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Squaramide Calix[4]pyrroles for Anion Transport

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Abstract: In this paper we demonstrate the transmembrane chloride transport properties of a series of calix[4]pyrroles appended with squaramide moieties for the first time. The flexibility imparted by the methylene group between the squaramide and macrocycle allowed for changes between 1 : 1 and 1 : 2 (host : guest) binding modes at high chloride concentrations. Electroneutral transport was observed in the substituted compounds compared to the parent calix[4]pyrrole, with an improvement in transport activity when distal electron withdrawing groups were appended. These results highlight the important role of the squaramide in facilitating transmembrane transport for the substituted macrocycles.

Keywords: calix[4]pyrrole, squaramide, chloride, transmembrane transport.

INTRODUCTION

TRANSMEMBRANE chloride transport within biological systems is an important process responsible for regulating critical physiological functions.[1,2] Disruption of natural chloride transport has been linked to several diseases, including cystic fibrosis and cancer. Cystic fibrosis is the result of a genetic defect in the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride ion channel within the lungs. The defect results in impaired channel functionality, causing a buildup of mucus that leads to breathing difficulties and infection.[3,4] Small molecule anionophores have been proposed as a potential future therapy for cystic fibrosis and similar conditions, where they act to restore the missing chloride transport. Separately, the co-transport of chloride and H^+ was shown to dissipate lysosomal pH levels in HeLa cancer cells, leading to apoptosis. Studies into the mechanisms of cell death indicated that H^+ / Cl^- co–transport disrupted autophagy, while chloride uniport induced apoptosis.[5,6] Therefore, research into synthetic anion receptors capable of chloride transport may open up novel avenues for their use in the remediation of life-threatening diseases.

Calix[4]pyrroles are a well-known class of neutral anion receptors with strong affinities for anions in organic solvents[7–9] The strong binding is driven by a conformational change in the macrocycle in the presence of an anionic guest, where the four pyrrole ring N–H groups rearrange to provide a convergent hydrogen bond donor site. In particular, halides such as fluoride and chloride are strongly bound.[10] Efforts to enhance the binding affinity of *meso*-octamethylcalix[4]pyrrole (OMCP) have included extending the binding cavity via substitutions at the β position with halogenated or aromatic substituents. A β-substituted calix[4]pyrrole prepared by Sessler and co-workers, formed from pyrrole fused to *N*-tosylpyrrolidine, was found to recognise halides more effectively than OMCP.[11] The same researchers used 3,4–difluoropyrrole as the subunit for a fluorinated calix[4]pyrrole, which resulted in a two-fold increase in the affinity towards fluoride and chloride, and a seven-fold increase in affinity to dihydrogen phosphate compared to OMCP.[8]

Substitutions at the *meso*-position of calix[4]pyrrole with other functional groups can also yield improved anion binding and enhanced functionality. Ballester and co-workers extended the walls of OMCP to include four aromatic surfaces in a "four-walled" calix[4]pyrrole, which displayed

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higher affinities to chloride with the aid of significant anion-π interactions.[12] Similar improvements in the affinity toward halides have been achieved through other substitutions at the *meso*-position, including photoswitchable groups, nucleic acids, and aliphatic groups.[13–15] The binding properties of OMCP can also be improved through the addition of a bridge across the macrocycle, forming a bicyclic "strapped" system. Strapped calix[4]pyrroles display enhanced anion binding affinities, selectivity, and augmented chloride transport behaviour depending on the nature of the strap.[16–18] OMCP is incapable of performing transmembrane chloride transport on its own and will only perturb chloride concentrations via a coupled process with caesium.[19,20] However, the installation of various substituents has alleviated this limitation while also allowing for the transport of phosphate and fluoride, anions that were historically difficult to transport.[21,22]

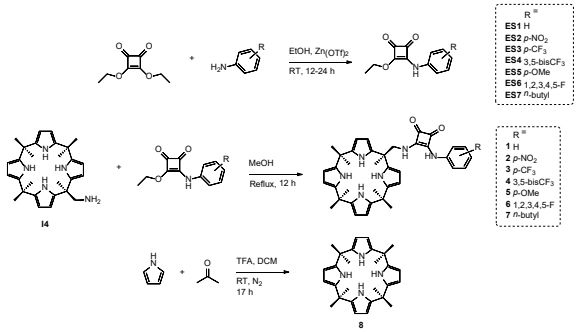
The design of small molecule anionophores for chloride transport often incorporates dual hydrogen bond donor groups such as ureas, thioureas, and squaramides.[23–27] Squaramides have attracted considerable interest as anion receptors and transporters due to their dual hydrogen bond donor sites. The rigidity of the cyclobutene ring, the convergent directionality of NH bonds, and the NH bond angles are key elements that often make squaramides better receptor motifs than urea analogues.[28] While a variety of functional groups have been explored and appended onto the OMCP scaffold, there is a lack of exploration in the conjugation of squaramides onto the macrocycle. Recently, Wang and co-workers explored the anion binding ability of a single-walled *meso*-substituted calix[4]pyrrole involving an aliphatic-squaramide unit.[29] Their initial investigation highlighted that the addition of a squaramide unit to OMCP provided an additional hydrogen-bond donor site for an anion, thus increasing the binding affinity of the compound towards fluoride, chloride, and dihydrogen phosphate.[29] Molecular modelling also suggested the formation of an encapsulated anion binding site with the methylene $-CH_2$ group imparting the necessary flexibility.

We sought to investigate whether the increased binding affinity to chloride afforded by adding a squaramide unit to OMCP would translate to enhanced chloride transport capability. It was envisaged that the presence of aromatic substituents on the squaramides would form a more encapsulated binding pocket around the anion. Previous work in our group highlighted the benefits of a strapped calix[4]pyrrole in enhancing fluoride transport. However, it was challenging to modify the bridging triazole with alternative functional groups.[30] The electron-withdrawing groups (EWGs) on the aromatic substituents will also allow a comparison to be made between the effects of preorganization on anion transport versus the enhancement caused by appending electron-withdrawing derivatives. To the best of our knowledge, this work represents the first investigation into the transport properties of a calix[4]pyrrole with a squaramide substituent. The chloride transport activity of the molecules was explored in a comprehensive series of assays. Several of the compounds displayed moderate transport activity in the HPTS assay, whereas OMCP was inactive, demonstrating that H^+ / Cl^- cotransport could be achieved through the addition of a squaramide unit.

EXPERIMENTAL

Chemicals and Materials

Chemicals were purchased from commercial sources and used without purification unless otherwise stated. Synthesis of the squaramide-substituted calix[4]pyrroles studied in this work is outlined in Scheme 1. The calix[4]pyrrole intermediates (**I1–I4**), compound **7**, and OMCP **8** were prepared according to literature procedures.[29] Pyrrole oligomers formed during the synthesis of **I2**, which resulted in the need for additional washes with hot *n*-hexane after column chromatography. These oligomeric side products were also responsible for reduced yields in this step.[31] The mono-substituted ethyl squarates **ES1–ES7** were prepared by treating diethyl squarate with one equiv. of the appropriate aniline in the presence of zinc triflate. For **ES6** and **ES7**, additional washes with *n*-hexane were necessary after column chromatography to fully remove residual diethyl squarate. Finally, **ES1–ES7** were treated with aminomethyl calix[4]pyrrole (**I4**) to give the final squaramide-substituted calix[4]pyrroles (**1–7**). Compounds **1–5** slowly precipitated out of the reaction mixture over several hours during cooling, eliminating the need for further workup. OMCP **8** was prepared from distilled pyrrole and acetone according to literature procedures.



Scheme 1. Synthesis of ethyl squarates **ES1–ES7**, final compounds **1–7**, and OMCP **8** studied in this work.

Transport Studies

Vesicles were prepared using 1–palmitoyl–2-oleoyl–*sn*–glycerol–3–phosphocholine (POPC) lipids and loaded with NaCl (487 mM) solution, buffered to pH 7.2 with sodium phosphate salts (5 mM). The lipids were suspended in a $NaNO_3$ external solution (487 mM), also buffered to pH 7.2. The addition of the transporter as a DMSO solution induced chloride efflux out of the vesicles, which was detected using the ISE over 300 s before lysing the vesicles with detergent to obtain a 100 % chloride efflux reading.

RESULTS AND DISCUSSION

Crystallography

Crystals of **6** that were suitable for single crystal X-ray diffraction were grown from a DMSO solution of the compound. Compound **6** crystallised in the *P–1* space group with three formula units in the unit cell (CCDC deposition number: 2499222).

The pyrrole subunits for **6** were oriented in a partial cone configuration where three subunits were face up and one face down (Figure 1). Hydrogen bonds were observed between the three face-up pyrrole subunits (N-H···O 2.05 Å) and the singular face-down subunit (N-H···O 2.04 Å) to the oxygen of nearby DMSO molecules, which could have acted as a guest and induced a geometric change within the macrocycle. Hydrogen bonds were also observed between the two squaramide NH groups and a singular DMSO molecule. The pentafluorobenzene subunit was observed in a non-planar orientation compared to the squaramide moiety, most likely due to steric interactions between the fluorine atoms and squaramide oxygens if both groups were aligned within the same plane.

Binding Studies

To assess the binding affinity of the compounds for chloride, titrations with tetrabutylammonium chloride (TBACl) salt were performed and monitored via ^{1}H NMR. Chloride binding can be visualised and tracked via shifts in the resonances corresponding to protons involved in the binding event**.** The ^{1}H NMR titrations were performed with TBACl guest in DMSO-d_6 / 0.5 % H_2O. In all cases, the addition of chloride resulted in simultaneous downfield shifts of resonances corresponding to the squaramide NHs and the pyrrolic NHs of the macrocycle unit (Figure 2). Furthermore, the pyrrolic NH resonance peaks showed a marked change in shape from a doublet to a broad singlet following the addition of 1 equiv. of chloride, which indicated strong hydrogen binding interactions between the anion and macrocycle.[29]

At higher equivalents of chloride (> 20 equiv.), the pyrrolic NH signals resolved back into sharp doublets and no longer shifted downfield, which suggested that binding saturation was reached. Notably, both squaramide NH signals continued to shift downfield to the maximum of 100 equiv. during the titration. This behaviour suggested that the squaramide initially formed an encapsulated binding site for chloride within the macrocycle but rearranges at higher equiv. to swing out and form a separate, secondary binding site. The methylene $–CH_2$ signal was also observed to move downfield in a similar fashion to the pyrrole NH signals, with a saturation point at approx. 20 equiv. of chloride. Due to the proximity of this $–CH_2$ group to the central macrocycle cavity, it was likely that chloride has a weak binding interaction with these protons.

The chemical shifts of the pyrrolic NHs, two squaramide NHs, and methylene $–CH_2$ were plotted against the equivalents of chloride throughout the titration. The data set was fitted to both a 1 : 1 and 1 : 2 (host : guest) binding model using the BindFit v0.5 software.[33] As a 1 : 2 binding model considers more parameters and naturally gives a better fit to the data, a subsequent analysis of the

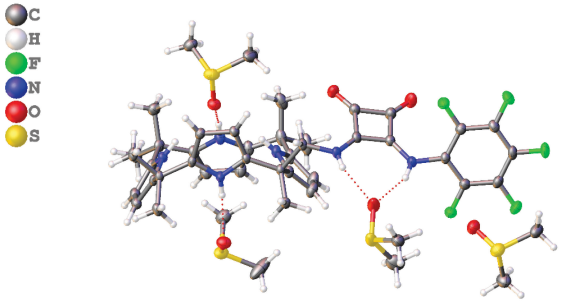


Figure 1. Crystal structure of compound **6** with hydrogen bonds shown in red. Thermal ellipsoids are shown at 50 % probability.

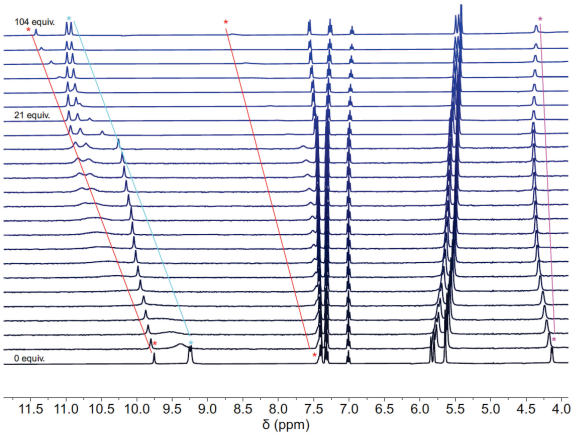


Figure 2. ^{1}H NMR titration of compound **1** at 400 MHz with TBACl in DMSO-d_6 / 0.5 % H_2O. Movement of the two squaramide NH (red), pyrrolic NH (cyan), and methylene -CH_2 signals (purple) are highlighted with a straight line.

covariance of fit for both models was performed, and the ratio ($F\,cov_{fit}$) between 1 : 1 and 1 : 2 models was compared. A $F\,cov_{fit} > 5$ is usually required to confirm that a 1 : 2 binding model is preferred.[34] For all compounds, the 1 : 2 binding model was confirmed as the best fit (Table 1).

The binding constants and preference for a 1 : 2 binding sequence further reinforce the hypothesis that the macrocycle and squaramide unit act as separate binding sites at high equiv. of chloride. The high K_{11} value (~ 500 M^{-1}) in compounds **1–7** is indicative of chloride binding to the macrocycle, which is comparable to the binding constant of **8**. The high error (23 %) in the binding constant for **8** can be attributed to the difficulty in accurately quantifying the pyrrole NH peaks due to significant broadening upon anion binding. For the second binding event, compounds **1–6** exhibited a stronger affinity than **7** due to the presence of the aromatic phenyl group which has been shown to enhance the hydrogen bonding donicity within squaramides.[36] The geometry of the squaramide also allows the aromatic C_α–H to participate in hydrogen bonding with the anion.[28] This was reflected in the NMR shifts, where the corresponding signal shifts slightly downfield in response to chloride. The lack of a similar secondary interaction in **7** accounts for the lower K_{12} value compared to the other compounds.

While the K_{12} binding constants are relatively small, there is still a trend in compounds **1–4** where the more potent EWGs (*p*-NO_2, *p*-CF_3, and 3,5-bisCF_3) displayed higher affinities. This was a result of the EWGs ability to increase the acidity of the NH proton, leading to stronger complexation with an anionic guest. While **6** also contained electron-withdrawing fluorine groups, the replacement of the hydrogen at C_α with a fluorine atom may result in steric hindrance, leading to a lower K_{12} value. A similar hindrance was seen in the crystal structure of **6**, where the pentafluorobenzene subunit could not be aligned in the same plane as the squaramide group due to steric hindrance between the fluorine and oxygen atoms. The compound library displayed negative binding cooperativity throughout the titration, reflected in the lower K_{12} values. The negative cooperativity was likely to be caused by the electrostatic repulsion of a second chloride following the first binding event.

An additional titration with 4 equiv. of fluoride was attempted for compounds **2**, **4**, and **7** as OMCP **8** is known to have a strong affinity for fluoride.[37] These three compounds were chosen as the *p*-NO_2 and 3,5-bis(CF_3) groups are strongly electron-withdrawing, leading to increased acidity of the squaramide NH proton. In contrast, the *n*-butyl group is likely to reduce acidity as a result of hyperconjugation.[38] The addition of 0.25 equiv. of fluoride resulted in the formation of a new set of peaks in all three compounds and slow exchange was observed on the NMR timescale. However, the proton shifts could not be fit to a binding model due to complex binding events which suggested the presence of multiple species in solution. At higher equiv., deprotonation of the compounds was suspected due to the disappearance of the squaramide NH peaks (Figure 3) in addition to a colour change within the NMR solution. This deprotonation was confirmed by the addition of one drop of 1 M hydrochloric acid to the NMR solution after 4 equiv. of fluoride had been added. The addition resulted in a reversal of the colour change, as well as regeneration of the squaramide NH peaks.

Table 1. Binding constants (M^{-1}) for compounds **1–8**. Binding studies were performed in DMSO-*d*6 / 0.5 % H2O with TBACl. Affinity errors are < 6 % for compounds **1–7** and 23 % error for 8.

Compound	K_{11}	K_{12}	$\alpha^{(a)}$	$F\,cov_{fit}{}^{(b)}$
1	549	9.28	0.07	99
2	510	16.4	0.13	57
3	518	12.7	0.10	98
4	491	17.7	0.14	77
5	513	8.96	0.07	62
6	529	6.77	0.05	117
7	568	3.67	0.03	121
8	740	N/A	N/A	N/A

(a) The interaction parameter (α) was calculated by multiplying K_{12} by four and dividing by K_{11}. An α value < 1 indicates negative cooperativity, a value > 1 indicates positive cooperativity, while a value of 1 describes non-cooperative binding.

(b) Factor of covariance of fit ($F\,cov_{fit}$), calculated by dividing the 1 : 1 cov_{fit} by 1 : 2 cov_{fit}. 1 : 2 binding is the preferred model if $F\,cov_{fit} > 5$.[35]

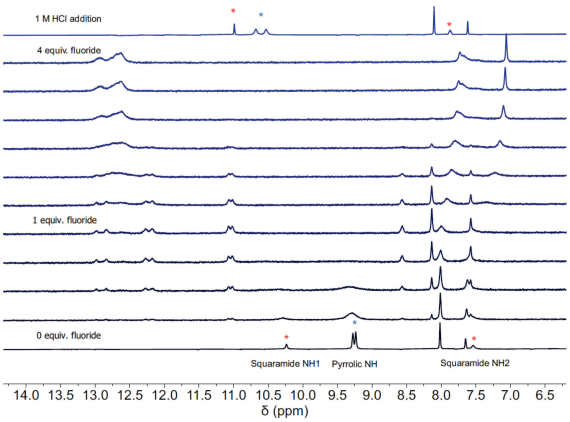


Figure 3. ^{1}H NMR titration of compound **4** at 400 MHz with four equivalents of TBAF in DMSO-d_6 / 0.5 % H_2O. Addition of one drop of 1 M HCl regenerates the squaramide peaks, indicating deprotonation. A comparison spectrum of **4** titrated with 8 equiv. of TBACl demonstrates chloride binding to **4** following regeneration of the squaramide peaks.

Chloride/Nitrate Exchange

The transport properties of the compounds were initially tested using an ion-selective electrode (ISE) chloride/nitrate exchange assay. Despite the modest binding affinities of **1–7** towards chloride, the compounds showed little activity in this assay. Compounds **3** and **4** demonstrated the highest activity overall, reaching approx. 40 % efflux after 300 s. This assay relies on the exchange of chloride with nitrate to sustain continued chloride efflux (Figure 4a). As the calix[4]pyrrole macrocycle does not bind nitrate strongly, it is unlikely to participate in the exchange process.[39] Thus, the transport activity observed could be attributed solely to the squaramide unit. Previous work with squaramides showed that the mono- and bis–CF_3 functionalised derivatives had the highest transport activity, which is reflected in the activities of **3** and **4**, which were comparatively highest in this series.[24,36]

Early work in our group indicated that OMCP **8** is capable of co-transporting caesium chloride as an ion-pair due to conformational changes that occur in the macrocycle following anion binding.[10,20] As a result, we repeated the chloride/nitrate exchange assay with an internal solution of caesium chloride in order to check for CsCl co-transport. Under these conditions, there was no improvement in the transport activity for any of compounds **1–7** compared to the NaCl conditions (Figure 4b). The lack of transport improvement is likely due to poor solubility of the squaramide derivatives under the conditions of the chloride/nitrate exchange assay, which hinders the incorporation of the macrocycles into the vesicle bilayer. The transport activity also remained the same when the internal cation of the chloride salt was replaced with potassium.

We further modified the chloride / nitrate exchange assay to investigate whether a chloride uniport mechanism was possible. This involved the use of potassium gluconate instead of nitrate in the external solution. Gluconate is a large hydrophilic anion and cannot be transported or diffuse independently across the lipid bilayer. This alteration was made to assess whether a lack of nitrate back-transport was was responsible for a depreciation in the chloride transport capabilities of the compounds. In this new assay, chloride transport is only possible in the presence of a cationophore, such as valinomycin or monensin. Valinomycin strictly uniports potassium, whereas monensin can exchange potassium with a proton. Coupling to valinomycin requires the ability to uniport chloride, known as electrogenic transport, while coupling to monensin requires H^+ / Cl^- co-transport, known as electroneutral transport. Across all compounds, the transport activities showed coupling to both ionophores, but no improvement over the activity in chloride/nitrate exchange conditions. The results indicated the compounds have no preference between electrogenic or electroneutral transport, which may arise as a result of chloride transport being rate-limiting in all processes studied. In the presence of valinomycin, it is possible that electrogenic transport arises from chloride binding to the macrocycle.

HPTS Assay for HCl Co-transport

As the compounds exhibited HCl co-transport, we further investigated their activity using the 8-hydroxypyrene-1,3,6-

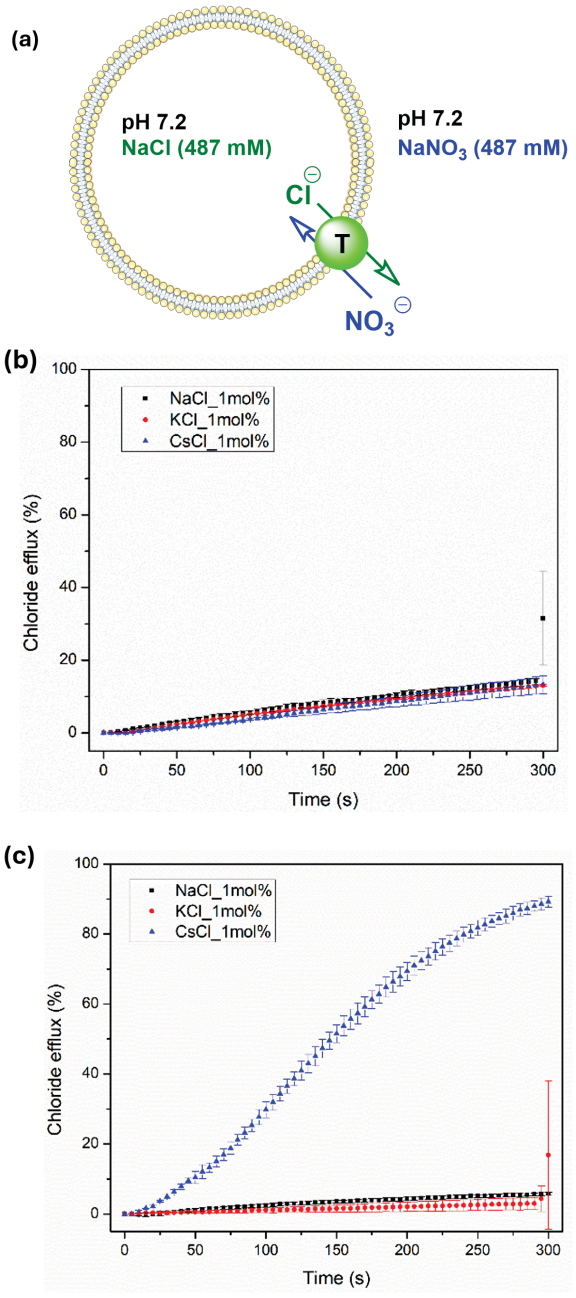


Figure 4. (a) Schematic of the ISE chloride/nitrate exchange assay. Comparison of transport activities at 1 mol% in the Cl^-/NO_3^- exchange assay with NaCl, KCl, and CsCl internal solutions for: (b) compound **1** and (c) OMCP **8** .

trisulfonic acid trisodium salt (HPTS) assay.[40] Here, POPC vesicles were loaded with KCl (100 mM) and the fluorescent dye HPTS (1 mM), balanced to pH 7.0 with HEPES buffer (10 mM). The vesicles were suspended in an otherwise identical solution without HPTS. The excitation wavelength of HPTS changes after deprotonation at pH 8, but not the emission wavelength. As a result, changes in the ratio of excitation / emission can be monitored as an indicator of H^+ / Cl^- transport. The addition of a NaOH base pulse generates the necessary pH gradient across the lipid bilayer to drive transport. Due to the lower amount of chloride transport (100 mM) in this assay, the compounds were much more active and displayed stronger transport activity. Consequently, Hill analysis and the corresponding EC_{50} values could be calculated for compounds **1–5** and **7** (Table 2). The transport activity for compound **6** plateaued at approx. 60 % of the maximal concentration, thus an EC_{50} could not be accurately calculated.

The activity of the compounds aligns with a trend in the potency of the EWGs attached to the squaramide. Compounds **2–4** exhibited much lower EC_{50} values compared to **1** and **7**. Similarly, compound 5, containing an electron-donating methoxy group, had a higher EC_{50} (similar to that of **7**). The EWGs in **2–4** improve the charge-delocalisation of the chloride complexes, allowing rapid shuttling through the lipid bilayer. Furthermore, previous work has shown the importance of lipophilicity in predicting the activity of an anion transporter.[41] The correlation between lipophilicity and activity can be seen in the c*Log*P values in Table 2, where the more active compounds **2–4** have higher lipophilicity values. The decreased activity of compounds **1** and **5** could be attributed to the electron-donating methoxy and *n*-butyl groups, which hindered the effective delocalisation of the negative charge. The effects of the EWGs on the EC_{50} clearly demonstrated that the squaramide group exerted a substantial influence on the transport activity of the compounds.

The Hill coefficient (an indicator of anion transport stoichiometry) is close to 1.0 for compounds **2–4**, indicating a transport stoichiometry of one transporter per chloride. In contrast, the coefficient is closer to 2.0 for compounds **1**, **5**, and **7**, suggesting transport via higher order aggregates.

The rate-limiting step during H^+ / Cl^- symport can be determined through the addition of valinomycin or a protonophore, carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP). An increase in activity in the presence of valinomycin indicates chloride transport is the rate-limiting step, whereas activity increases in the presence of CCCP indicate proton transport is the rate-limiting step.

Across the tested compounds, enhanced activity was seen after adding valinomycin but not CCCP, indicating H^+ > Cl^- selectivity in these compounds (Figure 5). As the squaramide unit was the major contributor to transport activity, the chloride rate-limiting step could be a result of the comparatively weaker chloride binding affinities of the squaramide moiety, which prevented the effective capture and release of the anion during transport.

Squaramide–based transporters have previously been shown to facilitate proton transport via a fatty acid flip–flop mechanism. This process involves the transporter binding to the anionic carboxylate headgroup of a fatty acid before diffusing over the bilayer together.[42] Commercially available POPC lipids contain fatty-acid impurities, which provide an alternative pathway to proton efflux.[43] To investigate the effect of fatty acids on transport activity, the

Table 2. EC_{50} values and Hill coefficients (*n*) for compounds **1–8** calculated from the HPTS assay. Calculated lipophilicity values (cLogP) were also obtained for all compounds

Compound	EC_{50}[a] (mol%)	*N*[b]	cLogP[c]
1	0.861 ± 0.11	1.5	6.57
2	0.084 ± 0.014	0.95	6.56
3	0.098 ± 0.012	1.1	6.95
4	0.043 ± 0.005	0.98	7.25
5	1.33 ± 0.12	1.7	6.56
6	–[d]	–[d]	5.93
7	1.17 ± 0.07	2.4	6.23
8	N/A	N/A	6.40

[a] EC_{50} at 200 s in the HPTS assay, presented as a transporter : lipid percentage.

[b] Hill coefficient is an indicator of the transport stoichiometry between the compound and chloride.

[c] cLogP values were calculated using VCC Lab's online tool.

[d] Compound **6** was not active enough in the HPTS assay to calculate an EC_{50} value.

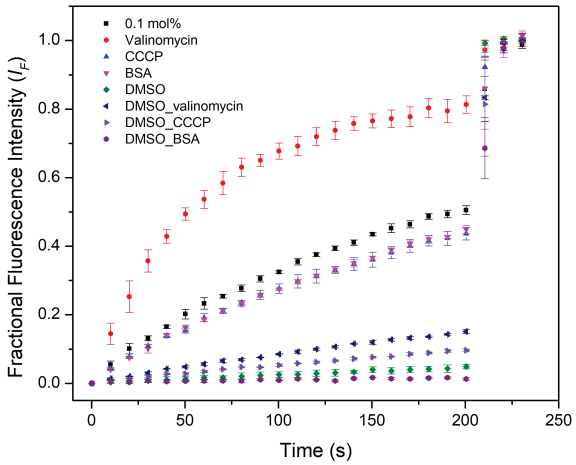


Figure 5. Comparison of transport activities for compound **3** at 0.1 mol % in the HPTS assay in the presence of ionophores and BSA.

assay was repeated in the presence of bovine serum albumin (BSA), oleic acid (OA), and a combination of both additives. BSA sequesters fatty acid impurities and halts the fatty acid flip-flop process. If a fatty acid flip-flop mechanism is involved, the addition of OA would restore transport activity.

The addition of 2 mol% OA resulted in a decrease in activity in all compounds (Figure 6), which indicated competitive binding between oleate and the compounds in place of chloride. BSA binds fatty acids in a 1 : 6 molar ratio, thus the addition of 1 mol% BSA to the POPC vesicles results in the sequestration of all fatty acid impurities within the POPC. Under these conditions, the transport activity for **7** was significantly attenuated, while a small decrease in transport was observed for compounds **1–6**. Overall, these results suggested that the compounds had minimal reliance on the fatty acid flip-flop mechanism. This may be due to the inability of the compounds to form a convergent binding site around the carboxylate head groups, preventing movement through the bilayer. Higher binding ratios, such as one transporter to two head groups, would also be unfavourable due to high entropic energy costs.

For compound **4**, there was no change in activity after adding BSA, which was unusual due as it suggested H^+ / Cl^- transport independent of the flip-flop cycle. One possibility for this might be the ability of **4** to self-deprotonate to facilitate H^+ / Cl^- symport. The presence of fatty acids act as a hindrance by competitively binding to **4**. Given that the presence of 2 mol% OA only resulted in a small decrease in activity for **4**, it is possible that the affinity of **4** for oleate is lower than the other compounds. Finally, the addition of 10 mol% OA after adding 1 mol% BSA gives a final concentration of approx. 4 mol% fatty acid in the vesicles. Restoration of transport activity under these conditions provides evidence for the flip–flop mechanism. In this assay, however, the transport activity decreased even more for all compounds than with only 2 mol% OA. This behaviour provides further evidence that oleate interferes and binds competitively with the compounds.

The anion selectivity of the compounds was investigated via a modified HPTS assay, using a method reported recently by our group.[44] The preparation of POPC vesicles was the same as for the standard HPTS assay, with the exception of a NaCl (100 mM) internal solution instead of KCl. They were then suspended in an external buffer with Cl^-, Br^-, NO_3^-, I^-, or ClO_4^- anions as the respective NaX salts (100 mM). None of compounds **1–7** showed preferential chloride selectivity over the other anions. Compound **5** showed slight anti-Hofmeister behaviour, with a small preference for iodide over perchlorate. The other compounds followed the Hofmeister series for anion selectivity. This was not unexpected, as anti-Hofmeister selectivity is difficult to achieve and typically requires substantial pre-organisation of the binding cavity.[45]

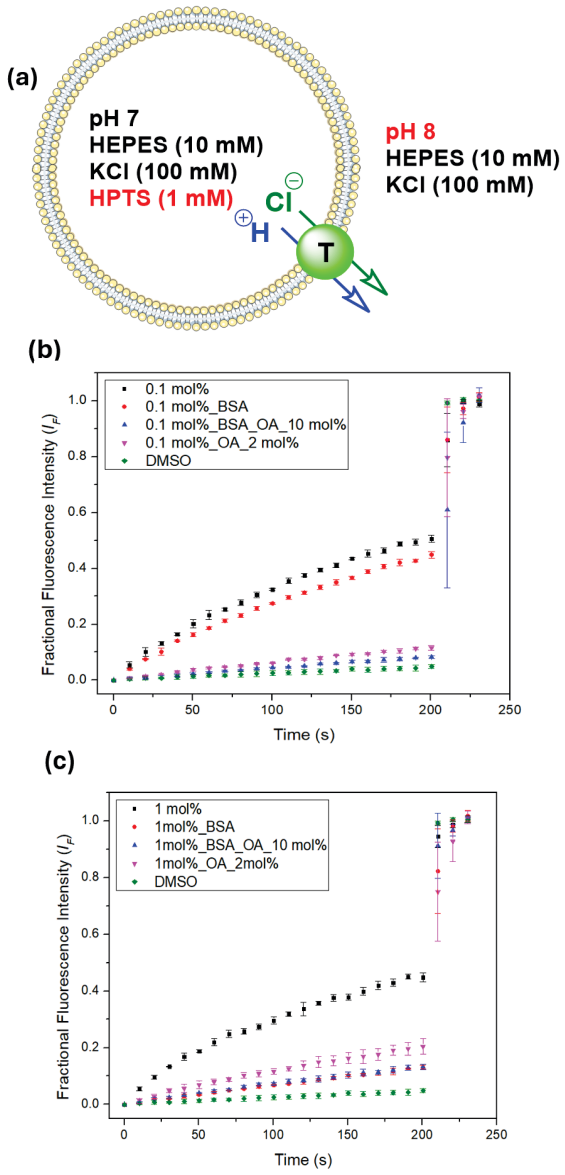


Figure 6. (a) Schematic of the HPTS assay and comparison of the effects of oleic acid (OA), bovine serum albumin (BSA), and a combination of both on (b) compounds **3** and (c) compound **7**.

CONCLUSIONS

We present the first investigations into the anion transport activity of a series of squaramide-substituted calix[4]pyrroles. The effect of a single-walled substitution on the conformational flexibility of the compounds was evident, as the calix[4]pyrroles displayed a change in binding geometry from a 1 : 1 binding mode to a 1 : 2 mode in the presence of high chloride concentrations. We have shown the importance of the squaramide moiety in

facilitating anionophoric activity in the ISE and HPTS assays. Despite their modest transport activities, the influence of EWGs on activity was still evident in the HPTS assay. Further modifications with squaramide-substituted calix[4]pyrroles may provide more functionalities for this class of compounds.

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Supplementary Information. Supporting information to the paper is attached to the electronic version of the article at: https://doi.org/10.5562/cca4201.

PDF files with attached documents are best viewed with Adobe Acrobat Reader which is free and can be downloaded from Adobe's web site.

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