**Anion Transport**

Squaramides as Potent Transmembrane Anion Transporters**

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The transmembrane transport of anions, particularly chloride and bicarbonate, is an important biological process that is normally regulated by transmembrane ion channels. A range of diseases, known as "channelopathies",[1] of which cystic fibrosis is the most common example, are caused by malfunctioning ion channels.[2] There has therefore been significant interest recently in the development of small, drug-like molecules that can facilitate the transport of anions across lipid bilayers, thereby restoring anion permeability to membranes containing faulty channel proteins.[3] Supramolecular chemists have developed ion channels (where molecules form pores in the membrane, through which anions can diffuse) and mobile carriers (where an anion complex is formed that diffuses through the membrane) using peptides,[4] calixarenes,[5] anion π-slides,[6] isophthalamides,[7] calixpyrroles[8] and, recently, also many ureas and thioureas.[9,10] However, to our knowledge, there have not been any reports of the use of squaramides in transmembrane anion transport. There are an increasing number of examples of anion receptors based on the squaramide motif. Costa and co-workers have used a range of computational and experimental techniques to provide compelling evidence that squaramide-based molecules are capable of both cation[11] and anion binding.[12] More recently, the groups of Taylor,[13] Fabbrizzi,[14] and others[15] have used squaramide-based receptors for the binding of various anions, including chloride, sulfate and carboxylates. Squaramides are also being developed as non-covalent organocatalysts,[16]

because the considerations applied to the design of hydrogen-bonding anion receptors and hydrogen-bonding organocatalysts are closely related.[17] Furthermore, there has been recent interest by medicinal chemists in introducing squaramido functionalities into drug candidates, using them as isosteres for ureas, guanidines, and other groups.[18] Squaramides are believed to be stable to nucleophilic attack, which could mean they have reduced toxicity[18] and make them suitable candidates for potential drugs, including for the treatment of channelopathies. Herein we report the anion-transport properties of a series of small, fluorinated squaramides and compare their transport abilities with analogous ureas and thioureas.

All of the tested compounds have been previously reported and can be easily synthesized from adapted literature procedures (see Supporting Information for details).[19] Squaramides **1**, **4**, and **7** were prepared using the zinc triflate-mediated reaction of squarate esters with the appropriate aniline, developed by Taylor and co-workers,[13a] whereas
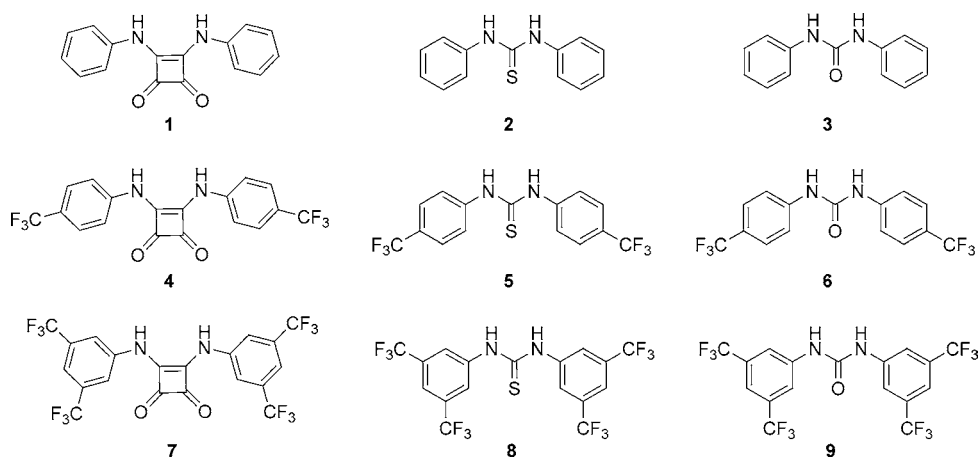
thioureas **2**, **5**, **8**, and ureas **3**, **6**, and **9** were synthesized by the reaction of the appropriate anilines with isothiocyanates or isocyanates, respectively.

Some of these compounds have been shown to possess excellent anion binding properties (**4** and **6**)[14b] or are attractive organocatalysts (e.g. Schreiner's thiourea **8**).[20] We have previously shown that fluorinated substituents can greatly improve the transport ability of an anion receptor.[10b] Compounds **1**–**9** were therefore considered good candidates for anion transport studies. To assess the chloride transport abilities of **1**–**9**, a series of unilamellar 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) liposomes loaded with NaCl (489 mM) was prepared and suspended in an isotonic,

chloride-free NaNO₃ solution (489 mM), according to previously reported methodologies (see Supporting Information).[21] A small volume of the putative transporter **1–9** in DMSO was added to these vesicles, and the resulting chloride efflux was monitored using an ion selective electrode (ISE) for 300 s. The results for compounds **1–6** are given in Figure 1 and demonstrate that squaramides **1** and **4** are able to transport chloride out of liposomes much faster than the analogous thioureas (**2**, **5**) and ureas (**3**, **6**) (additional results in Supporting Information).
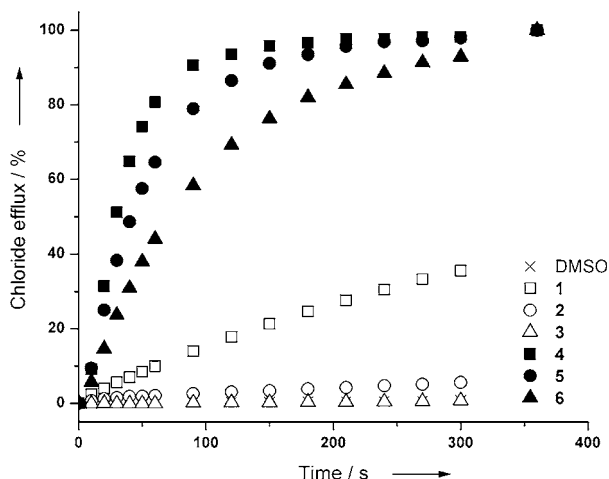

Figure 1. Chloride efflux promoted by receptors **1–6** (1 mol% receptor to lipid) from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts and dispersed in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment (300 s), detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents the average of three trials. DMSO alone was used as a control experiment.

The transport activity seen in Figure 1 results from either a Cl⁻/NO₃⁻ antiport process (anion exchange) or a H⁺/Cl⁻ or Na⁺/Cl⁻ symport (co-transport) process. To test which of these processes is predominant, POPC vesicles containing NaCl were prepared and suspended in a solution of Na₂SO₄, and a sample of **1–6** in DMSO was added to initiate the experiment (1 mol% transporter to lipid). After 2 min a pulse of bicarbonate was added to the external solution and the chloride efflux was monitored for a further 5 min using an ISE (Figure 2). Under the initial conditions of the experiment (i.e. before the addition of bicarbonate), no chloride efflux could be detected, whereas the addition of bicarbonate leads to an immediate increase in the release of chloride mediated by **1–6**. This strong dependence on the nature of the external anion is indicative of an anion antiport process. The strong hydrophilic nature of sulfate precludes lipid bilayer transport of this anion and therefore no significant chloride efflux is seen when sulfate is the only external anion. However, when nitrate (Figure 1) or bicarbonate (Figure 2) are present in the extravesicular solution, release of chloride from the vesicles is observed, indicative of a chloride/nitrate and the biologically more relevant[22] chloride/bicarbonate antiport process.
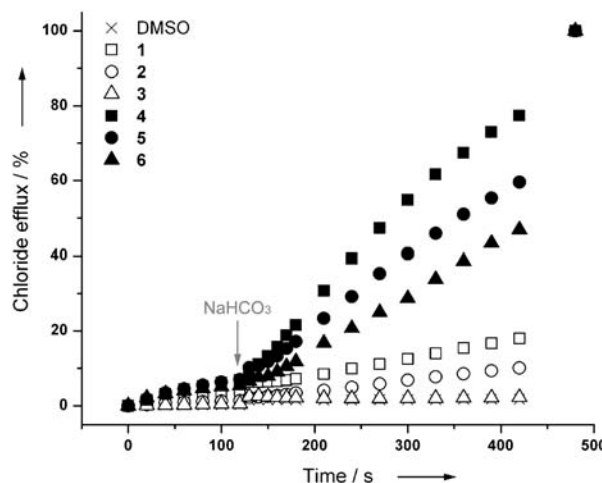

Figure 2. Chloride efflux promoted by receptors **1–6** (1 mol% receptor to lipid) from unilamellar POPC vesicles loaded with 450 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts and dispersed in 162 mM Na₂SO₄ buffered to pH 7.2 with 20 mM sodium phosphate salts. At $t = 120$ s, a solution of NaHCO₃ was added to give a final concentration of 40 mM. At the end of the experiment (420 s) detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents the average of three trials. DMSO alone was used as a control experiment.

Figure 2 also shows that for chloride/bicarbonate transport the squaramide-based compounds **1** and **4** display superior transport abilities compared to analogous ureas and thioureas (similar results were obtained for **7–9**, Supporting Information).

To quantify the extent to which the squaramides transport chloride more efficiently than ureas and thioureas, extensive Hill analyses were performed for both Cl⁻/NO₃⁻ and Cl⁻/HCO₃⁻ assays (see Supporting Information for details). In these tests, the vesicle experiments are repeated at various concentrations of transporter to obtain EC_{50} values, that is, the concentration of transporter needed to achieve 50% chloride efflux in 270 s, and Hill coefficients n that reveal the stoichiometry of the transport process[23] (Table 1, values for Cl⁻/HCO₃⁻ assays can be found in the Supporting Information). It can be seen from the EC_{50} values in Table 1 that the squaramide-based compounds (**1**, **4**, and **7**) are nearly one order of magnitude better than the analogous thioureas and ureas, allowing detectable chloride efflux at much lower concentrations of transporter.[24]

Because **1–9** have planar, aromatic substituents, it can be postulated that anion transport occurs through channels formed by stacking of these molecules, although this would be entropically unfavorable. The Hill coefficients n (Table 1) suggest that the transport of one chloride anion through the membrane requires only one or two molecules, which are not enough to span a POPC lipid bilayer. To test this idea, the liposome experiments were repeated using vesicles made of 7:3 POPC:cholesterol. It has often been suggested that cholesterol can increase the viscosity of a lipid bilayer,[25] thereby decreasing the ability of a mobile carrier to diffuse through the membrane, whereas the transport ability of ion

Table 1: Summary of the chloride binding (K_a) and anion transport (EC$_{50}$ and n) properties of compounds **1–9**. Calculated log P and TPSA [Å^2] are also presented.

Compound	K_a[a] [M^{-1}]	EC$_{50}$[b] [mol%]	n[c]	c log P[d]	TPSA[e] [Å^2]
1	260	1.38	1.7	2.02	48.03
2	15	_[f]	_[f]	3.66	21.30
3	31	_[f]	_[f]	2.51	32.74
4	458	0.06	1.2	3.87	47.61
5	43	0.22	1.6	5.50	21.04
6	75	0.42	2.2	4.35	32.28
7	643	0.01	1.1	5.71	47.94
8	41	0.16	2.0	7.34	21.61
9	88	0.30	1.4	6.20	32.69

[a] Association constant [M^{-1}] for **1–9** with Bu$_4$NCl in [D$_6$]DMSO/0.5% water at 298 K. [b] Concentration of transporter (mol% carrier to lipid) needed to obtain 50% chloride efflux in 270 s during the Cl$^-$/NO$_3^-$ experiments. [c] Hill coefficient for Cl$^-$/NO$_3^-$ experiments. [d] c log P calculated using Spartan '10 for Macintosh (Ghose–Crippen model). [e] Total polar surface area (TPSA) calculated using Spartan '10 for Macintosh. The receptors were minimized using AM1 semi-empirical methods. Values are given for the *cis/cis* conformer, which is believed the most stable conformer for *N*-phenyl squaramides.[30] [f] Transporter was not active enough to perform Hill analysis.

channels should remain unaffected. However, the cholesterol test gave inconclusive results, with some compounds (e.g. **1**) displaying slower transport activity, indicative of a mobile carrier mechanism, whereas most other compounds showed significantly faster transport (Supporting Information). This increase might be due to increased partitioning of the receptors into a lipid bilayer containing cholesterol, but this could not be verified. We then performed U-tube experiments using nitrobenzene as the organic phase to discriminate between ion channel and mobile carrier behavior. Significant chloride transport through the U-tube could be detected for most of the compounds, which can only be the result of a mobile carrier mechanism, because ion channel formation is impossible owing to the sheer size of the U-tube organic phase. Once again, it was the fluorinated squaramide compounds **4** and **7** that proved to be the most efficient transporters. These experiments provide supporting evidence that the mobile-carrier activity seen in the U-tube experiment is also present in liposomes (Supporting Information).

There could be several possible reasons why the squaramides outperform the ureas and thioureas in the anion-transport studies. We have reported previously that thioureas are better transporters than ureas and that fluorinated compounds are better than unfluorinated ones because of their higher lipophilicity and hence the enhanced partitioning into a lipid bilayer.[10] Although it has been suggested that squaramides are more lipophilic than ureas,[26] our calculations of the Ghose–Crippen log P and TPSA (total polar surface area) values[27] of compounds **1–9** (Table 1) suggest that the squaramide-based compounds are less lipophilic than their urea and thiourea analogues, which has also been proposed by Storer et al.,[18] and others.[28] This observation makes it unlikely that the enhanced transport behavior of the squaramides is due to their lipophilicity. On the other hand, the fact that squaramides have enhanced transport abilities

without high lipophilicity is useful in the development of drug-like transport systems with therapeutic potential. This potential is because the lower lipophilicity of squaramides keeps them within the boundaries defined by Lipinski's rule of five, which dictates that log $P < 5$ for drug-like systems.[29]

It could also be that squaramide-based compounds are better anion transporters because the two oxygen atoms provide a metal binding site (complexes of squaramides with alkylammonium cations have been reported)[11] and therefore, transport could possibly occur through the binding of a neutral ion pair (for example as NaCl/NaNO$_3$ exchange or NaCl/NaHCO$_3$ exchange). However, when the vesicle studies were repeated with K$^+$ salts, or when a Cs$^+$ gradient was used, the same transport rates were found as with Na$^+$ salts (Supporting Information). Hence, it is unlikely that the metal ions play an important role in the transport of anions by squaramides **1**, **4**, and **7**.

Therefore, the most likely explanation for the superior transport abilities of the squaramides is due to the enhanced anion-binding properties of squaramide-based compounds. It has been suggested that squaramides possess exceptional anion-binding abilities, because ion binding increases the aromaticity in the four-membered ring.[12b] Fabbrizzi and co-workers have shown that squaramides (including **4**) display higher anion binding constants in acetonitrile than the analogous ureas, which was attributed to the more convergent hydrogen-bond array and a larger participation of the *ortho* CH protons in hydrogen bonding to the anion.[14] To show that the same is true for all of the transporters, ^{1}H NMR spectroscopic titrations at 298 K in [D$_6$]DMSO containing 0.5% water were performed for all anions relevant in the transport studies (using the tetrabutylammonium (Bu$_4$N) or tetraethylammonium (Et$_4$N) salts). No interaction with NO$_3^-$ was found in this solvent mixture, whereas the interaction with HCO$_3^-$ proved to be difficult to interpret because of deprotonation of the squaramides and thioureas by HCO$_3^-$ (as confirmed by the addition of aliquots of strong base Bu$_4$NOH, which resulted in similar NMR spectra as the addition of Et$_4$NHCO$_3$, Supporting Information). The titration data with Bu$_4$NCl could be fitted to a 1:1 model using the WinEQNMR2 computer program,[31] and the results are summarized in Table 1. It is clear from Table 1 that the squaramide-based compounds **1**, **4**, and **7** all display association constants with chloride that are one order of magnitude larger than the association constants of the ureas and thioureas. These data imply that the enhanced anion binding is the most likely reason for the order of magnitude better transport activity displayed by the squaramides.

The ability of these compounds to bind anions was also confirmed by single-crystal X-ray diffraction (Figure 3; details in the Supporting Information). Crystals of the chloride complexes could be obtained for all squaramide-based compounds ([**1**-Cl]$^-$, [**4**-Cl]$^-$, and [**7**-Cl]$^-$) by the slow evaporation of a DMSO solution containing the receptor and excess Bu$_4$NCl (at 50°C). All chloride complexes show a 1:1 stoichiometry and have two, near linear N−H···Cl hydrogen bonds (all N−H···Cl angles are >160° and N···Cl distances vary from 3.029(19) Å to 3.201(18) Å), providing evidence for a convergent hydrogen-bond array of the squaramides that is

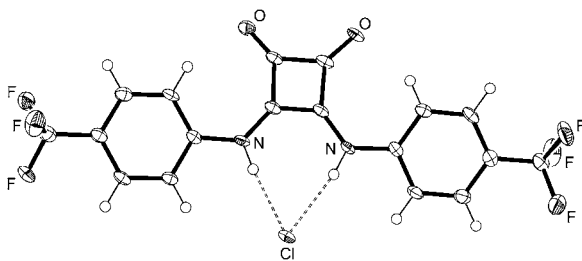**Figure 3.** An ORTEP view of [**4**-Cl][Bu₄N] with thermal ellipsoids set at 50% probability. Hydrogen bonds are represented by dashed lines. The counterion (Bu₄N) is omitted for clarity.

suitable for interaction with halide anions (Figure 3). Even though the interactions with Bu₄NNO₃ and Et₄NHCO₃ could not be detected in DMSO solutions, we were able to obtain crystal structures of complexes with these anions ([**3**-HCO₃]⁻, [(**6**)₃-CO₃]²⁻, [(**9**)₃-CO₃]²⁻, and [**3**-NO₃]⁻; Supporting Information). This result provides evidence that the urea compounds can interact with nitrate and (bi)carbonate and thereby supports the idea that chloride/nitrate and chloride/bicarbonate exchange occurs across lipid bilayers.

In summary, we have presented the first examples of squaramide-based compounds that can transport anions across lipid bilayers, presumably via mobile-carrier and anion-exchange mechanisms. It was shown that the squaramides possess better anion-transport activities than analogous ureas and thioureas, and this was mainly because of the enhanced anion-binding properties of the squaramide-based receptors. Since the squaramide functionality provides a way to increase the transport ability of a receptor without significantly increasing the lipophilicity, it offers an ideal platform for designing future anion transporters. More complex squaramide-containing transporters are currently being investigated in our laboratory and will be published in due course.

[1] F. M. Ashcroft, *Ion Channels and Disease*, Academic Press, San Diego and London, **2000**.

[2] a) C. Higgins, *Nature* **1992**, *358*, 536; b) J. Y. Choi, D. Muallem, K. Kiselyov, M. G. Lee, P. J. Thomas, S. Muallem, *Nature* **2001**, *410*, 94–97.

[3] For recent reviews see: a) J. T. Davis, O. Okunola, R. Quesada, *Chem. Soc. Rev.* **2010**, *39*, 3843–3862; b) P. R. Brotherhood, A. P. Davis, *Chem. Soc. Rev.* **2010**, *39*, 3633–3647; c) P. A. Gale, *Acc. Chem. Res.* **2011**, *44*, 216–226; d) S. Matile, A. Vargas Jentzsch, J. Montenegro, A. Fin, *Chem. Soc. Rev.* **2011**, *40*, 2453–2474.

[4] For selected examples see: a) L. P. Shank, J. R. Broughman, W. Takeguchi, G. Cook, A. S. Robbins, L. Hahn, G. Radke, T. Iwamoto, B. D. Schultz, J. M. Tomich, *Biophys. J.* **2006**, *90*, 2138–2150; b) L. You, R. Ferdani, R. Li, J. P. Kramer, R. E. K. Winter, G. W. Gokel, *Chem. Eur. J.* **2008**, *14*, 382–396.

[5] For recent examples see: a) A. Vargas Jentzsch, D. Emery, J. Mareda, P. Metrangolo, G. Resnati, S. Matile, *Angew. Chem.* **2011**, *123*, 11879–11882; *Angew. Chem. Int. Ed.* **2011**, *50*, 11675–11678; b) I. Izzo, S. Licen, N. Maulucci, G. Autore, S. Marzocco, P. Tecilla, F. De Riccardis, *Chem. Commun.* **2008**, 2986–2988.

[6] For recent examples see: a) V. Gorteau, M. D. Julliard, S. Matile, *J. Membr. Sci.* **2008**, *321*, 37–42; b) J. Mareda, S. Matile, *Chem. Eur. J.* **2009**, *15*, 28–37.

[7] For recent examples see: a) J. T. Davis, P. A. Gale, O. A. Okunola, P. Prados, J. C. Iglesias-Sánchez, T. Torroba, R. Quesada, *Nat. Chem.* **2009**, *1*, 138–144; b) X. Li, B. Shen, X.-Q. Yao, D. Yang, *J. Am. Chem. Soc.* **2009**, *131*, 13676–13680; c) C. R. Yamnitz, S. Negin, I. A. Carasel, R. K. Winter, G. W. Gokel, *Chem. Commun.* **2010**, *46*, 2838–2840.

[8] For recent examples see: a) S. J. Moore, M. G. Fisher, M. Yano, C. C. Tong, P. A. Gale, *Chem. Commun.* **2011**, *47*, 689–691; b) M. G. Fisher, P. A. Gale, J. R. Hiscock, M. B. Hursthouse, M. E. Light, F. P. Schmidtchen, C. C. Tong, *Chem. Commun.* **2009**, 3017–3019; c) P. A. Gale, C. C. Tong, C. J. E. Haynes, O. Adeosun, D. E. Gross, E. Karnas, E. M. Sedenberg, R. Quesada, J. L. Sessler, *J. Am. Chem. Soc.* **2010**, *132*, 3240–3241.

[9] For recent examples see: a) B. A. McNally, A. V. Koulov, T. N. Lambert, B. D. Smith, J.-B. Joos, A. L. Sisson, J. P. Clare, V. Sgarlata, L. W. Judd, G. Magro, A. P. Davis, *Chem. Eur. J.* **2008**, *14*, 9599–9606; b) L. W. Judd, A. P. Davis, *Chem. Commun.* **2010**, *46*, 2227–2229; c) S. Hussain, P. R. Brotherhood, L. W. Judd, A. P. Davis, *J. Am. Chem. Soc.* **2011**, *133*, 1614–1617.

[10] For recent examples see: a) N. Busschaert, P. A. Gale, C. J. E. Haynes, M. E. Light, S. J. Moore, C. C. Tong, J. T. Davis, W. A. Harrell, Jr., *Chem. Commun.* **2010**, *46*, 6252–6254; b) N. Busschaert, M. Wenzel, M. E. Light, P. Iglesias-Hernández, R. Pérez-Tomás, P. A. Gale, *J. Am. Chem. Soc.* **2011**, *133*, 14136–14148; c) N. J. Andrews, C. J. E. Haynes, M. E. Light, S. J. Moore, C. C. Tong, J. T. Davis, W. A. Harrell, Jr., P. A. Gale, *Chem. Sci.* **2011**, *2*, 256–260.

[11] a) S. Tomàs, M. C. Rotger, J. González, P. M. Deyà, P. Ballester, A. Costa, *Tetrahedron Lett.* **1995**, *36*, 2523–2526; b) D. Quiñonero, A. Frontera, G. A. Suñer, J. Morey, A. Costa, P. Ballester, P. M. Deyà, *Chem. Phys. Lett.* **2000**, *326*, 247–254; c) S. Tomàs, R. Prohens, M. Vega, M. C. Rotger, P. M. Deyà, P. Ballester, A. Costa, *J. Org. Chem.* **1996**, *61*, 9394–9401.

[12] Examples from the group of A. Costa: a) R. Prohens, S. Tomàs, J. Morey, P. M. Deyà, P. Ballester, A. Costa, *Tetrahedron Lett.* **1998**, *39*, 1063–1066; b) D. Quiñonero, R. Prohens, C. Garau, A. Frontera, P. Ballester, A. Costa, P. M. Deyà, *Chem. Phys. Lett.* **2002**, *351*, 115–120; c) C. Garau, A. Frontera, P. Ballester, D. Quiñonero, A. Costa, P. M. Deyà, *Eur. J. Org. Chem.* **2005**, 179–183; d) A. Frontera, J. Morey, A. Oliver, M. N. Piña, D. Quiñonero, A. Costa, P. Ballester, E. V. Anslyn, *J. Org. Chem.* **2006**, *71*, 7185–7195.

[13] a) A. Rostami, A. Colin, X. Y. Li, M. G. Chudzinski, A. J. Lough, M. S. Taylor, *J. Org. Chem.* **2010**, *75*, 3983–3992; b) A. Rostami, C. J. Wei, G. Guérin, M. S. Taylor, *Angew. Chem.* **2011**, *123*, 2107–2110; *Angew. Chem. Int. Ed.* **2011**, *50*, 2059–2062.

[14] a) V. Amendola, G. Bergamaschi, M. Boiocchi, L. Fabbrizzi, M. Milani, *Chem. Eur. J.* **2010**, *16*, 4368–4380; b) V. Amendola, L. Fabbrizzi, L. Mosca, F.-P. Schmidtchen, *Chem. Eur. J.* **2011**, *17*, 5972–5981.

[15] For recent examples see: a) V. Ramalingam, M. E. Domaradzki, S. Jang, R. S. Muthyala, *Org. Lett.* **2008**, *10*, 3315–3318; b) M. H. Al-Sayah, N. R. Branda, *Thermochim. Acta* **2010**, *503–504*, 28–32; c) D. Quiñonero, K. A. López, P. M. Deyà, M. N. Piña, J. Morey, *Eur. J. Org. Chem.* **2011**, 6187–6194; d) G. Ambrosi, M. Formica, V. Fusi, L. Giorgi, A. Guerri, M. Micheloni, P. Paoli, R. Pontellini, P. Rossi, *Chem. Eur. J.* **2007**, *13*, 702–712; e) G. Ambrosi, M. Formica, V. Fusi, L. Giorgi, E. Macedi, M. Micheloni, P. Paoli, R. Pontellini, P. Rossi, *Chem. Eur. J.* **2011**,

17, 1670–1682; f) C. E. Silva, H. L. F. Dos Santos, N. L. Speziali, R. Diniz, L. F. C. de Oliveira, *J. Phys. Chem. A* **2010**, *114*, 10097–10109.

[16] For a recent review about the use of squaramides in organocatalysis see: J. Alemán, A. Parra, H. Jiang, K. A. Jørgensen, *Chem. Eur. J.* **2011**, *17*, 6890–6899.

[17] Z. Zhang, P. R. Schreiner, *Chem. Soc. Rev.* **2009**, *38*, 1187–1198.

[18] For a recent review about squaramides and their use in medicinal chemistry see: R. I. Storer, C. Aciro, L. H. Jones, *Chem. Soc. Rev.* **2011**, *40*, 2330–2346.

[19] The urea and thiourea compounds herein have been used extensively before in various subjects. For a list of the exact references used during synthesis, see Supporting Information.

[20] M. Kotke, P. R. Schreiner, *Tetrahedron* **2006**, *62*, 434–439.

[21] a) B. D. Smith, T. N. Lambert, *Chem. Commun.* **2003**, 2261–2268; b) A. V. Koulov, T. N. Lambert, R. Shukla, M. Jain, J. M. Boon, B. D. Smith, H. Li, D. N. Sheppard, J.-B. Joos, J. P. Clare, A. P. Davis, *Angew. Chem.* **2003**, *115*, 5081–5083; *Angew. Chem. Int. Ed.* **2003**, *42*, 4931–4933.

[22] E. Cordat, J. R. Casey, *Biochem. J.* **2009**, *417*, 423–439.

[23] a) A. V. Hill, *Biochem. J.* **1913**, *7*, 471–480; b) S. Bhosale, S. Matile, *Chirality* **2006**, *18*, 849–856.

[24] It was observed that the transport activity of the squaramides was dependent on the concentration of the stock DMSO solution, possibly because of self-aggregation or precipitation. See Supporting Information for details and discussion. However, it was only a problem at high concentrations of transporter. At lower concentrations, the transport mediated by the squaramides was stable and still significantly higher than for the ureas and thioureas.

[25] W. F. D. Bennett, J. L. MacCallum, D. P. Tieleman, *J. Am. Chem. Soc.* **2009**, *131*, 1972–1978.

[26] K. Urbahns, M. Härter, M. Albers, D. Schmidt, B. Stelte-Ludwig, U. Brüggemeier, A. Vaupel, J. Keldenich, K. Lustig, H. Tsujishita, C. Gerdes, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6151–6154.

[27] A. K. Ghose, A. Pritchett, G. M. Crippen, *J. Comput. Chem.* **1988**, *9*, 80–90.

[28] R. C. Young, G. J. Durant, J. C. Emmett, C. R. Ganellin, M. J. Graham, R. C. Mitchell, H. D. Prain, M. L. Roantree, *J. Med. Chem.* **1986**, *29*, 44–49.

[29] C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv. Drug Delivery Rev.* **1997**, *23*, 3–25.

[30] R. S. Muthyala, G. Subramaniam, L. Todaro, *Org. Lett.* **2004**, *6*, 4663–4665.

[31] M. J. Hynes, *J. Chem. Soc. Dalton Trans.* **1993**, 311–312.