Hz, 1H, Ar CH); **13C NMR (75MHz, DMSO-d₆, δ = ppm):** 152.8 (C0), 143.6(Ar C), 129.3 (Ar C), 129.2 (Ar C), 126.0 (Ar CH), 125.2 (Ar CH), 124.6 (q, J= 269.1Hz, CF₃), 123.2 (Ar C), 121.6 (q, J= 32.01Hz, Ar C) 118.9 (Ar CH), 117.9 (Ar CH), 116.2 (Ar CH), 114.4 (Ar CH), 101.5 (Ar CH); **19F NMR (282MHz, DMSO-d₆, δ = ppm):** -60.08 (CF₃).
Synthesis of 1-(1H-indol-7-yl)-3-(4-(trifluoromethyl)phenyl)thiourea, 8

7-nitroindole (0.457g, 2.82mmol) was dissolved in methanol (25mL) with palladium on carbon (∼0.05g) and the reaction vessel flooded with hydrogen. The mixture was stirred for ninety minutes at room temperature. The palladium catalyst was removed by filtration and the filtrate reduced in vacuo to yield a white solid (0.412g). A portion of the solid (0.211g, 1.60mmol) was dissolved in pyridine (5mL) with 4-(trifluoromethyl)phenylisothiocyanate (0.307g, 1.51mmol) and stirred under nitrogen for eighteen hours at room temperature. Pyridine was removed in vacuo to yield a cream coloured solid. The crude product was purified by column chromatography (1:50 methanol/DCM). Product fractions were combined and reduced in vacuo to yield the product as a white solid (0.109g). 20% yield. Mp: 158-160°C; IR (Golden Gate) \( \nu_{\text{max}}/\text{cm}^{-1} \): 3390 (indole NH stretch), 3140, 2950 (thiourea NH stretches), 1100 (trifluoromethyl CF stretch); LRMS (ES) \( m/z \): 334.1 [M-H]⁻; HRMS (ES⁺) \( m/z \): act: 358.0591 [M+Na]⁺, cal: 358.0602; \(^1\)H NMR (400MHz, DMSO-d₆, \( \delta = \text{ppm} \)): 10.99 (s, 1H, indole NH), 10.05 (s, 1H, thiourea NH), 9.80 (s, 1H, thiourea NH), 7.84 (d, J=8.60Hz, 2H, Ar CH), 7.67 (d, J=7.08Hz, 2H, Ar CH), 7.46 (d, J=7.60Hz, 1H, Ar CH), 7.32 (s, 1H, Ar CH), 7.08-6.95 (m, 2H, Ar CH), 6.47 (br. s, 1H, Ar CH), \(^{13}\)C NMR (100MHz, DMSO-d₆, \( \delta = \text{ppm} \)): 180.4 (CS), 143.7 (Ar C), 131.6 (Ar C) 129.4 (Ar C), 125.5 (Ar CH), 125.4 (Ar CH), 125.4 (Ar CH), 124.4 (q, J = 269.1Hz, CF₃), 123.9 (q, J = 32.0Hz, Ar C), 123.4 (Ar C), 123.1 (Ar CH), 118.8 (Ar CH), 118.5 (Ar CH), 101.6 (Ar CH); \(^{19}\)F NMR (282MHz, DMSO-d₆, \( \delta = \text{ppm} \): -60.42 (CF₃).

Synthesis of 1-hexyl-3-phenylurea, 9

A solution of hexylamine (1.2mL, 9.08mmol) in DCM (15mL) was added dropwise to a solution of phenylisocyanate (1mL, 9.20mmol) in DCM (15mL) over 30 minutes, under nitrogen. After 18 hours water (7.5mL) was added. The reaction mixture was washed with 2M HCl (2x7.5mL) and then with 1M NaOH (2x15mL), dried over anhydrous magnesium sulphate, filtered and the solvent removed in vacuo to yield a white solid (1.953g). 98% yield. Mp: 58°C; IR (Golden Gate) \( \nu_{\text{max}}/\text{cm}^{-1} \): 3330, 3310 (urea NH stretches), 1630 (urea CO stretch); LRMS (ES⁺) \( m/z \): 436.2 [2M+Na]⁺; HRMS (ES⁺)
m/z: act: 221.1648 [M+H]^+, cal: 221.1654; ^1^H NMR (400MHz, DMSO-d_6, δ = ppm): 8.34 (s, 1H, urea NH), 7.37 (dd, J=7.56, 1.04Hz, 2H, Ar CH), 7.20 (app. t, J=7.60Hz, 2H, Ar CH), 6.87 (t, J=7.60Hz, 1H, Ar CH), 6.08 (t, J=5.56Hz, 1H, urea NH), 3.06 (dt, J=6.56, 6.04Hz, 2H, CH_2), 1.50-1.35 (m, 2H, CH_2), 1.35-1.20 (m, 6H, CH_2), 0.87 (t, J=6.56Hz, 3H, CH_3);

^13^C NMR (100MHz, DMSO-d_6, δ = ppm): 155.1 (CO), 140.6 (Ar C), 128.6 (Ar CH), 120.8 (Ar CH), 117.5 (Ar CH), 39.0 (CH_2), 31.0 (CH_2), 29.7 (CH_2), 26.0 (CH_2), 22.1 (CH_2), 13.9 (CH_3).

Synthesis of 1-hexyl-3-(4-(trifluoromethyl)phenyl)urea, 11

Hexylamine (0.47mL, 3.56mmol) was dissolved in dry DCM (10mL) under nitrogen. 4-(trifluoromethyl)phenyl isocyanate (0.50mL, 3.50mmol) was added to the reaction mixture. The reaction mixture was allowed to stir for 1 hour whereupon a white precipitate formed. The precipitate was removed by filtration and washed with DCM (2x25mL) to yield a white solid (0.693g). 69% yield. Mp: 103°C; IR (Golden Gate) ν_max/cm⁻¹: 3340, 3330 (urea NH stretches), 1640 (urea CO stretch), 1110 (trifluoromethyl CF stretch); LRMS (ES⁺) m/z: 289.1 [M+H]^+, 311.1 [M+Na]^+, 327.1 [M+K]^+, 599.3 [2M+Na]^+; HRMS (ES⁺) m/z: act: 289.1523 [M+H]^+, cal: 289.1528; ^1^H NMR (400MHz, DMSO-d_6, δ = ppm): 8.81 (s, 1H, urea NH), 7.58 (d, J=9.12Hz, 2H, Ar CH), 7.54 (d, J=9.12Hz, 2H, Ar CH), 6.25 (t, J=5.56Hz, 1H, urea NH), 3.08 (dt, J=7.04, 5.56Hz, 2H, CH_2), 1.50-1.35 (m, 2H, CH_2), 1.35-1.20 (m, 6H, CH_2), 0.87 (t, J=7.08Hz, 3H, CH_3);

^13^C NMR (100MHz, DMSO-d_6, δ = ppm): 154.8 (CO), 144.3 (Ar C), 125.9 (Ar CH), 124.6 (q, J = 269.1Hz, CF_3), 120.8 (q, J = 31.8Hz, Ar C) 117.1 (Ar CH), 39.0 (CH_2), 31.0 (CH_2), 29.6 (CH_2), 26.0 (CH_2), 22.1 (CH_2), 13.9 (CH_3); ^19^F NMR (282MHz, DMSO-d_6, δ = ppm): -60.05 (CF_3)

Synthesis of 1-hexyl-3-(4-(trifluoromethyl)phenyl)thiourea (12)

Hexylamine (0.33mL, 2.50mmol) was dissolved in dry DCM (10mL) under nitrygen. 4-(trifluoromethyl)phenyl isothiocyanate (0.509g, 2.51mmol) was added to the reaction mixture. The reaction mixture was allowed to stir for 18 hours. The reaction mixture was reduced in vacuo to yield an oily solid. The solid was washed with cold DCM (2x10mL) to yield a white solid (0.355g). 47% yield. Mp: 94-95°C; IR
(Golden Gate) $v_{\text{max}}$/cm$^{-1}$: 3220 (thiourea NH stretch), 1560 (thiourea CO stretch), 1110 (trifluoromethyl CF stretch); LRMS (ES$^+$) m/z: 305.2 [M+H]$^+$, 327.1 [M+Na]$^+$, 343.2 [M+K]$^+$, 631.0 [2M+Na]$^+$; HRMS (ES$^+$) m/z: act: 305.1293 [M+H]$^+$, cal: 305.1299; $^1$H NMR (400MHz, DMSO-$d_6$, $\delta$ = ppm): 9.77 (s, 1H, urea NH), 8.03 (s, 1H, urea NH), 7.73 (d, J=8.60Hz, 2H, Ar CH), 7.63 (d, J=8.60Hz, 2H, Ar CH), 3.55-3.40 (m, 2H, CH$_2$), 1.65-1.50 (m, 2H, CH$_2$), 1.40-1.25 (m, 6H, CH$_2$), 0.87 (t, J=6.56Hz, 3H, CH$_3$); $^{13}$C NMR (100MHz, DMSO-$d_6$, $\delta$ = ppm): 180.2 (CS), 143.5 (Ar C), 125.5 (Ar CH), 124.7 (q, J = 269.1Hz, CF3), 123.2 (q, J = 29.1Hz, Ar C), 121.6 (Ar CH), 43.8 (CH$_2$), 31.0 (CH$_2$), 28.2 (CH$_2$), 26.1 (CH$_2$), 22.0 (CH$_2$), 13.8 (CH$_3$); $^{19}$F NMR (282MHz, DMSO-$d_6$, $\delta$ = ppm): -60.49 (CF$_3$)
Figure S1: $^1$H NMR spectrum of compound 2 in DMSO-$d_6$.

Figure S2: $^{13}$C NMR spectrum of compound 2 in DMSO-$d_6$. 
**Figure S3:** $^1$H NMR spectrum of compound 3 in DMSO-$d_6$.

**Figure S4:** $^{13}$C NMR spectrum of compound 3 in DMSO-$d_6$. 
Figure S5: $^{19}$F NMR spectrum of compound 3 in DMSO-$d_6$.

Figure S6: $^1$H NMR spectrum of compound 4 in DMSO-$d_6$. 
Figure S7: $^{13}$C NMR spectrum of compound 4 in DMSO-$d_6$.

Figure S8: $^{19}$F NMR spectrum of compound 4 in DMSO-$d_6$. 
Figure S9: $^1$H NMR spectrum of compound 6 in DMSO-$d_6$.

Figure S10: $^{13}$C NMR spectrum of compound 6 in DMSO-$d_6$. 
Figure S11: $^1$H NMR spectrum of compound 7 in DMSO-$d_6$.

Figure S12: $^{13}$C NMR spectrum of compound 7 in DMSO-$d_6$. 
Figure S13: $^{19}$F NMR spectrum of compound 7 in DMSO-$d_6$.

Figure S14: $^1$H NMR spectrum of compound 8 in DMSO-$d_6$. 
**Figure S15:** $^{13}$C NMR spectrum of compound 8 in DMSO-$d_6$.

**Figure S16:** $^{19}$F NMR spectrum of compound 8 in DMSO-$d_6$. 
Figure S17: $^1$H NMR spectrum of compound 9 in DMSO-$d_6$.

Figure S18: $^{13}$C NMR spectrum of compound 9 in DMSO-$d_6$. 
Figure S19: $^1$H NMR spectrum of compound 11 in DMSO-$d_6$.

Figure S20: $^{13}$C NMR spectrum of compound 11 in DMSO-$d_6$. 
Figure S21: $^{19}$F NMR spectrum of compound 11 in DMSO-$d_6$.

Figure S22: $^1$H NMR spectrum of compound 12 in DMSO-$d_6$. 
Figure S23: $^{13}$C NMR spectrum of compound 12 in DMSO-$d_6$.

Figure S24: $^{19}$F NMR spectrum of compound 12 in DMSO-$d_6$. 
**NMR titrations**

Known volumes of a 0.15M solution of guest containing 0.01M receptor were added to a solution of 0.01M receptor. $^1$H NMR spectrum were recorded after each addition. Chemical shifts were calibrated to the solvent peak, and these values were then fitted to a relevant binding model using WinEQNMR 2 in order to generate values for the binding constants (Figures S22-S41).

**Figure S25:** NMR titration of compound 2 with TBACl in 0.5% H$_2$O/DMSO-$d_6$. $K_a = 17$ M$^{-1}$ (11% error).
Figure S26: NMR titration of compound 2 with TEAHCO$_3$ in 0.5% H$_2$O/DMSO-$d_6$. $K_a = 414\text{M}^{-1}$ (6% error).

Figure S27: NMR titration of compound 3 with TBACl in 0.5% H$_2$O/DMSO-$d_6$. $K_a = 154\text{M}^{-1}$ (6% error).
Figure S28: NMR titration of compound 3 with TEAHCO$_3$ in 0.5% H$_2$O/DMSO-$d_6$. $K_a > 10^4$ M$^{-1}$.

Figure S29: NMR titration of compound 4 with TBACL in 0.5% H$_2$O/DMSO-$d_6$. $K_a = 40$M$^{-1}$ (7% error).
**Figure S30:** NMR titration of compound 4 with TEAHCO₃ in 0.5% H₂O/DMSO-d₆. $K_a = 2150\text{M}^{-1}$ (10% error).

**Figure S31:** NMR titration of compound 5 with TBACl in 0.5% H₂O/DMSO-d₆. $K_a = 95\text{M}^{-1}$ (2% error).
**Figure S32:** NMR titration of compound 5 with TEA\(\text{HCO}_3\) in 0.5% H\(_2\)O/DMSO-\(d_6\). \(K_a = 3860\text{M}^{-1}\) (9% error).

**Figure S33:** NMR titration of compound 6 with TBACl in 0.5% H\(_2\)O/DMSO-\(d_6\). \(K_a = 25\text{M}^{-1}\) (8% error).
Figure S34: NMR titration of compound 7 with TBACl in 0.5% H$_2$O/DMSO-$d_6$. $K_a = 101\text{M}^{-1}$ (3% error).

Figure S35: NMR titration of compound 7 with TEAHCO$_3$ in 0.5% H$_2$O/DMSO-$d_6$. $K_a = 4050\text{M}^{-1}$ (19% error).
**Figure S36:** NMR titration of compound 8 with TBACl in 0.5% H₂O/DMSO-d₆. $K_a = 26M^{-1}$ (12% error).

**Figure S37:** NMR titration of compound 9 with TBACl in 0.5% H₂O/DMSO-d₆. $K_a = 16M^{-1}$ (14% error).
Figure S38: NMR titration of compound 9 with TEAHCO₃ in 0.5% H₂O/DMSO-d₆. $K_a = 121M^{-1}$ (3% error).

Figure S39: NMR titration of compound 10 with TBACl in 0.5% H₂O/DMSO-d₆. $K_a = 14M^{-1}$ (12% error).
**Figure S40**: NMR titration of compound 10 with TEAHCO$_3$ in 0.5% H$_2$O/DMSO-$d_6$. $K_a = 262$M$^{-1}$ (8% error).

**Figure S41**: NMR titration of compound 11 with TBACl in 0.5% H$_2$O/DMSO-$d_6$. $K_a = 23$M$^{-1}$ (8% error).
**Figure S42:** NMR titration of compound 11 with TEAHCO$_3$ in 0.5% H$_2$O/DMSO-$d_6$. $K_a = 329M^{-1}$ (6% error).

**Figure S43:** NMR titration of compound 12 with TBACl in 0.5% H$_2$O/DMSO-$d_6$. $K_a = 26M^{-1}$ (4% error).
Figure S44: NMR titration of compound 12 with TEAHCO₃ in 0.5% H₂O/DMSO-d₆. Kₐ = 931 M⁻¹ (8% error).

Figure S45: Job plot of compound 6 with TEA bicarbonate in 0.5% H₂O/DMSO-d₆.
**Figure S46:** Job plot of compound 8 with TEA bicarbonate in 0.5% H$_2$O/DMSO-$d_6$.

**Vesicle studies**

**General method**

A chloroform solution of 1-palmitoyl-2-oleoyl-$sn$-glycero-3-phosphocholine (POPC) (22.32 mg/mL) (Genzyme) was evaporated under reduced pressure to give a thin film. The lipid film was dried under high vacuum for at least 2 hours and rehydrated with the *internal* solution 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.) to obtain unilamellar vesicles. The liposomes underwent dialysis in the *external* solution. The vesicles were diluted to 5mL with the *external* solution to form a stock solution of lipid.

Samples for assay were prepared by diluting lipid stock solution to 5mL (using the *external* solution) to give a solution of 1mM lipid. Chloride efflux was monitored using a chloride selective electrode (Accumet). To initiate the experiment compounds were added as solutions in DMSO, to give a 1:50 compound to lipid ratio (2mol%). At the end of the experiment detergent (octaethylene glycol monododecyl ether) was added to allow the determination of 100% chloride efflux. Experiments were repeated in triplicate and all traces presented are the average of three trials. The chloride electrode was calibrated against sodium chloride solutions of known concentration.
**Chloride/nitrate antiport assay**

**Internal solution:** 489mM sodium chloride buffered to pH 7.2 with 5mM sodium phosphate salts.

**External solution:** 489mM sodium nitrate buffered to pH 7.2 with 5mM sodium phosphate salts.

Compound was added at t=0. Detergent was added at t=5mins.

**CsCl replacement chloride/nitrate antiport assay**

**Internal solution:** 489mM caesium chloride buffered to pH 7.2 with 5mM sodium phosphate salts.

**External solution:** 489mM sodium nitrate buffered to pH 7.2 with 5mM sodium phosphate salts.

Compound was added at t=0. Detergent was added at t=5mins.

**Chloride/bicarbonate antiport assay**

**Internal solution:** 450mM sodium chloride buffered to pH 7.2 with 20mM sodium phosphate salts.

**External solution:** 162mM sodium sulphate buffered to pH 7.2 with 20mM sodium phosphate salts.

Compound was added at t=0. A ‘pulse’ of sodium bicarbonate in external solution was added at t=2mins, such that the external concentration of bicarbonate is 40mM. Detergent was added at t=7mins

**Chloride/sulphate ‘blank’ assay**

**Internal solution:** 450mM sodium chloride buffered to pH 7.2 with 20mM sodium phosphate salts.
**External solution:** 162mM sodium sulphate buffered to pH 7.2 with 20mM sodium phosphate salts.

Compound was added at t=0. Detergent was added at t=7mins.

**Chloride/sulphate pH gradient assay**

**Internal solution:** 450mM sodium chloride buffered to pH 4.0 with 20mM sodium citrate salts.

**External solution:** 162mM sodium sulphate buffered to pH 7.2 with 20mM sodium phosphate salts.

Compound was added at t=0s. Detergent was added at t=5mins.

**HPTS fluorescence assay**

**Internal solution:** 450mM sodium chloride and 1mM HPTS buffered to pH 7.2 with 20mM sodium phosphate salts.

**External solution:** 162mM sodium sulphate buffered to pH 7.2 with 20mM sodium phosphate salts.

Vesicles were prepared as described previously. Instead of dialysis, vesicles were purified by size exclusion chromatography using sephadex gel (G-50). Samples for assay were prepared by diluting the lipid stock solution to 3mL (using the external solution). Fluorescence spectra were obtained using a Varian Cary Eclipse Fluorescence Spectrophotometer. HPTS fluorescence was monitored by excitation at 405nm and 460nm, and emission at 506nm. To initiate the experiment the test compound was added as a solution in DMSO, to give a 1:50 compound to lipid ratio (2mol%). Experiments were repeated in triplicate and all traces presented are the average of three trials.

**Lucigenin fluorescence assay for sulphate transport**

**Internal solution:** 100mM sodium chloride and 2mM lucigenin dye buffered to pH 7.2 with 20mM sodium phosphate salts.
**External solution:** 100mM sodium chloride buffered to pH 7.2 with 20mM sodium phosphate salts.

Vesicles were prepared as described previously. Instead of dialysis, vesicles were purified by size exclusion chromatography using sephadex gel (G-50). Samples for assay were prepared by diluting the lipid stock solution to 3mL (using the **external** solution). Fluorescence spectra were obtained using a Varian Cary Eclipse Fluorescence Spectrophotometer. Lucigenin fluorescence was monitored by excitation at 455nm and emission at 506nm. At \( t=30s \) a solution of sodium sulphate was added, such that the external concentration of sulphate was 40mM. At \( t=1\text{min} \) compounds were added as solutions in methanol, to give a 1:50 compound to lipid ratio (2mol%). Experiments were repeated in triplicate and all traces presented are the average of three trials.
Figure S47: Chloride efflux from unilamellar POPC vesicles containing 489mM sodium chloride, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 489mM sodium nitrate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of DMSO solutions of compounds 1-4 (2mol%). Each point represents an average of three trials.
**Figure S48:** Chloride efflux from unilamellar POPC vesicles containing 489mM sodium chloride, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 489mM sodium nitrate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of DMSO solutions of compounds 5-8 (2mol%). Each point represents an average of three trials.
Figure S49: Chloride efflux from unilamellar POPC vesicles containing 489mM sodium chloride, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 489mM sodium nitrate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of DMSO solutions of compounds 9-12 (2mol%). Each point represents an average of three trials.
Figure S50: Chloride efflux from unilamellar POPC vesicles containing 489mM sodium chloride, buffered to pH 7.2 with 20mM sodium phosphate salts and suspended in 162mM sodium sulphate, buffered to pH 7.2 with 20mM sodium phosphate salts, upon addition of DMSO solutions of compounds 1-4 (2mol%). At t=2mins a ‘pulse’ of sodium bicarbonate was added such that the external concentration of bicarbonate was 40mM. Each point represents an average of three trials.
**Figure S51**: Chloride efflux from unilamellar POPC vesicles containing 489mM sodium chloride, buffered to pH 7.2 with 20mM sodium phosphate salts and suspended in 162mM sodium sulphate, buffered to pH 7.2 with 20mM sodium phosphate salts, upon addition of DMSO solutions of compounds 5-8 (2mol%). At t=2mins a ‘pulse’ of sodium bicarbonate was added such that the external concentration of bicarbonate was 40mM. Each point represents an average of three trials.
Figure S52: Chloride efflux from unilamellar POPC vesicles containing 489mM sodium chloride, buffered to pH 7.2 with 20mM sodium phosphate salts and suspended in 162mM sodium sulphate, buffered to pH 7.2 with 20mM sodium phosphate salts, upon addition of DMSO solutions of compounds 9-12 (2mol%). At t=2mins a ‘pulse’ of sodium bicarbonate was added such that the external concentration of bicarbonate was 40mM. Each point represents an average of three trials.
**Figure S53**: Hill plot of chloride efflux promoted by varying concentrations of receptor 2 from unilamellar POPC vesicles loaded with 489mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM NaNO₃ buffered at pH 7.2 with 5mM sodium phosphate salts. Each point represents an average of 3 trials.
**Figure S54:** Hill plot of chloride efflux promoted by varying concentrations of receptor 3 from unilamellar POPC vesicles loaded with 489mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM NaNO$_3$ buffered at pH 7.2 with 5mM sodium phosphate salts. Each point represents an average of 3 trials.
Figure S55: Hill plot of chloride efflux promoted by varying concentrations of receptor 4 from unilamellar POPC vesicles loaded with 489mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM NaNO₃ buffered at pH 7.2 with 5mM sodium phosphate salts. Each point represents an average of 3 trials.
**Figure S56**: Hill plot of chloride efflux promoted by varying concentrations of receptor 7 from unilamellar POPC vesicles loaded with 489mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM NaNO₃ buffered at pH 7.2 with 5mM sodium phosphate salts. Each point represents an average of 3 trials.
Figure S57: Hill plot of chloride efflux promoted by varying concentrations of receptor 8 from unilamellar POPC vesicles loaded with 489mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM NaNO₃ buffered at pH 7.2 with 5mM sodium phosphate salts. Each point represents an average of 3 trials.
The low concentration dependency of Cl\textsuperscript{-}/NO\textsubscript{3}\textsuperscript{-} antiport by receptor 10 resulted in reduced reproducibility of the EC\textsubscript{50} value determined for this transport process. The shape of the curve for this Hill analysis is relatively flat, meaning that small variations in the experimentally observed parameter (chloride efflux at 270 s) results in large variation of the calculated parameter (the EC\textsubscript{50} value). As such, a range of EC\textsubscript{50} values from four repeat experiments is reported. We propose that this situation is most likely to occur when testing compounds of relatively low transport activity.

**Figure S58:** Hill plot of chloride efflux promoted varying concentrations of receptor 10 from unilamellar POPC vesicles loaded with 489mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM NaNO\textsubscript{3} buffered at pH 7.2 with 5mM sodium phosphate salts. Each point represents an average of three trials. Calculated EC\textsubscript{50} values are 2.0 %, 2.3 %, 2.6 % and 3.0 % respectively.
**Figure S59:** Hill plot of chloride efflux promoted varying concentrations of receptor 11 from unilamellar POPC vesicles loaded with 489mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM NaNO₃ buffered at pH 7.2 with 5mM sodium phosphate salts. Each point represents an average of 3 trials.
**Figure S60:** Hill plot of chloride efflux promoted varying concentrations of receptor 12 from unilamellar POPC vesicles loaded with 489mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM NaNO₃ buffered at pH 7.2 with 5mM sodium phosphate salts. Each point represents an average of 3 trials.
Figure S61: Hill plot of chloride efflux promoted varying concentrations of receptor 2 from unilamellar POPC vesicles loaded with 450mM NaCl buffered to pH 7.2 with 20mM sodium phosphate salts upon addition of a bicarbonate ‘pulse’, bringing the external concentration of bicarbonate to 40mM. The vesicles were dispersed in 162mM Na$_2$SO$_4$ buffered to pH 7.2 with 20mM sodium phosphate salts. Each point represents an average of 3 trials.
Figure S62: Hill plot of chloride efflux promoted varying concentrations of receptor 3 from unilamellar POPC vesicles loaded with 450mM NaCl buffered to pH 7.2 with 20mM sodium phosphate salts upon addition of a bicarbonate ‘pulse’, bringing the external concentration of bicarbonate to 40mM. The vesicles were dispersed in 162mM Na₂SO₄ buffered to pH 7.2 with 20mM sodium phosphate salts. Each point represents an average of 3 trials.
Figure S63: Hill plot of chloride efflux promoted varying concentrations of receptor 4 from unilamellar POPC vesicles loaded with 450mM NaCl buffered to pH 7.2 with 20mM sodium phosphate salts upon addition of a bicarbonate ‘pulse’, bringing the external concentration of bicarbonate to 40mM. The vesicles were dispersed in 162mM Na₂SO₄ buffered to pH 7.2 with 20mM sodium phosphate salts. Each point represents an average of 3 trials.
**Figure S64:** Hill plot of chloride efflux promoted varying concentrations of receptor 10 from unilamellar POPC vesicles loaded with 450mM NaCl buffered to pH 7.2 with 20mM sodium phosphate salts upon addition of a bicarbonate ‘pulse’, bringing the external concentration of bicarbonate to 40mM. The vesicles were dispersed in 162mM Na$_2$SO$_4$ buffered to pH 7.2 with 20mM sodium phosphate salts. Each point represents an average of 3 trials.
Figure S65: Hill plot of chloride efflux promoted varying concentrations of receptor 12 from unilamellar POPC vesicles loaded with 450mM NaCl buffered to pH 7.2 with 20mM sodium phosphate salts upon addition of a bicarbonate ‘pulse’, bringing the external concentration of bicarbonate to 40mM. The vesicles were dispersed in 162mM Na₂SO₄ buffered to pH 7.2 with 20mM sodium phosphate salts. Each point represents an average of 3 trials.
Figure S66: Chloride efflux from unilamellar POPC vesicles containing 450mM sodium chloride, buffered to pH 7.2 with 20mM sodium phosphate salts and suspended in 162mM sodium sulphate, buffered to pH 7.2 with 20mM sodium phosphate salts, upon addition of DMSO solutions of compounds 1-12 (2mol%). Each point represents an average of three trials.
Figure S67: Chloride efflux from unilamellar POPC vesicles containing either i) 450mM sodium chloride, buffered to pH 7.2 with 20mM sodium phosphate salts, or ii) 450mM sodium chloride, buffered to pH 4.0 with 20mM sodium citrate salts, upon addition of DMSO solutions of compound 4 (2mol%). Vesicles were suspended in 162mM sodium sulphate, buffered to pH 7.2 with 20mM sodium phosphate salts. Each point represents an average of three trials.
Figure S68: Chloride efflux from unilamellar POPC vesicles containing either 489 mM sodium chloride or 489 mM caesium chloride, buffered to pH 7.2 with 5 mM sodium phosphate salts and suspended in 489 mM sodium nitrate, buffered to pH 7.2 with 5 mM sodium phosphate salts, upon addition of DMSO solutions of compound 1 (2 mol%). Each point represents an average of three trials.
Figure S69: Chloride efflux from unilamellar POPC vesicles containing either 489mM sodium chloride or 489mM caesium chloride, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 489mM sodium nitrate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of DMSO solutions of compound 2 (2mol%). Each point represents an average of three trials.
Figure S70: Chloride efflux from unilamellar POPC vesicles containing either 489mM sodium chloride or 489mM caesium chloride, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 489mM sodium nitrate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of DMSO solutions of compound 3 (2mol%). Each point represents an average of three trials.
**Figure S71:** Chloride efflux from unilamellar POPC vesicles containing either 489mM sodium chloride or 489mM caesium chloride, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 489mM sodium nitrate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of DMSO solutions of compound 4 (2mol%). Each point represents an average of three trials.
Figure S72: Chloride efflux from unilamellar POPC vesicles containing either 489mM sodium chloride or 489mM caesium chloride, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 489mM sodium nitrate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of DMSO solutions of compound 5 (2mol%). Each point represents an average of three trials.
**Figure S73:** Chloride efflux from unilamellar POPC vesicles containing either 489mM sodium chloride or 489mM caesium chloride, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 489mM sodium nitrate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of DMSO solutions of compound 6 (2mol%). Each point represents an average of three trials.
Figure S74: Chloride efflux from unilamellar POPC vesicles containing either 489mM sodium chloride or 489mM caesium chloride, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 489mM sodium nitrate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of DMSO solutions of compound 7 (2mol%). Each point represents an average of three trials.
Figure S75: Chloride efflux from unilamellar POPC vesicles containing either 489mM sodium chloride or 489mM caesium chloride, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 489mM sodium nitrate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of DMSO solutions of compound 8 (2mol%). Each point represents an average of three trials.
Figure S76: Chloride efflux from unilamellar POPC vesicles containing either 489mM sodium chloride or 489mM caesium chloride, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 489mM sodium nitrate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of DMSO solutions of compound 9 (2mol%). Each point represents an average of three trials.
Figure S77: Chloride efflux from unilamellar POPC vesicles containing either 489mM sodium chloride or 489mM caesium chloride, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 489mM sodium nitrate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of DMSO solutions of compound 10 (2mol%). Each point represents an average of three trials.
Figure S78: Chloride efflux from unilamellar POPC vesicles containing either 489mM sodium chloride or 489mM caesium chloride, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 489mM sodium nitrate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of DMSO solutions of compound 11 (2mol%). Each point represents an average of three trials.
Figure S79: Chloride efflux from unilamellar POPC vesicles containing either 489 mM sodium chloride or 489 mM caesium chloride, buffered to pH 7.2 with 5 mM sodium phosphate salts and suspended in 489 mM sodium nitrate, buffered to pH 7.2 with 5 mM sodium phosphate salts, upon addition of DMSO solutions of compound 12 (2 mol%). Each point represents an average of three trials.
**Figure S80:** Change in intravesicular pH upon addition of a DMSO solution of receptor 4 (2mol% with respect to lipid) from unilamellar POPC vesicles loaded with 450mM NaCl and 1mM HPTS, buffered to pH 7.2 with 20mM sodium phosphate salts. The vesicles were dispersed in 162mM Na₂SO₄ buffered to pH 7.2 with 20mM sodium phosphate salts. Each trace represents an average of 3 trials.
**Figure S81:** Lucigenin fluorescence intensity of unilamellar POPC vesicles containing 100mM sodium chloride and 2mM lucigenin dye buffered to pH 7.2 with 20mM sodium phosphate salts upon addition of compounds 2, 3, 4, 10 and 12. Vesicles were suspended in a solution containing 100mM sodium chloride buffered to pH 7.2 with 20mM sodium phosphate salts. A sulphate ‘pulse’ was added at t=30s such that the external concentration of sulphate was 40mM. Compounds (2mol%) were added as solutions in methanol at t=60s. Experiments were repeated in triplicate and all traces presented are the average of three trials.

**U-tube assays for mobile carrier activity**

**Source phase:** 488mM sodium chloride solution buffered to pH 7.2 with 5mM sodium phosphate salts.

**Receiving phase:** 488mM sodium nitrate solution buffered to pH 7.2 with 5mM sodium phosphate salts.

The source phase and the receiving phase (7.5mL each) were placed in u-tube apparatus, separated by the organic phase (1mM compound and 1mM tetrabutylammonium)
hexafluorophosphate in nitrobenzene, 15mL). The organic phase was stirred at room temperature and the chloride concentration in the receiving phase was determined using a chloride selective electrode (Accumet) after 24hrs, 60hrs and 96hrs.

![Chloride concentration graph](image)

**Figure 82:** Chloride concentration in a receiving phase of 489mM sodium nitrate buffered to pH 7.2 with 5mM sodium phosphate salts in a series of U-tube mobility assays for compounds 2, 3, 4, 7, 8, 11 and 12, after 24hrs, 60hrs and 96hrs.

**Cell lines and Culture Conditions**

Human lung cancer cell line GLC4 was obtained from the laboratory of Dr. N.H. Mulder and was cultured in RPMI media (Biological Industries, Beit Haemek, Israel). Human melanoma (A375), human colon adenocarcinoma (SW480), human oral adenosquamous carcinoma (CAL27) and mammary epithelial (MCF-10A) cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA). Cells were cultured in DMEM Medium (Biological Industries) supplemented with 10% heat-inactivated foetal bovine serum (FBS; Life Technologies, Carlsbad, CA), 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 mM L- glutamine, all from Biological Industries. MCF10A cell line
was cultured in DMEM:F12 media (1:1, Biological Industries) supplemented with 5% horse serum (Life Technologies), 20 ng/ml EGF, 0.5 µg/ml Hydrocortisone, 100 ng/ml Cholera toxin, 10 µg/ml insulin all from Sigma-Aldrich Chemical Co. (St. Louis, MO) and 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 mM L- glutamine (Biological Industries). Cells were grown at 37°C in a 5% CO₂ atmosphere.

**Cell Viability Assay**

Adherent cells (1x10⁵ cells/ml) were seeded in 96-well plates and allowed to grow for 24 h. Afterwards, they were treated with 10 µM of different receptors for 48 h. Floating cells (2x10⁵ cells/ml GLC4) were seeded and then treated. Cell viability was determined by MTT assay. After treatment, 10 µM of 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma-Aldrich) was added to each well for an additional 4 h. DMSO was added as a control. The blue MTT formazan precipitate was dissolved in 100 µl of isopropanol: 1N HCl (24:1). The absorbance at 570 nm was measured on a multiwell plate reader. Cell viability was expressed as a percentage of the control, and data are shown as the mean value ± S.D. of two independent experiments performed in triplicate.

**Acridine Orange Staining**

Cells (2x10⁵ cells/ml) were seeded on glass slices and 24 h later they were treated with 10 µM of diverse receptors during 1 h. Afterwards, cells were washed twice with PBS and incubated in 5 µg/ml acridine orange solution during 30 min at room temperature. Finally, they were washed with PBS-10% FBS three times and examined by fluorescence in a NIKON eclipse E800 microscope.

**Hoechst Staining**

Cells (1x10⁵ cells/ml) were seeded in 24-well plates, allowed to grow for 24 h and then treated or not with cytotoxic receptors for 48 h. They were washed in PBS, resuspended in 2 µg/ml Hoechst 33342 (Sigma-Aldrich Chemical Co.) and incubated for 30 min at 37°C in the dark. Finally, cells were washed in PBS and examined by fluorescence in a NIKON eclipse E800 microscope.


References


