DOI: 10.1002/anie.201207535

Anion Transport

Small-Molecule Lipid-Bilayer Anion Transporters for Biological Applications

*Nathalie Busschaert and Philip A. Gale**

anions · hydrogen bonding · ion transport · membranes · supramolecular chemistry

In memory of Hiroshi Tsukube

The development of small-molecule lipid-bilayer anion transporters for potential future use in channel replacement therapy for the treatment of diseases caused by dysregulation of anion transport (such as cystic fibrosis), and in treating cancer by perturbing chemical gradients within cells, thus triggering apoptosis, is an area of intense current interest. This Minireview looks at recent developments in the design of small-molecule transmembrane anion transporters and focuses on the progress so far in employing these compounds in biological systems.

Introduction

The movement of anions across phospholipid-bilayer membranes relies on the facilitated transport of the ions through channel proteins that span the membrane. These processes are important in regulating pH value, maintaining osmotic balance, and in cellular signaling pathways. However, defects in anion-transport proteins can lead to a number of diseases known as "channelopathies."[1] This class of disease includes cystic fibrosis (CF), a condition that is caused by dysregulation of anion transport through the CFTR anion channel in epithelial cell membranes. Both chloride and bicarbonate flux through the CFTR channel is impaired in CF, which affects the transport of water through the membrane and thus results in the formation of sticky mucus in organs that contain epithelial cells (such as the lungs, causing chronic lung disease in most CF patients).[2] It has been proposed that synthetic channels could replace the function of the CFTR channel.[3] Seminal independent work from Tomich[4] and Gokel[5] and their respective co-workers on peptide-based anion channels and their application in biological systems are important steps toward this goal, while the elegant anion–π slide compounds reported by Matile[6] and co-workers demonstrate that wholly synthetic structures can also function as anion channels. Another recent approach by Smith and co-workers has been to develop phospholipids functionalized with urea groups which, when present in both leaflets of the membrane, can 'hand' anions across the bilayer in a relay-type system.[7] However, many of these channel-like compounds are too large to be considered drug-like. There has been a recent interest in developing small synthetic compounds capable of carrying anions across lipid bilayers (mobile carrier mechanism) by employing the supramolecular concepts of anion binding (Figure 1). Anion-receptor chemistry is now a maturing field.[8] A wide variety of receptors have been reported that employ electrostatic interactions, hydrogen bonds, anion–π interactions, halogen bonds, and anion–dipole interactions to complex anionic guests.[9] However, our understanding of how to design small-molecule anion transporters that function by binding an anionic guest and diffusing through a lipid bilayer to release the guest on the other side of the membrane is less well developed.[10] More work is required in order to understand the molecular parameters that must be optimized to achieve efficient transport by small molecules at low concentrations. Herein, we focus on the progress that has been made toward the development of small-molecule drug-like anion transporters that have shown promising biological activity. The reader is referred elsewhere for a full overview of transmembrane anion transport.[10]

Natural Products

The design of small-molecule anion transporters can be guided by taking inspiration from the structure of natural products capable of facilitating anion transport. However, there are only a few examples of natural products that are capable of mediating transmembrane anion transport, and there is currently debate about the mode of action of these compounds within biological systems.[11] The most notable and most studied family of natural products capable of transmembrane anion transport are the prodigiosins,[12] a class

---

[*] N. Busschaert, Prof. Dr. P. A. Gale
Chemistry, University of Southampton
Highfield, Southampton, SO17 1BJ (UK)
E-mail: philip.gale@soton.ac.uk
Homepage: http://www.supramolecularchemistry.net

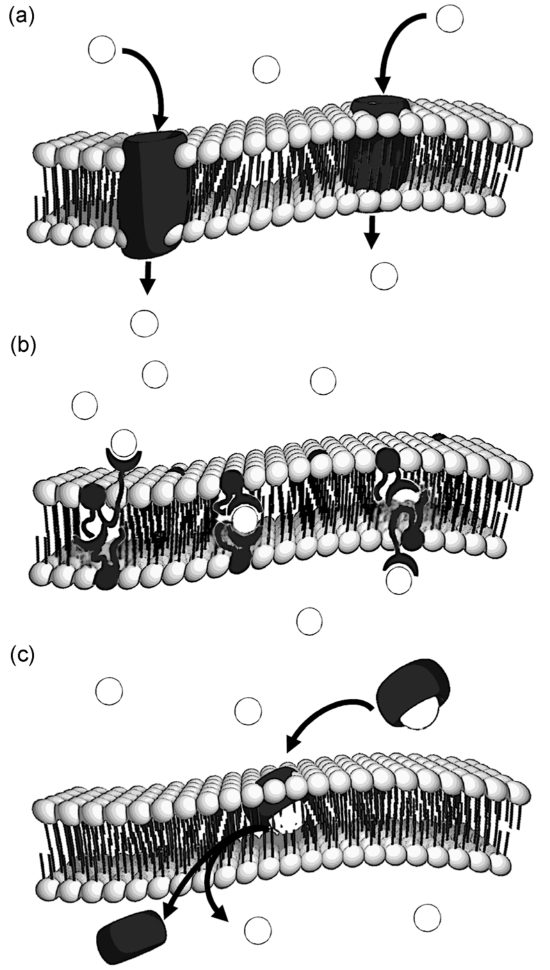
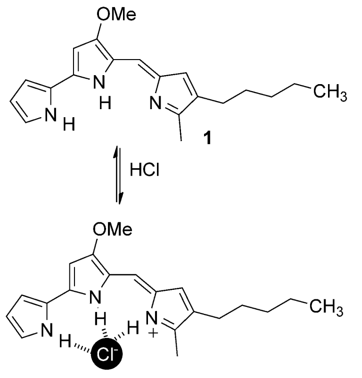


Figure 1. Overview of the various anion-transport mechanisms. a) Channel mechanism: receptor forms a pore that spans the membrane through which anions can diffuse. b) Relay mechanism: modified phospholipid binds to the anion and passes it onto another modified phospholipid. c) Mobile-carrier mechanism: receptor binds to the anion and the complex diffuses to the other side of the membrane where the anion is released.



Scheme 1. Prodigiosin (**1**) binds HCl through three hydrogen bonds and electrostatic interactions.

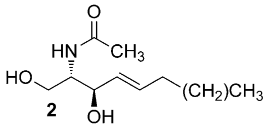
of natural products produced by *Streptomyces* and *Serratia*. The structure of the parent prodigiosin **1** is shown in Scheme 1. These tripyrrolic compounds have anticancer properties. A number of hypotheses have been advanced to account for this, including the ability of these compounds to deacidify acidic compartments within cells by HCl co-transport,[11] thereby decreasing the pH value of the cytoplasm within cancer cells and triggering apoptosis. The protonated form of **1** can coordinate to chloride through three hydrogen-bonding interactions. This complex is lipid soluble and can function as a symporter for H^+/Cl^-, thereby providing a mechanism for the depolarization of acidic compartments within cells. More recent studies have demonstrated the ability of prodigiosin to function as a chloride transporter through an anion-exchange (or antiport) process[13] and to transport bicarbonate anions.[14] This compound functions at low concentrations (transport of chloride and bicarbonate was observed in POPC liposomes at concentrations of 0.005% carrier to lipid),[14] thus outperforming many of the synthetic systems described below. Many of the studies reported in this area are conducted in POPC or POPC/cholesterol membranes. While these are useful models in early-stage studies, it should be highlighted that they are only model systems, and that the behavior of transporters may differ in biological membranes.

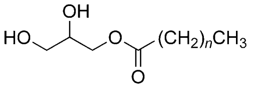
Recently, Davis and co-workers have discovered two further examples of natural products that are capable of transmembrane anion transport through a discrete molecular carrier mechanism; both natural products employ OH···anion interactions to transport anionic guests. The first example is sphingolipid ceramide **2**, which had previously been shown to form pores at high concentrations and trigger the release of

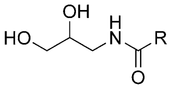
Philip A. Gale is professor of supramolecular chemistry and head of chemistry at the University of Southampton. He was an undergraduate and postgraduate student at the University of Oxford before moving to the University of Texas at Austin as a Fulbright Post-doctoral Fellow in 1995. He moved back to the University of Oxford in 1997 as a Royal Society University Research Fellow before accepting a lectureship at the University of Southampton in 1999. He has won a number of research awards, including the RSC Corday Morgan medal and prize. His research focuses on the recognition, sensing, and transmembrane lipid-bilayer transport of anionic species.

Nathalie Busschaert graduated magna cum laude from the University of Leuven (K.U.Leuven), Belgium, in 2010. She is currently pursuing a Ph.D. in the field of anion recognition and transmembrane anion transport under the supervision of Prof. Philip A. Gale (University of Southampton) and Prof. Young-Tae Chang (Singapore Bio-Imaging Consortium).

proapoptotic proteins from mitochondria.[15] By using experiments in EYPC liposomes, Davis and co-workers demonstrated that **2** is capable of binding and transporting chloride and bicarbonate in an antiport process at concentrations (1 mol% carrier to lipid) below which it self-associates to form pores. Proton NMR experiments in CD_2Cl_2 suggest that the chloride ion binds to the two OH groups and the NH group.[16] Similarly, the Davis group found that naturally occurring monoacylglycerols **3a** and **3b** were able to transport chloride across EYPC membranes at the same carrier to lipid ratio through a chloride/nitrate antiport process, binding the anions by $OH\cdots A^-$ hydrogen bonds. Simple modifications introducing an extra hydrogen-bond donor and a perfluorinated chain resulted in synthetic analogues **4a** and **4b**, which significantly outperformed the natural compound **3a** in terms of transmembrane-anion-transport ability. This example illustrates the usefulness of studying natural products and taking inspiration from Nature to develop new anionophores.[17]


2


3a $n = 6$
3b $n = 16$

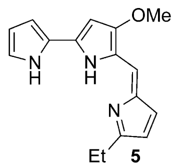

4a R = $(CH_2)_6CH_3$
4b R = $(CF_2)_6CF_3$

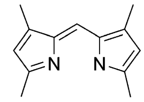
Synthetic Systems

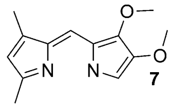
1. Prodigiosin Analogues

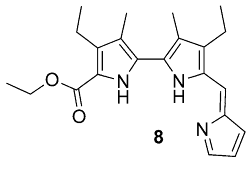
With the prodigiosin family being the most studied natural compounds with significant anion-transport ability and well-documented anticancer and immunosuppresive activity, it is no surprise that several research groups have taken their inspiration from this class of compounds. In 2005, Sessler, Magda, and co-workers studied the chloride-transport properties of a series of structurally related acyclic pyrrolic systems, including prodigiosins **5**, **8**, and **9** and dipyrromethenes **6** and **7**.[18] They found that the order of activity of the compounds for chloride transport in vesicle studies was $\mathbf{5} \gg \mathbf{8} \approx \mathbf{6} > \mathbf{7} \gg \mathbf{9}$, and that they functioned mainly through a H^+/Cl^- symport mechanism. Interestingly, prodigiosin **5** was significantly more active than the other analogues. The anticancer properties of the compounds were studied in A549 human lung cancer and PC3 human prostate cancer cell lines. Antiproliferative activity was observed for the compounds with a sequence of efficacy that generally matched the order of activity of the compounds for anion transport.

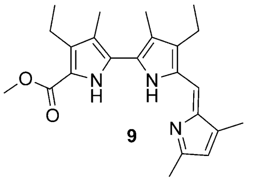
More recently, Quesada has shown that Obatoclax (GX15-070), which is a synthetic prodiginine that contains an indole ring (**10**) commercially developed by Gemin X (recently acquired by Cephalon) and has shown promising activity in pre-clinical and clinical trials across many different cancer types, is also capable of transporting chloride and bicarbonate ions through lipid-bilayer membranes, and can depolarize acidic compartments within GLC4 cancer cells (small-cell human lung carcinoma). These results provide further evidence that the anticancer activity of prodigiosins is due to their ability to alter the pH value of tumor cells through anion-transport processes, thereby triggering apoptosis.[19]

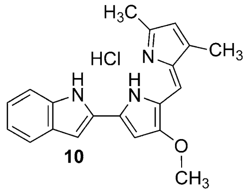

5


6

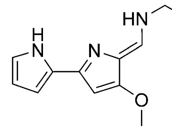

7

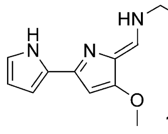

8

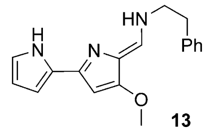

9

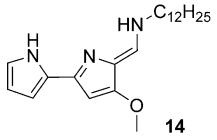

10

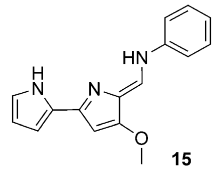
Quesada and co-workers have also shown that the structurally related and synthetically easier to access tambjamine alkaloids **11**–**16** are also capable of transmembrane anion transport.[20] In contrast to the prodigiosins, the pK_a value of these compounds is around 10 as opposed to 7.2 for prodigiosin. This means that these compounds will remain protonated at physiological pH value, and experiments with model membrane systems showed that, unlike the prodigiosins, these compounds are poor HCl co-transporters. However, the compounds were potent Cl^-/HCO_3^- antiport agents, with compound **16** showing the highest activity of the series. The biological and anticancer activity of these tambjamines was also studied using GLC4 cells stained with pH-sensitive dye acridine orange. It was shown that compounds **14** and **16** (the two most potent transporters) could deacidify the acidic compartments within these cells. Similar results were obtained with **11** and **15**, but compound **12**, which proved to be a poor anionophore, did not deacidify the compartments. The authors suggest that bicarbonate transport may be responsible for the pH changes in the cells. Recent studies on tambjamines has demonstrated a correlation between lipophilicity and transport ability in this class of compounds.[21]

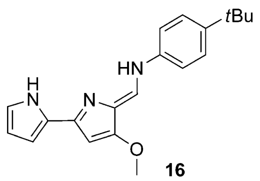

11


12


13


14


15


16

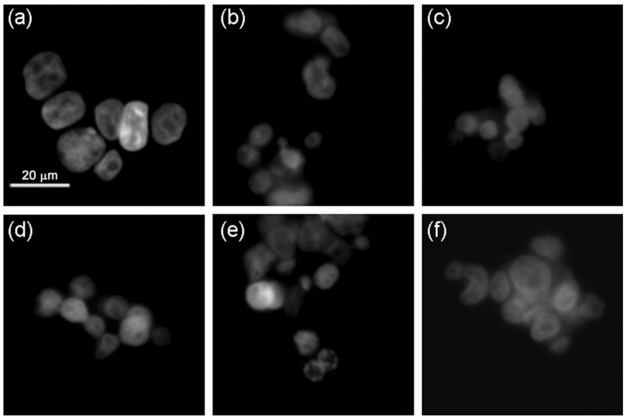
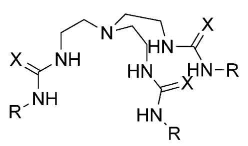


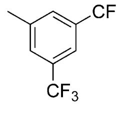
Figure 2. Hoechst 33342 staining of GLC4 cell line (blue staining of the cell nucleus) after exposure to different receptors for 24 h: a) untreated cells, b) **18**, c) **20**, d) **23**, e) **24**, f) **25**. (a) and (b): cells with normal nuclear morphology; (c)–(f): cells with condensation of the nuclei, and nuclei with a "bean shape", indicative of apoptosis. Reproduced with permission from Ref. [24].

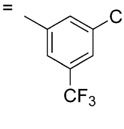
2. Anticancer Activity

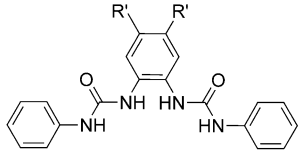
Inspired by the apparent link between anion transport and anticancer activity of prodigiosin and analogues, there has been a recent interest in developing other synthetic compounds with similar properties that are not based on the structure of the prodigiosins. Tris-(2-aminoethyl)amine (tren)-based systems have been particularly interesting in this respect. In 2009, D. K. Smith and co-workers showed that tren-based tris-amides are able to transport HCl across an organic layer in a series of U-tube experiments.[22] Gale has recently shown that tren-based tris-thioureas, such as compound **22**, significantly outperform urea analogues (e.g. compound **17**) as both chloride/nitrate and chloride/bicarbonate antiport agents.[23] Fluorination of the pendant phenyl rings increased their lipophilicity (a technique used by medicinal chemists to improve the bioavailability of their compounds). Fluorination resulted in significantly enhanced transport efficiency both in vesicles and in vitro, with the best transporters (e.g. compound **25**) depolarizing acidic compartments within GLC4 cells and reducing cell viability in a range of human cancer cell lines. Hoechst 33342 staining of GLC4 cells confirmed that the active transporters triggered apoptosis in these cells (Figure 2).[24]

In a recent paper, Gale and co-workers showed that bis-ureas based on *ortho*-phenylenediamine (OPD) **27**–**34** are effective transporters for a variety of anions, including chloride, nitrate, bicarbonate, fumarate, and maleate.[25] Tests performed in model phospholipid vesicles indicated that the addition of electron-withdrawing groups, such as NO_2 (**32**) or CN (**30**), can dramatically improve the anion-transport ability of this type of receptor. The authors also provide evidence that the OPD-based compounds display antitumor activity in vitro. Similar to the previously discussed tripodal receptors **17**–**26**, Acridine Orange staining of the A375 human melanoma cell line showed that receptors **28**, **29**, **31**, **32**, and **33** are able to deacidify cellular compartments, and Hoechst 33342 staining of the same cell line confirmed that the bis-ureas can induce apoptosis in cancer cells. The highest anticancer activity was observed for the most potent anion transporters, providing further evidence for the hypothesis that anion-transport-induced changes in pH values in tumor cells can result in promising anticancer activity. Compound **34** was found to possess anticancer activity, but not to deacidify organelles, and may thus function by an alternate mechanism.

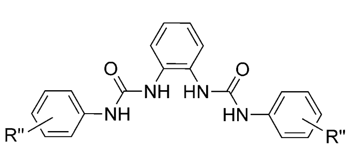


17 R = Ph ; X = O
18 R = p-F-C_6H_4; X = O
19 R = C_6F_5; X = O
20 R = p-CF_3-C_6H_4; X = O
21 R =  ; X = O

22 R = Ph ; X = S
23 R = p-F-C_6H_4; X = S
24 R = C_6F_5; X = S
25 R = p-CF_3-C_6H_4; X = S
26 R =  ; X = S



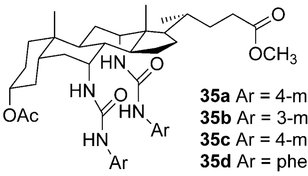
27 R' = H
28 R' = F
29 R' = Cl



30 R'' = p-CN
31 R'' = p-CF_3
32 R'' = p-NO_2
33 R'' = m-NO_2
34 R'' = o-NO_2

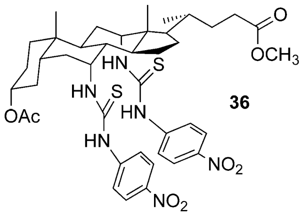
3. Cystic Fibrosis and Related Applications

In addition to the possible use in cancer therapy, the main focus of anion-transport chemistry lies in the development of channel replacement therapies for cystic fibrosis. In patients with CF, chloride and bicarbonate transport across the cell membrane is impaired, causing the formation of a thick mucous layer, especially on epithelial cells, and it has been postulated that synthetic drug-like molecules that function as effective transmembrane anion transporters can provide a possible treatment for this condition. In fact, some of the earliest and most effective synthetic anionophores were developed by A. P. Davis and show promising results in this respect. These systems are based on a cholic acid skeleton with appended hydrogen-bond-donor groups. In a seminal early paper Smith, Davis and Sheppard reported the anion binding and transport properties of a series of cholapods **35a**–**e**.[26] Affinity constants for chloride were measured in water-saturated chloroform solution and ranged from $3.4\times10^{6}\,\text{M}^{-1}$ for **35a** to $5.2\times10^{8}\,\text{M}^{-1}$ for **35e**. Chloride-transport studies were conducted using POPC/cholesterol 7:3 vesicles, which showed that **35e** was a significantly better transporter than the other compounds for chloride/nitrate antiport. The authors demonstrated that compound **35e** could also transport chloride in Madin Darby canine kidney (MDCK) epithelia by using the Ussing chamber technique, thus claiming initial evidence that this class of compounds may be useful in the treatment of cystic fibrosis.



35a Ar = 4-methoxyphenyl
35b Ar = 3-methoxyphenyl
35c Ar = 4-methylphenyl
35d Ar = phenyl
35e Ar = 4-nitrophenyl

More recently, Davis and Smith have conducted a structure–activity relationship study by varying the substituents on the cholapod skeleton and measuring the transport properties of the resulting compounds.[27] They found that compound **36** was capable of transporting chloride at carrier concentrations as low as 1:250000 carrier to lipid (which is an average of less than two transporter molecules per vesicle) through POPC/cholesterol 7:3 membranes. They found that increasing the lipophilicity of the cholapods had no effect on the rate of transport, and that positively charged steroids had low transport activity. In these studies, the cholapods were pre-incorporated into the vesicles prior to the transport experiments. In interesting recent work, Davis has used *trans*-decalin as a scaffold from which to append urea groups. These systems also showed high transport activity at low concentrations.[28] However, the biological activity of these newer systems have not yet been reported.

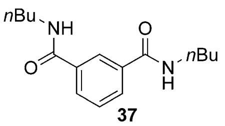


36

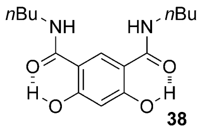
Over the last few years, other scaffolds have been developed that possess anion-transport ability, including series of calixpyrroles[29] and isophthalamides. Davis, Gale, Quesada, and co-workers have shown that preorganization of a transporter can have a dramatic effect on both its affinity for anions and its ability to transport.[30] Compounds **37**–**39** are isophthalamides with compound **38** containing hydroxy groups in the 4 and 6 positions that preorganize the amides into the *syn*–*syn* conformation suitable for anion complexation. Compound **39** contains methoxy groups in the 4 and 6 positions, which accept hydrogen bonds from the amide groups; hence, this compound does not bind anions and was used as a control. Stability-constant determinations by ^{1}H NMR titration techniques in CD_3CN revealed the affinity of **37** and **38** for chloride, bromide, and iodide, showing that the preorganized receptor has significantly higher affinity for anions (Table 1). The higher affinity may also be due to polarization of the amide bonds by the intramolecular hydrogen-bonding interactions in **38**. Compound **38** proved to be a potent chloride/nitrate exchanger in EYPC vesicles, whereas compounds **37** and **39** proved to be inactive.

Table 1: Association constants K_a [M^{-1}] for **37** and **38** binding chloride, bromide, and iodide (tetrabutylammonium salts) measured at 298 K in CD_3CN (Errors <10%).

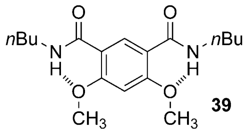
Compound	Cl^-	Br^-	I^-
37	195	60	15
38	5230	716	152



37



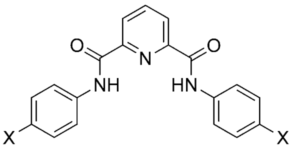
38



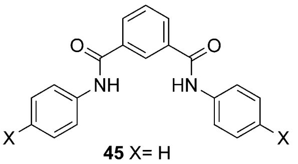
39

Gokel and co-workers have also studied the anion-transport ability of isophthalamides and their pyridine analogues **40**–**47**.[31] They showed that these compounds are able to transport chloride across DOPC membranes and that the compounds with the highest anion-binding ability also possessed the highest anion-transport ability. Furthermore, planar-lipid-bilayer techniques suggest that the potent transporter **43** functions by forming a channel or pore in the lipid bilayer. More recently, Gokel and co-workers have employed these compounds to mediate the transfer of plasmid DNA into *E. coli*. It was shown that both the isophthalamides and the pyridine analogues can function as transformation agents for *E. coli* cells, with the highest activity observed for **44** and **47**. Although the transformation efficiency is still modest, this research provides an interesting new goal for the biological application of anion transporters.

An isophthalamide scaffold containing two α-aminoxy acid units (**48**) was studied by Yang and co-workers.[32] This group showed that **48** can bind a variety of guests in $CDCl_3$ with a good selectivity for chloride over other common anions. The ability of the α-aminoxy-containing compound to transport chloride across lipid bilayers was shown in both model vesicles and in living Madin–Darby canine kidney (MDCK) cells. Patch-clamp techniques on giant liposomes suggest that the transport activity displayed by **48** is due to the formation of ion channels.[32] In a separate article, the authors studied the biological activity of **48** in more depth.[33] Using a variety of cell-based studies, it was shown that **48** can alter the membrane potential of living cells through chloride-transport processes. This is important because changes in the cell-membrane potential as a result of ion transport will affect the behavior of the other natural ion channels in living cells, which renders the effect of a synthetic anion transporter on biological systems hard to predict. A receptor that functions as an anion antiporter in vesicle studies does not necessarily cause antiport in living cells, as the chloride transport induced by the synthetic transporter can be compensated by a variety of other cellular processes. For example, it is known that high extracellular K^+ concentrations strongly alter the membrane potential of smooth muscle cells, and thereby activate voltage-gated Ca^{2+} channels, causing muscle constriction. Yang and co-workers were able to show that **48** can relax mouse aortic rings (preconstricted using high extracellular K^+ concentrations), presumably by repolarizing the membrane potential through chloride transport and thereby deactivating voltage-gated Ca^{2+} channels. This research implies that the anion-transport activity of synthetic receptors can have an effect on many other cellular processes and hints that artificial anion transport can be used in the treatment of hypertension (which relies on muscle relaxation).



40 X= CH_3
41 X = H
42 X = CN
43 X = NO_2
44 X = OCH_3

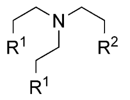


45 X= H
46 X = NO_2
47 X = OCH_3

4. Flippase Activity

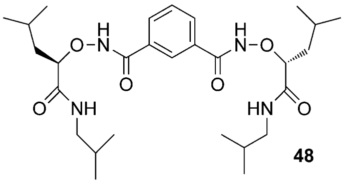
A process that can compete with or complement transmembrane anion transport is so-called “flippase” activity, where the receptor interacts with a phospholipid headgroup of the bilayer and subsequently translocates the phospholipid molecule from the outer to the inner leaflet of the membrane and vice versa. In biological systems, the phospholipids are asymmetrically distributed between the two layers of the membrane, and this asymmetry plays a vital role in a range of cellular functions, such as exocytosis, membrane fusion, blood coagulation, and signal transduction pathways.[34] This asymmetric distribution is normally maintained by specialized proteins, but the development of synthetic flippases can be useful for therapeutic purposes.

Smith and co-workers have conducted work in this area. Initially, this group developed tren-based sulfonamide **49** and showed that it can interact with phospholipids, and that it is capable of phosphatidylcholine (PC)–flippase activity in both model vesicles and in erythrocytes.[35] Later studies found that urea containing sulfonamides **50** and **51** were capable of translocating phosphatidylserine (PS) across the membranes of liposomes and erythrocytes, but not across the plasma membrane of nucleated cells. The authors propose that this is due to the poor membrane-residence time of the receptors, leading to fast distribution of **50** and **51** into the membranes of internal organelles in nucleated cells, a process that cannot occur in red blood cells.[35]

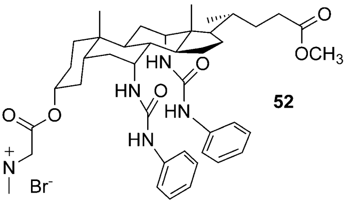


49 $R^1 = R^2 = NHSO_2C_6H_4CH_3$
50 $R^1 = NHSO_2C_6H_4CH_3$
$R^2 = NHCONHC_6H_5$
51 $R^1 = NHSO_2C_6H_4CH_3$
$R^2 = NHCONHC_6H_4NO_2$

Smith and Davis have also studied the flippase activity of cholapods **35d** and **52**.[36] It was shown that neutral cholapod **35d** is capable of translocating PC across vesicle membranes, but not PS. On the other hand, the cationic cholapod **52** was shown to strongly promote the translocation of PS across both liposome membranes and erythrocytes. The authors also investigated the potential therapeutic properties of cholapod **52**. The ability of **52** to influence the blood coagulation process was studied through the effect of **52** on the conversion of prothrombin to thrombin. They showed that **52** is able to significantly increase the amount of thrombin formed by the erythrocyte, and that this is due to the increased amount of PS on the cell surface of the red blood cell.[36]



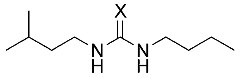
48



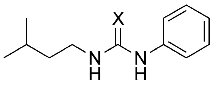
52

5. Lipinski's Rule of Five

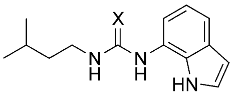
The previous discussion shows that synthetic anion transporters can have potential in pharmaceutical applications, and this has led to recent interest in applying guidelines used in drug design to the development of anionophores. Medicinal chemists use a number of rules of thumb, such as "Lipinski's rule of 5", to enhance the chances that compounds have acceptable ADME (absorption, distribution, metabolism, and excretion properties).[37] These include having a molecular weight of less that 500, not more than five hydrogen-bond donors, not more than ten hydrogen-bond acceptors and a $\log P$ (octanol–water partition coefficient) of not greater than five. In order to meet these criteria, the potential of smaller systems containing monourea or monothiourea groups was examined by Gale and co-workers. Initially, compounds **53**–**58** were prepared, and it was found that compound **58** (a thiourea with an appended indole ring) outperformed the other monourea and thiourea compounds and tris-thiourea **22** in a chloride/bicarbonate antiport process across a POPC membrane.[38] Consequently, a series of compounds of similar structure were synthesized, some of which contain CF_3 groups in order to enhance the lipophilicity of the transporters (**59**–**62**).[39] Chloride/nitrate antiport experiments showed that compound **59** was essentially inactive, but that the other compounds functioned as transporters, with compound **62** being the most effective followed by compound **60** and then compound **61**. The addition of the CF_3 group to the urea system effectively switched on transport by this system, whereas with the thiourea system it enhanced the rate of transport. Furthermore, in vitro studies on A375 melanoma cells showed that even these simple monoureas and thioureas containing fluorinated indoles (**61** and **62**) display promising anticancer behavior.



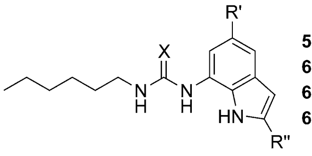
53 X = O
54 X = S



55 X = O
56 X = S

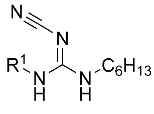


57 X = O
58 X = S

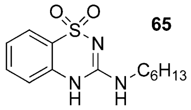


59 X = O, R' = H, R'' = H
60 X = S, R' = H, R'' = H
61 X = O, R' = CF_3, R'' = *n*Bu
62 X = S, R' = CF_3, R'' = *n*Bu

Thiourea groups therefore seem attractive motifs upon which anion-transporter designs can be based. However, there are concerns regarding the toxicity of this group.[40] Therefore, there is an ongoing quest for other simple hydrogen-bonding motifs that are effective transporters. In collaboration with Davis, Gale has reported that thiourea isosteres, such as cyanoguanidines **63** and **64** and 3-amino-1,2,4-benzothiadiazine-1,1-dioxide **65**, are capable of chloride/nitrate antiport, the rates of which are enhanced in the presence of valinomycin and a potassium gradient across a POPC membrane.[41]

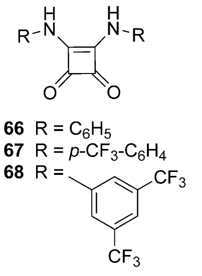


63 $R^1 = C_6H_5$
64 $R^1 = C_6F_5$

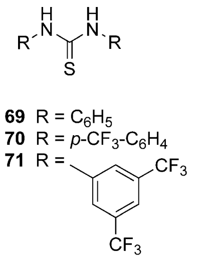


65

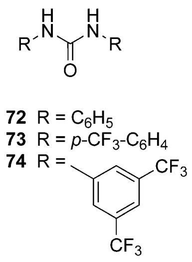
The anion-transport properties of squaramides were also studied, and in this case, for both chloride/nitrate and chloride/bicarbonate exchange, compounds **66**–**68** outperformed their urea and thiourea analogues **69**–**71**, and **72**–**74**, respectively. The addition of CF_3 groups to the parent compound was found to significantly enhance the rate of transport.[42] The lipophilicities of the squaramides are lower than those of analogous thioureas, which is an advantage when trying to stay within the boundaries of Lipinski's rule of five. The authors attribute the enhanced transport of chloride to the significantly higher affinity of the squaramides for chloride than either the urea or thiourea analogues.



66 R = C_6H_5
67 R = *p*-CF_3-C_6H_4
68 R =

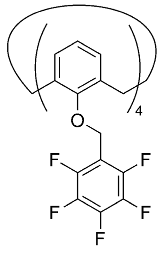


69 R = C_6H_5
70 R = *p*-CF_3-C_6H_4
71 R =

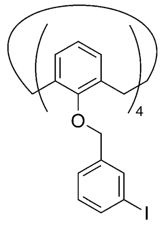


72 R = C_6H_5
73 R = *p*-CF_3-C_6H_4
74 R =

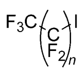
Recently, Matile and co-workers have employed halogen-bonding interactions in the design of small anionophores. Originally, this group studied calix[4]arenes-containing halogenated phenyl rings at the lower rim as transporters that bind anions through anion–π interactions (e.g. **75**) and halogen-bonding interactions (e.g. **76**).[43] Most recently, the same group has shown that very simple halogenated compounds **77**–**84** can mediate anion transport through lipid-bilayer membranes by halogen-bond formation with the transported anion. This is a remarkable finding, as these compounds are small, simple, commercially available, and within the guidelines of Lipinski's rule of five. Furthermore, the authors showed that **77** was also able to mediate transmembrane anion transport when added in the gas phase. This could have an important therapeutic benefit in the treatment of cystic fibrosis as the transporter could be administered directly to the lungs (the main site affected in CF patients) by the use of an inhaler.[44]



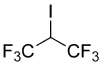
75



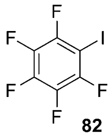
76



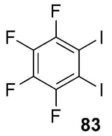
77 $n = 0$
78 $n = 2$
79 $n = 3$
80 $n = 5$



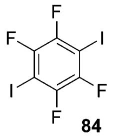
81



82



83



84

Summary and Outlook

Anion transporters have potential future application in the treatment of both cystic fibrosis and cancer. In order to achieve this goal, we must gain an understanding of how to optimize the molecular parameters of the transport compounds in order to effectively transport at low concentrations. Fluorination has boosted transport rates in a number of systems,[17,23,39,42] while designing compounds that fall within rules of thumb, such as Lipinski's rule of five, should optimize the biocompatibility of active systems that are tested in vitro and in vivo. Studies on correlating molecular properties to transport ability are currently underway in our laboratory. This work together with that of other groups is beginning to shed light on how to optimize the transport properties of anion receptors. Once this has been achieved, the next stage is to study the effect the transporters have in cells and biological systems. A future challenge will be to unambiguously elucidate the mechanism of action of these compounds in cells—a nontrivial problem. However, the potential benefit of developing these systems as future therapies drives this work forward.

Received: September 17, 2012

---

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

[2] P. M. Quinton, *Lancet* **2008**, *372*, 415–417; M. A. Garcia, N. Yang, P. M. Quinton, *J. Clin. Invest.* **2009**, *119*, 2613–2622.

[3] J. M. Tomich, U. Bukovnik, J. Layman, B. D. Schultz in *Cystic Fibrosis—Renewed Hopes Through Research* (Ed.: D. Sriramulu), InTech, Rijeka, Croatia, **2012**, pp. 291–332.

[4] U. Bukovnik, J. Gao, G. A. Cook, L. P. Shank, M. B. Seabra, B. D. Schultz, T. Iwamoto, J. Chen, J. M. Tomich, *Biochim. Biophys. Acta Biomembr.* **2012**, *1818*, 1039–1048.

[5] R. Pajewski, R. Garcia-Medina, S. L. Brody, W. M. Leevy, P. H. Schlesinger, G. W. Gokel, *Chem. Commun.* **2006**, 329–331.

[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] B. A. McNally, E. J. O'Neil, A. Nguyen, B. D. Smith, *J. Am. Chem. Soc.* **2008**, *130*, 17274–17275.

[8] J. L. Sessler, P. A. Gale, W.-S. Cho, *Anion Receptor Chemistry* (Ed.: J. F. Stoddart), Royal Society of Chemistry, London, **2006**.

[9] *Anion Coordination Chemistry* (Eds.: K. Bowman-James, A. Bianchi, E. García-España), Wiley-VCH, Weinheim, **2012**.

[10] 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.

[11] For an excellent overview of the chemistry of the prodigiosins related to their structural, anion transport, and anticancer properties, see: J. T. Davis in *Topics in Heterocyclic Chemistry, Vol 24* (Eds.: P. A. Gale, W. Dehaen), Springer, Berlin, **2010**, pp. 145–176.

[12] A. Fürstner, *Angew. Chem.* **2003**, *115*, 3706–3728; *Angew. Chem. Int. Ed.* **2003**, *42*, 3582–3603.

[13] J. L. Seganish, J. T. Davis, *Chem. Commun.* **2005**, 5781–5783.

[14] 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.

[15] a) L. J. Siskind, M. Colombini, *J. Biol. Chem.* **2000**, *275*, 38640–38644; b) L. J. Siskind, R. N. Kolesnick, M. Colombini, *J. Biol. Chem.* **2002**, *277*, 26796–26803; c) L. J. Siskind, A. Davoody, N. Lewin, S. Marshall, M. Colombini, *Biophys. J.* **2003**, *85*, 1560–1575; d) L. J. Siskind, R. N. Kolesnick, M. Colombini, *Mitochondrion* **2006**, *6*, 118–125.

[16] W. A. Harrell, Jr., M. L. Bergmeyer, P. Y. Zavalij, J. T. Davis, *Chem. Commun.* **2010**, *46*, 3950–3952.

[17] S. Bahmanjah, N. Zhang, J. T. Davis, *Chem. Commun.* **2012**, *48*, 4432–4434.

[18] J. L. Sessler, L. R. Eller, W.-S. Cho, S. Nicolaou, A. Aguilar, J. T. Lee, V. M. Lynch, D. J. Magda, *Angew. Chem.* **2005**, *117*, 6143–6146; *Angew. Chem. Int. Ed.* **2005**, *44*, 5989–5992.

[19] B. D. de Greñu, P. I. Hernández, M. Espona, D. Quiñonero, M. E. Light, T. Torroba, R. Pérez-Tomás, R. Quesada, *Chem. Eur. J.* **2011**, *17*, 14074–14083.

[20] P. I. Hernández, D. Moreno, A. A. Javier, T. Torroba, R. Pérez-Tomás, R. Quesada, *Chem. Commun.* **2012**, *48*, 1556–1558.

[21] V. Saggiomo, S. Otto, I. Marques, V. Félix, T. Torroba, R. Quesada, *Chem. Commun.* **2012**, *48*, 5274–5276.

[22] K. J. Winstanley, S. J. Allen, D. K. Smith, *Chem. Commun.* **2009**, 4299–4301.

[23] 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.

[24] N. Busschaert, M. Wenzel, M. E. Light, P. Iglesias-Hernández, R. Pérez-Tomás, P. A. Gale, *J. Am. Chem. Soc.* **2011**, *133*, 14136–14148.

[25] S. J. Moore, C. J. E. Haynes, J. González, J. L. Sutton, S. J. Brooks, M. E. Light, J. Herniman, G. J. Langley, V. Soto-Cerrato, R. Pérez-Tomás, I. Marques, P. J. Costa, V. Félix, P. A. Gale, *Chem. Sci.* **2013**, *4*, 103–117.

[26] 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.

[27] 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.

[28] S. Hussain, P. R. Brotherhood, L. W. Judd, A. P. Davis, *J. Am. Chem. Soc.* **2011**, *133*, 1614–1617.

[29] For recent examples, see: a) P. A. Gale, C. C. Tong, C. J. E. Haynes, O. Adeosun, D. E. Gross, E. Karnas, E. Sedenberg, R. Quesada, J. L. Sessler, *J. Am. Chem. Soc.* **2010**, *132*, 3240–3241; b) M. Yano, C. C. Tong, M. E. Light, F. P. Schmidtchen, P. A.

Gale, *Org. Biomol. Chem.* **2010**, *8*, 4356–4363; c) I.-W. Park, J. Yoo, B. Kim, S. Adhikari, S. K. Kim, Y. Yeon, C. J. E. Haynes, J. L. Sutton, C. C. Tong, V. M. Lynch, J. L. Sessler, P. A. Gale, C. H. Lee, *Chem. Eur. J.* **2012**, *18*, 2514–2523.

[30] P. V. Santacroce, J. T. Davis, M. E. Light, P. A. Gale, J. C. Iglesias-Sánchez, P. Prados, R. Quesada, *J. Am. Chem. Soc.* **2007**, *129*, 1886–1887.

[31] a) C. R. Yamnitz, S. Negin, I. A. Carasel, R. K. Winter, G. W. Gokel, *Chem. Commun.* **2010**, *46*, 2838–2840; b) J. L. Atkins, M. B. Patel, M. M. Daschbach, J. W. Meisel, G. W. Gokel, *J. Am. Chem. Soc.* **2012**, *134*, 13546–13549.

[32] X. Li, B. Shen, X.-Q. Yao, D. Yang, *J. Am. Chem. Soc.* **2007**, *129*, 7264–7265.

[33] a) X. Li, B. Shen, X.-Q. Yao, D. Yang, *J. Am. Chem. Soc.* **2009**, *131*, 13676–13680; b) B. Ma, H. Zha, N. Li, D. Yang, G. Lin, *Mol. Pharm.* **2011**, *8*, 1073–1082; B. Shen, X. Li, F. Wang, X. Yao, D. Yang, *PloS ONE* **2012**, *7*, e34694.

[34] a) N. Kato, M. Nakanishi, N. Hirashima, *Biochemistry* **2002**, *41*, 8068–8074; b) S. Manno, Y. Takakuwa, N. Mohandas, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 1943–1948; c) A. J. Verkleij, J. A. Post, *J. Membr. Biol.* **2000**, *178*, 1–10; d) R. F. Zwaal, A. J. Shroit, *Blood* **1997**, *89*, 1121–1132.

[35] a) J. M. Boon, B. D. Smith, *J. Am. Chem. Soc.* **1999**, *121*, 11924–11925; b) J. M. Boon, B. D. Smith, *J. Am. Chem. Soc.* **2001**, *123*, 6221–6226; c) J. M. Boon, T. N. Lambert, B. D. Smith, A. M. Beatty, V. Ugrinova, S. N. Brown, *J. Org. Chem.* **2002**, *67*, 2168–2174; d) Y. Sasaki, R. Shukla, B. D. Smith, *Org. Biomol. Chem.* **2004**, *2*, 214–219; e) K. M. DiVittorio, F. T. Hofman, J. R. Johnson, L. Abu-Esba, B. D. Smith, *Bioorg. Med. Chem.* **2009**, *17*, 141–148.

[36] a) T. N. Lambert, J. M. Boon, B. D. Smith, M. N. Pérez-Payán, A. P. Davis, *J. Am. Chem. Soc.* **2002**, *124*, 5276–5277; b) J. M. Boon, T. N. Lambert, A. L. Sisson, A. P. Davis, B. D. Smith, *J. Am. Chem. Soc.* **2003**, *125*, 8195–8201.

[37] C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv. Drug Delivery Rev.* **2001**, *46*, 3–26.

[38] 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.

[39] S. J. Moore, M. Wenzel, M. E. Light, R. Morley, S. J. Bradberry, P. Gómez-Iglesias, V. Soto-Cerrato, R. Pérez-Tomás, P. A. Gale, *Chem. Sci.* **2012**, *3*, 2501–2509.

[40] a) P. B. Smith, C. Crespi, *Biochem. Pharmacol.* **2002**, *63*, 1941–1948; b) R. C. A. Onderwater, J. N. M. Commandeur, E. J. Groot, A. Sitters, W. M. P. B. Menge, N. P. E. Vermeulen, *Toxicology* **1998**, *125*, 117–129; c) S. A. Svarovsky, R. H. Simoyi, S. V. Makarov, *J. Phys. Chem. B* **2001**, *105*, 12634–12643.

[41] M. Wenzel, M. E. Light, A. P. Davis, P. A. Gale, *Chem. Commun.* **2011**, *47*, 7641–7643.

[42] N. Busschaert, I. L. Kirby, S. Young, S. J. Coles, P. N. Horton, M. E. Light, P. A. Gale, *Angew. Chem.* **2012**, *124*, 4502–4506; *Angew. Chem. Int. Ed.* **2012**, *51*, 4426–4430.

[43] A. Vargas Jentzsch, D. Emery, J. Mareda, S. K. Nayak, P. Metrangolo, G. Resnati, S. Matile, *Angew. Chem.* **2011**, *123*, 11879–11882; *Angew. Chem. Int. Ed.* **2011**, *50*, 11675–11678.

[44] A. Vargas Jentzsch, D. Emery, J. Mareda, S. K. Nayak, P. Metrangolo, G. Resnati, N. Sakai, S. Matile, *Nat. Commun.* **2012**, *3*, 905.