Supporting Information

Anion binding and transport properties of cyclic 2,6-bis(1,2,3-triazol-1-yl)pyridines

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S1. Anion transport studies

S1.1. General procedures

Procedures described below are typical membrane transport studies, external and internal solutions may vary and are described in the captions of figures. An Accumet chloride-selective electrode was used during the transport experiments to determine the chloride concentrations. Polyoxymethylene(8)lauryl ether (0.232 mM in 7:1 water:DMSO v/v) was used as the detergent to lyse the vesicles and was supplied by TCI. The vesicles we made using POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) which was supplied by Genzyme, it was stored at -20 °C in chloroform (1 g of POPC in 35 mL).

S1.1.1. Vesicle preparation

A thin POPC lipid film was afforded from a chloroform solution under reduced pressure and was dried under vacuum for more than 4 hours. The film was rehydrated using a metal chloride salt solution (489 mM MCl, 5 mM phosphate buffer at pH 7.2) in a vortex. The suspension then underwent nine freeze thaw cycles, using a liquid nitrogen bath followed by a warm water bath. The lipid was left to acclimatise at room temperature for 30 min. Following this the lipid suspension was extruded 25 times through a Nucleopore™ 200 nm polycarbonate membrane resulting in the formation of unilamellar vesicles. The vesicles were then dialyzed for a minimum of 2 hours using Spectra/Por® 2 Membrane MWCO 12-14 kD against the external medium to remove the unencapsulated metal chloride salts.

S1.1.2. Transport assay- chloride/nitrate at pH 7.2

POPC unilamellar vesicles prepared as above containing 489 mM NaCl solution were suspended in an external medium of 489 mM NaNO₃ buffered to pH 7.2 using sodium phosphate salts (5 mM buffer using NaH₂PO₄ and Na₂HPO₄). A DMSO solution of the receptor was added to start the experiment and the chloride efflux was measured using the chloride selective electrode. After 5 min the vesicles were lysed using 50 µL of detergent, a final reading of chloride efflux was taken at 7 min to determine 100% efflux. All other points were calibrated to this and the initial reading (0 %).

S1.1.3. Transport assay- chloride/bicarbonate at pH 7.2

POPC unilamellar vesicles prepared as above containing 450 mM NaCl solution were buffered to pH 7.2 using sodium phosphate salts (20 mM buffer using NaH₂PO₄ and Na₂HPO₄). The vesicles were suspended in a 162 mM Na₂SO₄ external medium which was buffered to pH 7.2 using sodium phosphate salts (20 mM buffer using NaH₂PO₄ and Na₂HPO₄). A DMSO solution of the receptor was added to start the experiment and the chloride efflux was measured using a chloride selective electrode. After 2 min a NaHCO₃ solution was added so the external medium
contained 40 mM NaHCO₃. After 7 min the vesicles were lysed using 50 µL of detergent, a final reading of chloride efflux was taken at 9 min to determine 100 % chloride efflux. All other points were calibrated to this and the initial reading (0%).

S1.1.4. Transport assay- caesium chloride at pH 7.2
POPc unilamellar vesicles prepared as above containing 450 mM CsCl solution were buffered to pH 7.2 using sodium phosphate salts (20 mM buffer using NaH₂PO₄ and Na₂HPO₄). The vesicles were suspended in 162mM Na₂SO₄ external medium which was buffered to pH 7.2 using sodium phosphate salts (20 mM buffer using NaH₂PO₄ and Na₂HPO₄). A DMSO solution of the receptor was added to start the experiment and the chloride efflux was measured using the chloride selective electrode. After 5 min the vesicles were lysed using 50 µL of detergent, a final reading of chloride efflux was taken at 7 min to determine 100% efflux. All other points were calibrated to this and the initial reading (0 %).

S1.1.5. Lucigenin assays- chloride/sulfate antiport at pH 7.2
A thin POPC lipid film was afforded from a chloroform solution under reduced pressure and was dried under vacuum for more than 6 hours. The film was rehydrated using a NaCl solution (2 mM lucigenin, 100mM NaCl, 20mM phosphate buffer at pH 7.2) in a vortex. The suspension then underwent nine freeze thaw cycles, using a liquid nitrogen bath followed by a warm water bath. The lipid was left to acclimatise at room temperature for 30 min. Following this the lipid suspension was extruded 25 times through a Nucleopore™ 200 nm polycarbonate membrane resulting in the formation of unilamellar vesicles. The unencapsulated lucigenin was removed by size exclusion chromatography on a sephadex G-25 column using a NaCl solution as eluent (100 mM NaCl, 20 mM phosphate buffer at pH 7.2).

The POPC unilamellar vesicles containing NaCl and lucigenin, prepared as above, were suspended in a NaCl solution buffered to pH 7.2 with sodium phosphate salts (20 mM buffer using NaH₂PO₄ and Na₂HPO₄). The sample had a lipid concentration of 0.5 mM. The internal chloride concentration was monitored by fluorescence of intravesicular lucigenin following excitation at 372 nm and recording the emission at 503 nm. At t = 10 s, a stock salt solution of the appropriate anion was added so the external concentration was 40 mM of the new salt (NaNO₃, Na₂SO₄, NaCl). At t = 40s a DMSO solution of the receptor was added to start transport. After 240 s the vesicles were lysed with detergent. A blank was run using Na₂SO₄ and DMSO.

S1.1.6 HPTS assays- HCl co-transport
A thin POPC lipid film was afforded from a chloroform solution under reduced pressure and was dried under vacuum for more than 6 hours. The film was rehydrated using a NaCl solution (1 mM HPTS, 489mM NaCl, 5 mM phosphate buffer at pH 7.2) in a vortex. The suspension then underwent nine freeze thaw cycles, using a liquid nitrogen bath followed by a warm water bath. The lipid was left to acclimatise at room temperature for 30 min. Following this the lipid suspension was extruded
25 times through a *Nucleopore™* 200 nm polycarbonate membrane resulting in the formation of unilamellar vesicles. The unencapsulated lucigenin was removed by size exclusion chromatography on a sephadex G-25 column using a Na$_2$SO$_4$ solution as eluent (162 mM Na$_2$SO$_4$, 5 mM phosphate buffer at pH 7.2).

The POPC unilamellar vesicles containing NaCl and HPTS, prepared as above, were suspended in a 162 mM Na$_2$SO$_4$ solution buffered to pH 7.2 with sodium phosphate salts (20 mM buffer using NaH$_2$PO$_4$ and Na$_2$HPO$_4$). The sample had a lipid concentration of 1.0 mM. At t = 10 s, a DMSO solution of the receptor was added to start transport. The fluorescence of intravesicular HPTS was monitored by excitation at 403 nm and 460 nm and recording the emission at 510 nm. After 310 s the vesicles were lysed with detergent. The internal pH was obtained by fitting the data to the following equation:

$$pH = \frac{-1}{1.796} \ln \left( \frac{4.2055}{I_{460\,\text{nm}}/I_{403\,\text{nm}} - 1} \right) + 7.6142$$
S1.2. Chloride/nitrate transport

**Figure 1.** Chloride efflux at 270 s promoted by thioureas 6a-f (2 % molar carrier to lipid) from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 489 mM NaNO$_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, detergent as added to lyse the vesicles and calibrate the ISE to 100 % chloride efflux. Each point represents the average of three trials.
S1.3. Hill Plots

The above chloride/nitrate transport assays are performed for various concentrations of receptor with respect to the lipid. The chloride efflux 270s after addition of the receptor is plotted as a function of receptor concentration. These data points are then fitted to the hill equation using *Origin 9.1*:

\[ y = \frac{V_{\text{max}} \cdot x^n}{k + x^n} \]

Where \( y \) is the chloride efflux at 270 s (%) and \( x \) is the carrier concentration (mol\% carrier to lipid). \( V_{\text{max}} \), \( k \) and \( n \) are the parameters to be fitted. \( V_{\text{max}} \) is the maximum efflux possible (often fixed to 100\%, as this is physically the maximum chloride efflux possible), \( n \) is the Hill coefficient and \( k \) is the carrier concentration needed to reach \( V_{\text{max}}/2 \) (when \( V_{\text{max}} \) is fixed to 100\%, \( k \) equals \( EC_{50} \)).

So from the hill plot it is possible to obtain \( EC_{50} \) values (receptor concentration needed to obtain 50 \% chloride efflux) for 270s.
**Figure S2.** Hill plot for chloride efflux facilitated by 6a from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. Chloride efflux was measured at 270 s.

**Figure S3.** Hill plot for chloride efflux facilitated by 6b from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. Chloride efflux was measured at 270 s.
Figure S4. Hill plot for chloride efflux facilitated by 6c from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 489 mM NaNO\textsubscript{3} buffered to pH 7.2 with 5 mM sodium phosphate salts. Chloride efflux was measured at 270 s.

Figure S5. Hill plot for chloride efflux facilitated by 6d from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 489 mM NaNO\textsubscript{3} buffered to pH 7.2 with 5 mM sodium phosphate salts. Chloride efflux was measured at 270 s.
Figure S6. Hill plot for chloride efflux facilitated by 6e from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. Chloride efflux was measured at 270 s.

Figure S7. Hill plot for chloride efflux facilitated by 6f from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. Chloride efflux was measured at 270 s.
S1.4. Chloride/bicarbonate transport

**Figure S8.** Chloride efflux promoted by compound 6a, c-f (2 % molar carrier to lipid) from unilamellar POPC vesicles loaded with 450 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were dispersed in 162 mM Na$_2$SO$_4$ buffered to pH 7.2 with 20 mM sodium phosphate salts. At $t = 120s$, a solution of NaHCO$_3$ was added to give a 40 mM external concentration (blue markers) or the experiment was allowed to continue without the addition of NaHCO$_3$ (red markers). At the end of the experiment, detergent was added to lyse the vesicles and calibrate the ISE to 100 % chloride efflux. Each point represents the average of three trials.
S1.5. Cs/Na chloride transport- symport vs antiport

**Figure S9.** Chloride efflux promoted by 6a-6f (2 % molar carrier to lipid) after 270 s from unilamellar POPC vesicles loaded with 450 mM CsCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were dispersed in 162 mM Na₂SO₄ buffered to pH 7.2 with 20 mM sodium phosphate salts. At the end of the experiment, detergent was added to lyse the vesicles and calibrate the ISE to 100 % chloride efflux. Each point represents the average of three trials.
S1.6. Chloride/sulfate antiport

Figure S10. Unilamellar POPC vesicles were loaded with 100 mM NaCl and 2 mM lucigenin buffered to pH 7.2 with 20 mM sodium phosphate salts and dispersed in a 100 mM NaCl solution (buffered to pH 7.2). At t = 10 s, a solution of the appropriate anion was added (final concentration of 40 mM NaNO₃, 40 mM Na₂SO₄ or 40 mM NaCl). At t = 40 s, a DMSO solution of the compound 6a was added. At the end of the experiment (340 s), detergent was added to lyse the vesicles. The blank measurement refers to the addition of Na₂SO₄, followed by the addition of DMSO. Each point represents the average of three trials.

Figure S11. Unilamellar POPC vesicles were loaded with 100 mM NaCl and 2 mM lucigenin buffered to pH 7.2 with 20 mM sodium phosphate salts and dispersed in a 100 mM NaCl solution (buffered to pH 7.2). At t = 10 s, a solution of the appropriate anion was added (final concentration of 40 mM NaNO₃, 40 mM Na₂SO₄ or 40 mM NaCl). At t = 40 s, a DMSO solution of the compound 6c was added. At the end of the experiment (340 s), detergent was added to lyse the vesicles. The blank measurement refers to the addition of Na₂SO₄, followed by the addition of DMSO. Each point represents the average of three trials.
**Figure S12.** Unilamellar POPC vesicles were loaded with 100 mM NaCl and 2 mM lucigenin buffered to pH 7.2 with 20 mM sodium phosphate salts and dispersed in a 100 mM NaCl solution (buffered to pH 7.2). At t = 10 s, a solution of the appropriate anion was added (final concentration of 40 mM NaNO₃, 40 mM Na₂SO₄ or 40 mM NaCl). At t = 40 s, a DMSO solution of the compound 6d was added. At the end of the experiment (340 s), detergent was added to lyse the vesicles. The blank measurement refers to the addition of Na₂SO₄, followed by the addition of DMSO. Each point represents the average of three trials.

**S1.7 HCl co-transport**

**Figure S13.** Intravesicular pH change promoted by 6a-6d (2 % molar carrier to lipid) from unilamellar POPC vesicles loaded with 1 mM HPTS and 489 mM NaCl, buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 162 mM Na₂SO₄ buffered to pH 7.2 with 5 mM sodium phosphate salts. At t = 10 s, a DMSO solution of the putative transporters was added to start the experiment. At the end of the experiment (t = 310 s), detergent was added to lyse the vesicles. Each point represents the average of three trials.
S1.8 Stability constants of compound 8 with a variety of anions added as their tetrabutylammonium salts in DMSO-$d_6$/0.5% water

- **Cl⁻**: log $K = 1.76 \pm 0.0207$
  $K = 58 \pm 3 \text{ M}^{-1}$
- **Br⁻**: log $K = 1.34 \pm 0.0534$
  $K = 22 \pm 3 \text{ M}^{-1}$
- **nitrate**: log $K = /$
  $K = 0 \text{ M}^{-1}$
- **benzoate**: log $K = 2.17 \pm 0.04$
  $K = 150 \pm 16 \text{ M}^{-1}$
- **acetate**: log $K = 2.49 \pm 0.0484$
  $K = 310 \pm 34 \text{ M}^{-1}$
- **bisulfate**: log $K = 1.54 \pm 0.0497$
  $K = 35 \pm 4 \text{ M}^{-1}$