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Current Opinion in Chemical Biology

Therapeutic synthetic anion transporters

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This short review highlights recent examples of small-molecule anion transporters reported in the literature that have potentially useful biological activity. This includes anionophores with antibiotic or antifungal activity, anticancer activity, or the potential to treat channelopathies such as cystic fibrosis. Additionally selective and targeted anion transporters are also discussed.

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Current Opinion in Chemical Biology 2024, **83**:102535

This review comes from a themed issue on **Ion transport across biological membranes (2025)**

Edited by **Dr Robin Bon** and **Prof Matthew Langton**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.cbpa.2024.102535

Keywords
Anion transport, Cystic fibrosis, Antimicrobial resistance, Cancer.

Introduction

Anion transporters (or anionophores) have been developed for their potential application in the treatment of disease and as antimicrobials and antifungal agents (Figure 1). For example, transporters may offer a novel approach to the treatment of cancer that relies on supramolecular chemistry to perturb homeostasis and trigger apoptosis. Biological activity results from the function of the anion carrier rather than its specific structure, and consequently, a wide range of compounds with varying binding motifs are able to mediate the activity, resulting in a larger pool of potential therapeutics. Alternatively, transporters could be used to replace the function of faulty anion channels that cause diseases such as cystic fibrosis. A variety of different applications together with recent examples of new anion transporter systems are featured in this short review with the structures of the anionophores shown in Figures 2–4.

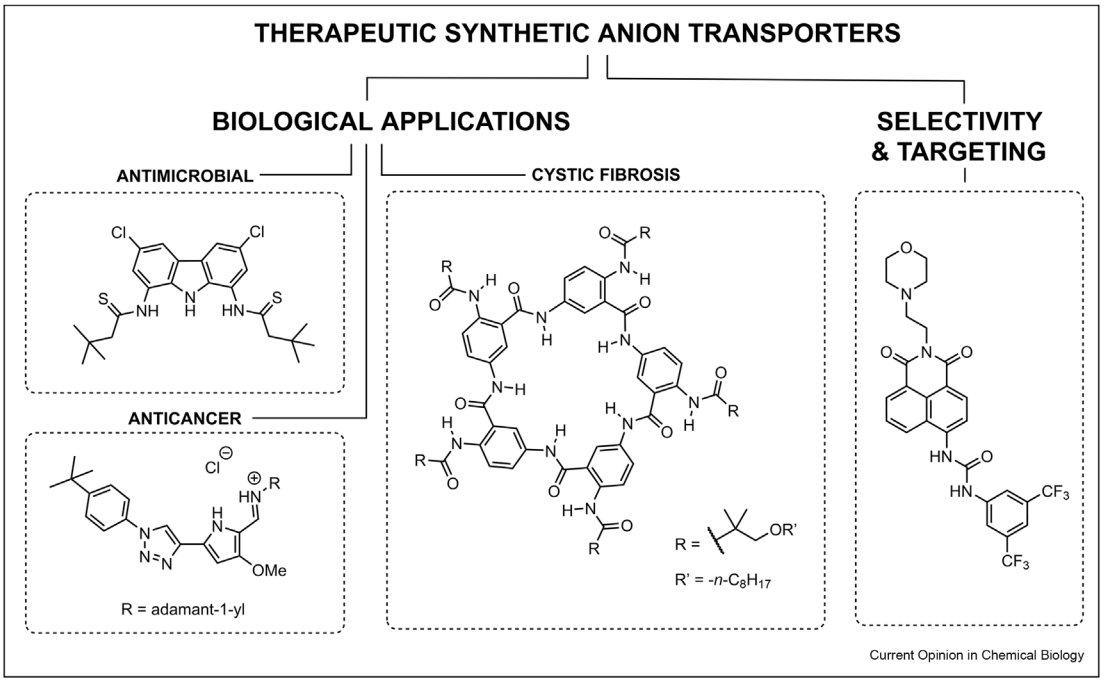
Anion transporters with antibiotic and/or antifungal activity

There is increasing pressure to develop new antibiotics to tackle the problem of antimicrobial resistance. 700,000 deaths occur worldwide each year due to antibiotic resistance, with this number estimated to rise to 10 million by 2050 [1,2]. Cationophores such as lasalocid, monensin, narasin, and salinomycin have been used extensively in animals as antibiotic agents and have been shown to be effective against Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) [3–5]. However, it is only in the last decade that the potential of synthetic anionophores as antibiotic and antifungal agents has begun to be explored, and efforts have been made to elucidate the modes of action [6–11].

Amendola, Davis, and co-workers reported the antimicrobial activity of a tris (benzimidazolium)-based organic cage $\mathbf{1}^{3+}$ [7]. The salts $\mathbf{1}(PF_6)_3$ and $\mathbf{1}(NO_3)_3$ exhibited minimal to no transport in 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) lipid vesicle transport experiments, which the authors attributed to the high positive charge increasing the hydrophilicity and hindering membrane permeability. The compounds were shown to be non-cytotoxic to SH-SY5Y cancer cells, but exhibited antimicrobial activity against the Gram-positive bacteria *S. aureus*. Previously, the antimicrobial properties of benzimidazolium complexes were related to their ability to depolarize and weaken bacterial membranes [6].

More recently Elmes, Kavanagh, Gale, Hawes, and coworkers conducted a detailed study on the mechanisms of action of a series of antimicrobial squindoles **2a-d** [8]. Compound **2b**, which showed the most potent chloride transport across POPC membranes, was evaluated in an antimicrobial susceptibility assay. It exhibited a clinically relevant IC_{50} of 2.5 μM against *S. aureus* and 2.35 μM against MRSA (IC_{50} of monensin against *S. aureus* and MRSA is 6 μM [5]) and showed a low acquired resistance compared with other clinical antimicrobials. *In vitro* cell viability studies in HaCaT cells showed the compound was non-cytotoxic up to 10 μM, which is 4x the IC_{50} concentration. *In vivo* toxicity in *Galleria mellonella* was also investigated, with no observed toxicity up to 300 μM. An *S. aureus* infection model in *G. mellonella* showed an increase in survival rate on

Figure 1



Overview of the topics discussed in this review, including the biological applications of synthetic anion transporters, as well as selective and targeted transporters.

treatment with the compound. From mechanistic studies it was determined that compound **2b** functions by perturbing the chloride homeostasis, which induces oxidative and osmotic stress, ultimately resulting in cell death. This mechanism of action can overcome existing resistance pathways in MRSA bacteria. Though the antimicrobial effect is linked to the anionophoric ability of the compound it is possible there are multiple influential factors as antimicrobial susceptibility assays of two potent squaramide transporter controls (**3** and **4**) showed significantly lower activity than **2b**.

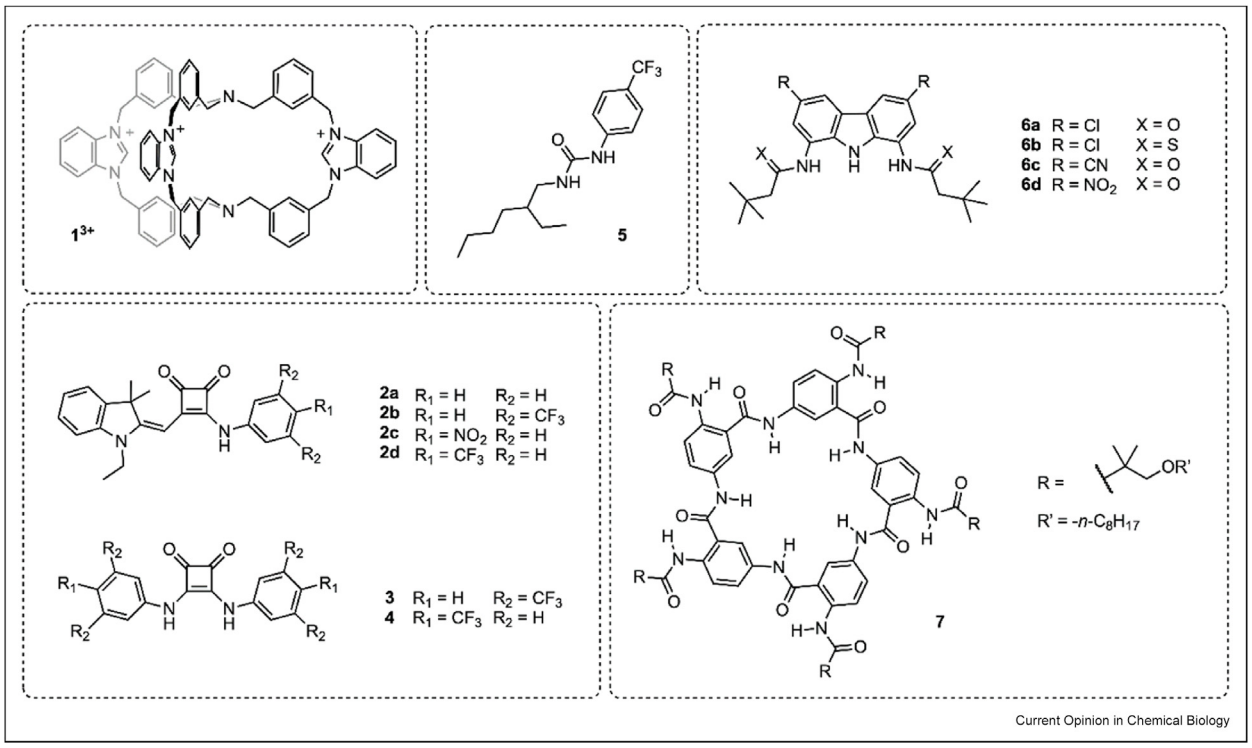
Further evidence for the role of anion transport in the antibiotic activity of anionophores was provided in a study by Busschaert and co-workers [12]. They performed bacterial cytological profiling on their previously reported bactericidal urea-based compound **5** [11], revealing a membrane-related mode of action distinct from that of other membrane-based antibiotics such as the cationophore Calcimycin and pore-forming nisin.

Valkenier, Chmielewski, and co-workers investigated the antibiotic properties of 1,8-di(thio)amidocarbazole bicarbonate transporters **6a-d** [13]. The compounds were tested against two Gram-positive bacteria, *Bacillus subtilis*, and *S. aureus*, and two Gram-negative bacteria, *Escherichia coli*, and *Acinetobacter baumannii*. All four compounds showed strong antibacterial activity against *B. subtilis*, with the trend following that of their H^+/Cl^- transport ability. Compound **6b** showed potent activity against *S. aureus* with an MIC (0.07 μM) almost five times greater than of the antibiotic vancomycin (0.345 μM). Negligible activity was seen with *E. coli* or *A. baumannii*, which is common for synthetic anionophores and is attributed to the more complex cell membrane of Gram-negative bacteria [7–9].

Cystic fibrosis

Another potential use for anionophores is as 'channel replacement therapies' for channelopathies such as cystic fibrosis (CF) [14–17]. CF is caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) protein gene, leading to a decrease or loss of function of the cystic fibrosis transmembrane conductance regulator (CFTR) protein [18]. The CFTR protein acts primarily as a chloride channel and is expressed in many epithelial and blood cells. Without effective chloride transport, chloride ions, and associated water accumulate within cells, resulting in dehydration and acidification of the airway surface liquid (ASL), compression of the cilia, and prevention of regular mucus clearance. Most current CF treatments consist of CFTR modulators, which correct malfunctioning CFTR proteins; however, they are not applicable in CF cases where CFTR proteins are absent [19]. As anionophores function independently of the existing channels, they

Figure 2



The structures of anion transporters $\mathbf{1}^{3+}$ - **7**.

have potential as therapeutics for CFTR-deficient epithelia [20,21]. Furthermore as anionophores can transport both into epithelial cells as well as disrupting bacterial membranes, they could also have additional antibiotic properties beneficial to CF treatment.

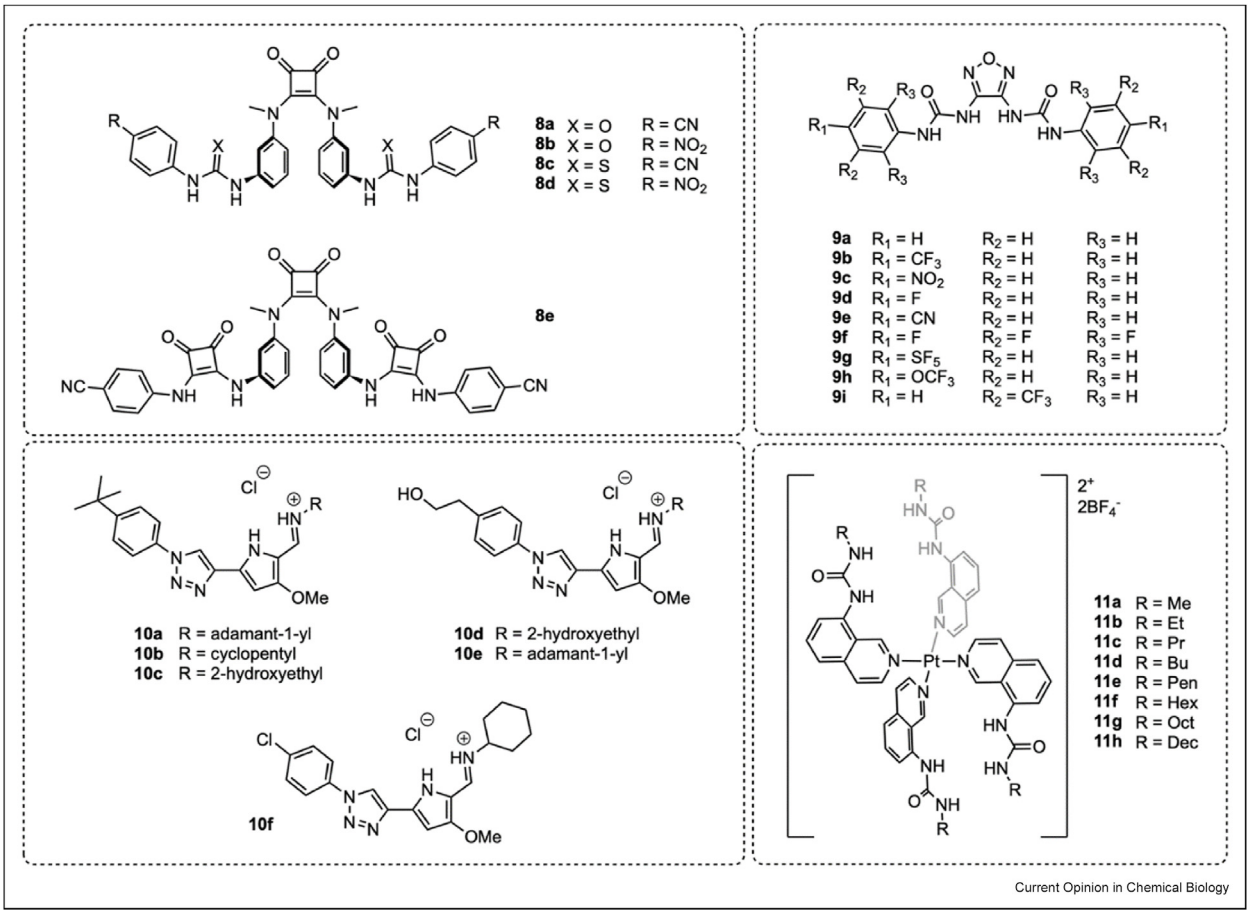
Gong, Shao, and co-workers have reported a C5-symmetric aromatic pentaamide macrocycle able to restore the hydration and volume of the ASL of human bronchial epithelial (HBE) cells taken from CF patients [22]. The macrocycle **7** was synthesized in a one-pot reaction and showed chloride selective transmembrane transport in the initial vesicle studies. Compound **7** was then applied apically for five days to HBE cells cultured on permeable supports, and the ASL layer was fluorescently labeled to allow the thickness to be determined *via* XZ confocal scanning microscopy. The ASL thickness of the treated cells (7.8 ± 1.7 μm) increased by over 50% compared to the control cells (5.1 ± 1.4 μm), a promising result for use of anionophores as CF therapeutics. Though there are limited studies regarding the use of synthetic anionophores for the treatment of CF, Burke, and Welsh have investigated the use of natural product anionophore amphotericin B for this purpose [20,21]. They have had positive initial *in vivo* results that encourage further clinical studies to assess its potential and reaffirm the value of investigating small-molecule anionophores for CF.

Transporters with anticancer properties

Synthetic anion transporters have been investigated for their potential as anti-cancer agents, due to their ability to disrupt ionic and pH gradients. Studies have shown anionophores can induce apoptosis via a caspase-dependent pathway, with some also able to interfere with autophagy [23–27].

Gale and co-workers developed an *N,N′*-dimethylated-*N,N′*-diphenylsquaramide scaffold to form a family of anionophores (**8a-e**) with dual hydrogen-bonding groups [28]. The transporters were shown to function as H^+/Cl^- co-transporters. The most active compound **8d** (EC_{50} of 0.03 mol% in Cl^-/NO_3^- exchange assay) outperformed control compound **4** (EC_{50} of 0.06 mol%), a squaramide previously reported for its efficiency as a Cl^- transporter [24,29]. Interestingly, compound **4** was shown to be highly cytotoxic, reducing the cell viability of A549 cancer cell lines to below 10 % at a concentration of 10 μM, whereas compound **8d**, a more efficient transporter, showed no cytotoxicity up to concentrations of 200 μM. This highlights one of the current main challenges in the development of anionophores as

Figure 3



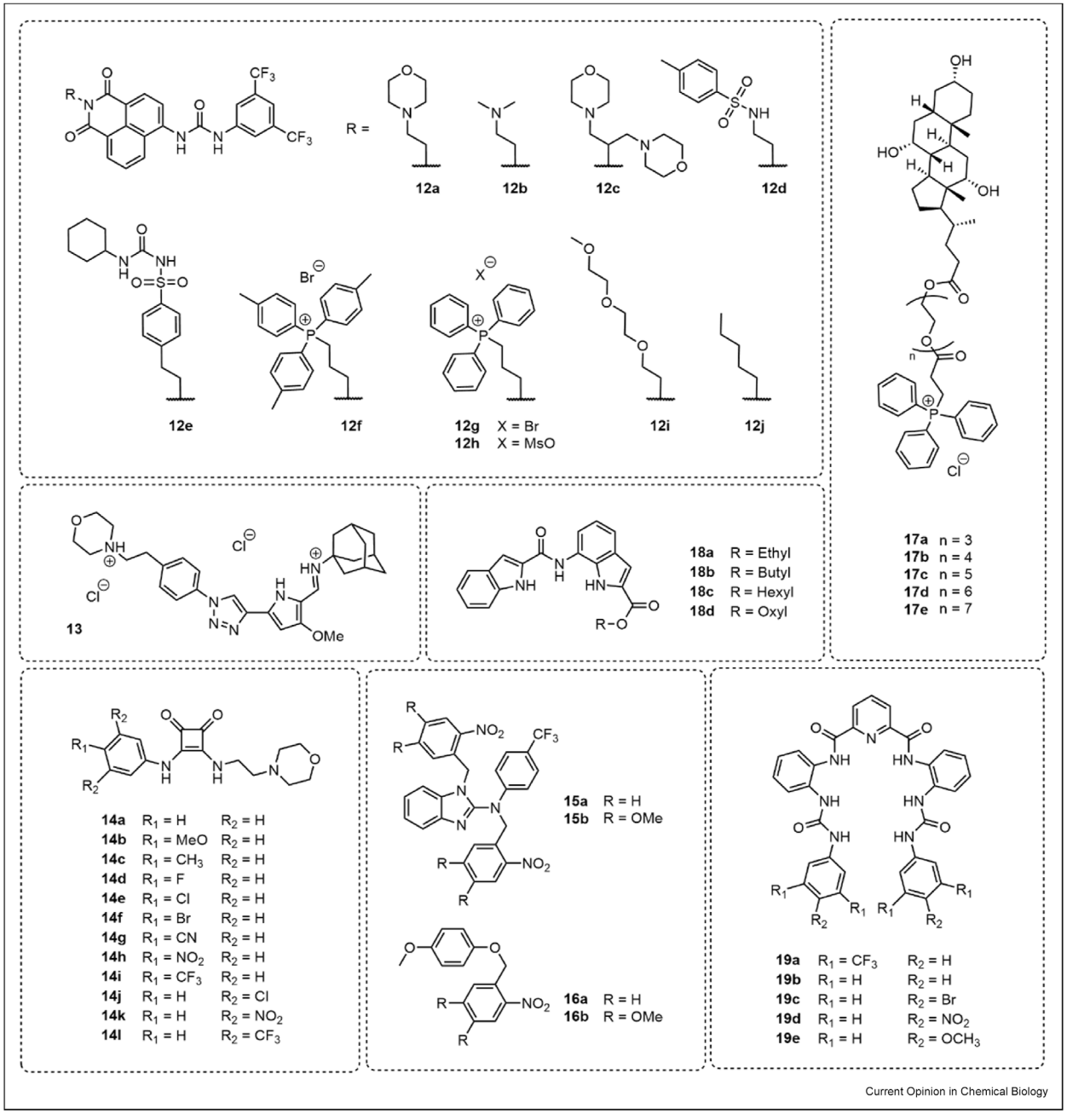
The structures of transporters **8**–**11**.

therapeutics, as anion transport activity does not always correlate with the observed physiological effects. A deeper understanding of the function of anionophores in cells is needed to evaluate other factors influencing the effectiveness of compounds.

Gale and co-workers have also studied a furazan bis-urea scaffold functionalized with a range of electron-withdrawing substituents to assess the effects of lipophilicity and hydrogen-bond strength on transport activity [30]. The compounds **9a-i** were made synthetically accessible one- or two-step procedures, an important consideration as the field of anion transporters moves more towards potential therapeutic applications. All bar one of the compounds (**9e**) had a cytotoxic effect in both HEK293 (normal) and 4T1 (cancer) cells, with no significant selectivity observed between the two lines. In the cancerous cells, the trend in cytotoxicity generally followed the trend in transport activity, though it is worth noting that the only compound shown to be nontoxic was the second most efficient transporter. The trend also broadly correlated with that of the Hammett constants of the substituents suggesting that the electron-withdrawing strength of the phenyl substituent, and thus the effect on the N-H acidity is an important factor in the biological activity of the transporters. The trends were less prominent in the normal cell line.

Pérez-Tomas, Quesada, and co-workers reported a series of click-tambjamine lactate transporters **10a-f** [31]. Evidence of lactate transport was obtained via ^{13}C nuclear magnetic resonance spectroscopy experiments, where efflux of L-lactate-1-^{13}C from POPC vesicles was observed via a broadening of the nuclear magnetic resonance signal on coordination to externally added paramagnetic $MnCl_2$. The cytotoxicity of the compounds in a variety of cancerous (HeLa, MCF7, CAL27) and normal (MCF10A) cell lines was assessed, with compound **10a** found to have the greatest cytotoxic effect (IC_{50} of 3.3−7.7 μM) across the different cell lines. Thus, **10a** was chosen to examine the lactate transport ability in HeLa cells. The lactate monocarboxylate transporter 1 (MCT1) and 4 (MCT4) dual

Figure 4



The structures of transporters **12**–**19**.

inhibitor syrosingopine was used in the assays to demonstrate lactate transport by the compound independently of MCT activity. Compound **10a** was also tested in combination therapy with the chemotherapeutic agent cisplatin in HeLa cells. At all doses tested, the effect of the combined therapy on cell viability was higher than for cisplatin on its own. The combination indices of different doses were analyzed and found that most of the combinations exhibited an additive effect, though a moderate synergic effect was seen with higher doses of **10a** and cisplatin. The potential use of anionophores in conjunction with existing therapeutics is a promising line of investigation.

Inspired by the anticancer activity of cisplatin and other platinum-based drugs, Gale, and co-workers developed a series of Pt(II) complexes with urea-appended isoquinoline ligands (**11a-h**), capable of transmembrane

chloride transport [32]. The cytotoxicity of the complexes in AGS, MCF-7, and MDA-MB-231 cancer cells was investigated, and four of the complexes (**11b-e**) were found to exhibit IC_{50} values lower than cisplatin. Further mechanistic studies on complexes **11b-d** showed they induce apoptosis with low necrotic activity. The complexes caused a significant decrease in reactive oxygen species in cells, which was surprising considering cisplatin is known to induce higher reactive oxygen species levels and can cause cell death as a direct result. This suggests the Pt(II) complexes are inducing apoptosis by an alternative, unknown mechanism.

Selectivity and targeting

One of the main challenges of using anionophores as therapeutic agents is their specificity. Exerting control over where and when these compounds work is crucial for them to be viable treatments. In recent years, this challenge has been addressed in a variety of ways, including targeting the anionophores to specific types of cells or subcellular compartments, and through the development of stimuli-responsive transporters.

The availability and extensive library of known subcellular targeting groups has allowed the development of anionophores that selectively localize in specific organelles. This is illustrated by Gale and coworkers' family of urea-based anionophores to target the lysosomes (**12a-c**), the endoplasmic reticulum (ER) (**12d-e**), and mitochondria (**12f-h**) [33]. The use of a fluorescent 4-amino-1,8-napthalimide unit within the transporters enabled the use of colocalization experiments to confirm the accumulation of the targeted anionophores within the intended organelles. AlamarBlue cell viability assays in A549 cancer cells showed mitochondrial-targeted compounds **12g** and **12h** (IC_{50} 11.9 μM, 11,4 μM) exhibited similar cytotoxicity compared to a nontargeted analogue **12j** (IC_{50} 12 μM), whereas lysosome-targeted compounds **12a-c** showed enhanced cytotoxicity ($IC_{50} < 5$ μM). Surprisingly the highest percentage of apoptotic cells, as determined by flow cytometry, was not observed for the compounds with the highest cytotoxicity, but rather for the ER (**12d** and **12e**) and mitochondrial (**12h**) targeted transporters. The disparity between cytotoxicity and percentage of apoptotic cells suggests that targeting different organelles may induce different cell death mechanisms. Developing more active ER and mitochondrial targeted transporters may help uncover novel mechanisms of cell death.

Chen and co-workers reported the use of squaramides appended with a lysosome targeting morpholine group (**14a-l**) to specifically modulate lysosomal pH via anion transport [34]. Though the squaramides showed negligible or minimal transport in a Cl^-/NO_3^- exchange assay, some were able to efficiently alkalize the lysosomes of HeLa cancer cells, possibly via chloride exchange with hydroxide or bicarbonate ions. The most effective compound **14l** induced a change in pH of 1.90 ± 0.01 within the lysosome, which is comparable to the change of 2.17 ± 0.13 caused by the known lysosomal pH-modulator chloroquinone phosphate. The trend in induced pH change followed that of the transport ability. The compounds showed no cytotoxicity or disturbance to the autophagy of cells, which may be due to the poor transport activity.

Soto-Cerrato, Pérez-Tomas, Quesada, and co-workers developed a lysosome-targeted late-stage autophagy inhibitor with a click-tambjamine scaffold [35]. They showed compound **13** deacidifies the lysosome while acidifying the cytosol, resulting in a blockage of cytoprotective autophagy in cancer cells. Compound **13** had an IC_{50} of 15.47 ± 0.79 μM in A549 cancer cells after 24 h of incubation, which was lower than that of chloroquine and 3-methyladenine, two well-known autophagy inhibitors. Western blots showed the compound initiates both apoptosis and necrosis. Compound **13** was also evaluated in combination therapy with cisplatin, and showed the compounds sensitize the tumor cells to the cisplatin treatment with a synergistic effect, resulting in lower cell viability when used in combination. Autophagy inhibitors are of particular interest as autophagy is one of the mechanisms that can confer treatment resistance to cancer cells [36,37].

Another strategy being explored to control the undesired activity of transporters in normal cells is the use of stimuli-responsive systems including photocaging. A photocleavable group can be used to block the anion binding site of a transporter, which is then activated in a specific location using external radiation. This has been explored by Talukdar and co-workers, who recently reported doubly o-nitrobenzyl-protected benzimidazoleamines **15a** and **15b** that exhibited photoinduced cytotoxicity in MCF-7 cancer cells [38]. The procarrier initially shows no cytotoxic effect, but on irradiation at 400 nm the release of the active transporter causes significant increase in cell death. To investigate the potential role of the *o*-nitrosobenzaldehyde by-product in the observed cytotoxicity, photoprotected control compounds (**16a** and **16b**), which release the same by-product, were tested. The controls showed minimal cytotoxicity on irradiation, confirming the anionophore is responsible for cell death.

An alternative approach to achieving selectivity toward cancer cells was employed in a paper by Ren and co-workers [39]. They propose that as the mitochondrial transmembrane potential (MMP) of cancer cells is approximately 60 mV greater than that of normal cells, a mitochondrial-targeted artificial ion channel, driven by

MMP, could allow for selective cytotoxicity to cancerous cells. They reported a novel class of adaptive artificial ion channels with the mitochondrial targeting triphenyl phosphine group attached via a polyethylene glycol linker to a cholic acid moiety (**17a-e**). At low concentrations, two molecules assemble into chloride channels, but at high concentrations form nanopores permeable to small molecules. All the compounds exhibited anticancer activity in HepG2 human liver cancer cells, with the trend approximately following that of the ability to form nanopores, as confirmed by a carboxyfluorescein dye permeation assay. The most potent compound **17c** had an IC_{50} of 3.8 μM, matching that of doxorubicin a chemotherapeutic agent currently in use. The compounds were also tested in normal human liver cells HHL-5, and selectivity indexes were determined. Remarkably **17c** exhibited a selectivity index of 4.37, which is 16.8 times higher than that of doxorubicin, cementing the potential of these compounds for use in selective anticancer therapy.

Some compounds show a natural selectivity for cancerous cells without intentional design. Talukdar and co-workers' bis-indole transporters **18a-d** cause cytotoxic effects in MCF-7 cancer cells *via* disruption of chloride ion homeostasis leading to oxidative stress, mitochondrial membrane depolarization, and lysosomal pH disruption [40]. However, negligible cytotoxicity was observed in noncancerous mouse embryonic fibroblasts (MEFs). Srivastava and co-workers reported a series of pyridine-2,6-dicarboxamide molecular clips with two spatially proximal urea groups (**19a-e**) that facilitate transmembrane H^+/Cl^- co-transport [41]. Preliminary cytotoxicity studies indicate selective toxicity toward cancerous HeLa cells over noncancerous HEK-293T cells, though no explanation was proposed.

Perspectives and conclusions

Anion transporters have the potential to be future treatments for a range of diseases and have other useful biological activity, resulting from their function as supramolecular carriers. Many advancements have been made in recent years to understand how these compounds can affect biological systems. Research into their antimicrobial properties has found a variety of anion transporters with bactericidal activity equal to or greater than that of existing antibiotics. Mechanistic studies suggest that the bactericidal activity of some transporters may arise from a mode of action distinct from other antibiotics. This is very encouraging for the potential use of anion transporters for treatment of antibiotic-resistant bacteria.

There has been much work in the exploration of anticancer anionophores. One of the main challenges that remains is the unpredictability of anion transporter cytotoxicity. Though cytotoxicity seems to follow the general trend of anion transport ability there are some exceptions that are poorly understood. Without a thorough understanding of the factors causing differences in cytotoxicity, it is difficult to effectively design and optimize novel anticancer transporters, limiting the rate at which advances can be made. However, there have nonetheless been considerable developments within the field. Several recent studies have highlighted the increased cytotoxicity observed with the use of anionophores and cisplatin in combination therapies. This is a promising avenue for further exploration as combination therapies are desirable to combat tumor resistance and improve treatment outcomes. Additionally, the ability of some anion transporters to act as autophagy inhibitors makes them particularly interesting, as autophagy is one of the mechanisms that can grant treatment resistance in cancer cells. Another challenge for the use of anion transporters as therapeutics is their lack of selectivity. The development of targeted and/or triggered transporters offers a pathway for a more restricted and controlled mode of action, which is necessary for them to be viable and worthwhile treatments for investigation.

There has been comparatively limited research into the use of synthetic anionophores for the treatment of cystic fibrosis and other channelopathies over the last few years. This is likely due to a range of factors. Designing CF therapeutics requires achieving a balance between efficient chloride transport and minimal cytotoxicity, which is particularly hard to achieve with the current lack of understanding of anion transporter cytotoxicity. Furthermore, the tests required to assess the effectiveness of CF treatments in initial stages are more complex than the cell viability assays used to test for anti-cancer activities, making it harder and more time-consuming to screen complexes for CF therapeutics. 3D cell cultures such as spheroids, organoids, and air-liquid interface systems are often required for *in vitro* CF models [42]. These may be some of the reasons the field has shifted more toward a focus on anticancer and antimicrobial activity, and away from channelopathies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

E.F. and P.A.G. acknowledge and pay respect to the Gadigal people of the Eora Nation, the traditional owners of the land on which we research, teach, and collaborate at the University of Technology Sydney. We thank the University of Technology Sydney for funding.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Review on Antimicrobial Resistance, Tackling drug-resistant infrections globally: final report and recommendations. https://amr-review.org/, 2016 (accessed 26 July 2024).

2. Miethke M, Pieroni M, Weber T, Brönstrup M, Hammann P, Halby L, Arimondo PB, Glaser P, Aigle B, Bode HB, *et al.*: **Towards the sustainable discovery and development of new antibiotics**. *Nat Rev Chem* 2021, **5**:726–749, https://doi.org/10.1038/s41570-021-00313-1.

3. Russell JB, Houlihan AJ: **Ionophore resistance of ruminal bacteria and its potential impact on human health1**. *FEMS Microbiol Rev* 2003, **27**:65–74, https://doi.org/10.1016/S0168-6445(03)00019-6.

4. Hickey EE, Wong HS, Khazandi M, Ogunniyi AD, Petrovski KR, Garg S, Page SW, O'Handley R, Trott DJ: **Repurposing Ionophores as novel antimicrobial agents for the treatment of bovine mastitis caused by Gram-positive pathogens**. *J Vet Pharmacol Therapeut* 2018, **41**:746–754, https://doi.org/10.1111/jvp.12674.

5. Hickey EE, Page SW, Trott DJ: **In vitro efficacy and pharmacodynamic profiles of four polyether ionophores against methicillin-resistant Staphylococcus spp**. *J Vet Pharmacol Therapeut* 2020, **43**:499–507, https://doi.org/10.1111/jvp.12871.

6. Elie CR, David G, Schmitzer AR: **Strong antibacterial properties of anion transporters: a result of depolarization and weakening of the bacterial membrane**. *J Med Chem* 2015, **58**:2358–2366, https://doi.org/10.1021/jm501979f.

7. * La Cognata S, Armentano D, Marchesi N, Grisoli P, Pascale A, Kieffer M, Taglietti A, Davis AP, Amendola V: **A benzimidazolium-based organic cage with antimicrobial activity**. *Chemistry* 2022, **4**:855–864, https://doi.org/10.3390/chemistry4030061.
First organic cage to be studied as both an anionophore and antimicrobial agent

8. ** Brennan LE, Kumawat LK, Piatek ME, Kinross AJ, McNaughton DA, Marchetti L, Geraghty C, Wynne C, Tong H, Kavanagh ON, O'Sullivan F, Hawes CS, Gale PA, Kavanagh K, Elmes RBP: **Potent antimicrobial effect induced by disruption of chloride homeostasis**. *Chem* 2023, **9**:3138–3158, https://doi.org/10.1016/j.chempr.2023.07.014.
In depth study into the underlying mechanisms of actions of antimicrobial anionophores.

9. Carreira-Barral I, Rumbo C, Mielczarek M, Alonso-Carrillo D, Herran E, Pastor M, Del Pozo A, García-Valverde M, Quesada R: **Small molecule anion transporters display in vitro antimicrobial activity against clinically relevant bacterial strains**. *Chem Commun* 2019, **55**:10080–10083, https://doi.org/10.1039/C9CC04304G.

10. Share AI, Patel K, Nativi C, Cho EJ, Francesconi O, Busschaert N, Gale PA, Roelens S, Sessler JL: **Chloride anion transporters inhibit growth of methicillin-resistant Staphylococcus aureus (MRSA) in vitro**. *Chem Commun* 2016, **52**:7560–7563, https://doi.org/10.1039/C6CC03645G.

11. Herschede SR, Gneid H, Dent T, Jaeger EB, Lawson LB, Busschaert N: **Bactericidal urea crown ethers target phosphatidylethanolamine membrane lipids**. *Org Biomol Chem* 2021, **19**:3838–3843, https://doi.org/10.1039/D1OB00263E.

12. ** Herschede SR, Salam R, Gneid H, Busschaert N: **Bacterial cytological profiling identifies transmembrane anion transport as the mechanism of action for a urea-based antibiotic**. *Supramol Chem* 2022, **34**:26–33, https://doi.org/10.1080/10610278.2023.2178921.
Showed antibiotic bis-urea functions via a membrane-related mode of action distinct from cationophore or pore-forming antibiotics.

13. Maslowska-Jarzyna K, Cataldo A, Marszalik A, Ignatikova I, Butler SJ, Stachowiak R, Chmielewski MJ, Valkenier H: **Dissecting transmembrane bicarbonate transport by 1,8-di(thio)amidocarbazoles**. *Org Biomol Chem* 2022, **20**:7658–7663, https://doi.org/10.1039/D2OB01461K.

14. Gianotti A, Capurro V, Delpiano L, Mielczarek M, García-Valverde M, Carreira-Barral I, Ludovico A, Fiore M, Baroni D, Moran O, *et al.*: **Small molecule anion carriers correct abnormal airway surface liquid properties in cystic fibrosis airway epithelia**. *Int J Mol Sci* 2020, **21**:1488, https://doi.org/10.3390/ijms21041488.

15. Li H, Valkenier H, Thorne AG, Dias CM, Cooper JA, Kieffer M, Busschaert N, Gale PA, Sheppard DN, Davis AP: **Anion carriers as potential treatments for cystic fibrosis: transport in cystic fibrosis cells, and additivity to channel-targeting drugs**. *Chem Sci* 2019, **10**:9663–9672, https://doi.org/10.1039/C9SC04242C.

16. Li H, Salomon JJ, Sheppard DN, Mall MA, Galietta LJV: **Bypassing CFTR dysfunction in cystic fibrosis with alternative pathways for anion transport**. *Curr Opin Pharmacol* 2017, **34**:91–97, https://doi.org/10.1016/j.coph.2017.10.002.

17. Davis JT, Gale PA, Quesada R: **Advances in anion transport and supramolecular medicinal chemistry**. *Chem Soc Rev* 2020, **49**:6056–6086, https://doi.org/10.1039/C9CS00662A.

18. O'Sullivan BP, Freedman SD: **Cystic fibrosis**. *Lancet* 2009, **373**:1891–1904, https://doi.org/10.1016/S0140-6736(09)60327-5.

19. Allan KM, Farrow N, Donnelley M, Jaffe A, Waters SA: **Treatment of cystic fibrosis: from gene- to cell-based therapies**. *Front Pharmacol* 2021, **12**, https://doi.org/10.3389/fphar.2021.639475.

20. Muraglia KA, Chorghade RS, Kim BR, Tang XX, Shah VS, Grillo AS, Daniels PN, Cioffi AG, Karp PH, Zhu L, Welsh MJ, Burke MD: **Small-molecule ion channels increase host defences in cystic fibrosis airway epithelia**. *Nature* 2019, **567**:405–408, https://doi.org/10.1038/s41586-019-1018-5.

21. Chorghade RS, Kim BR, Launspach JL, Karp PH, Welsh MJ, Burke MD: **Amphotericin B induces epithelial voltage responses in people with cystic fibrosis**. *J Cyst Fibros* 2021, **20**:540–550, https://doi.org/10.1016/j.jcf.2020.11.018.

22. ** Cao R, Rossdeutcher RB, Zhong Y, Shen Y, Miller DP, Sobiech TA, Wu X, Buitrago LS, Ramcharan K, Gutay MI, *et al.*: **Aromatic pentaamide macrocycles bind anions with high affinity for transport across biomembranes**. *Nat Chem* 2023, **15**:1559–1568, https://doi.org/10.1038/s41557-023-01315-w.
One-pot synthesis of a macrocycle able to restore the hydration and volume of the ASL of CF patient HBE cells.

23. Jowett LA, Howe ENW, Soto-Cerrato V, Van Rossom W, Pérez-Tomás R, Gale PA: **Indole-based perenosins as highly potent HCl transporters and potential anti-cancer agents**. *Sci Rep* 2017, **7**:9397, https://doi.org/10.1038/s41598-017-09645-9.

24. Busschaert N, Park S-H, Baek K-H, Choi YP, Park J, Howe ENW, Hiscock JR, Karagiannidis LE, Marques I, Félix V, *et al.*: **A synthetic ion transporter that disrupts autophagy and induces apoptosis by perturbing cellular chloride concentrations**. *Nat Chem* 2017, **9**:667–675, https://doi.org/10.1038/nchem.2706.

25. Yu X-H, Hong X-Q, Chen W-H: **Fluorinated bisbenzimidazoles: a new class of drug-like anion transporters with chloride-mediated, cell apoptosis-inducing activity**. *Org Biomol Chem* 2019, **17**:1558–1571, https://doi.org/10.1039/C8OB03036G.

26. Ko S-K, Kim SK, Share A, Lynch VM, Park J, Namkung W, Van Rossom W, Busschaert N, Gale PA, Sessler JL, Shin I: **Synthetic ion transporters can induce apoptosis by facilitating chloride anion transport into cells**. *Nat Chem* 2014, **6**:885–892, https://doi.org/10.1038/nchem.2021.

27. Park S-H, Park S-H, Howe ENW, Hyun JY, Chen L-J, Hwang I, Vargas-Zuñiga G, Busschaert N, Gale PA, Sessler JL, Shin I: **Determinants of ion-transporter cancer cell death**. *Chem* 2019, **5**:2079–2098, https://doi.org/10.1016/j.chempr.2019.05.001.

28. McNaughton DA, York E, Rawling T, Gale PA:N: **N′-Dimethylsquaramide as a central scaffold for anionophore design**. *Org Biomol Chem* 2024, https://doi.org/10.1039/D4OB00703D.

29. Busschaert N, Kirby IL, Young S, Coles SJ, Horton PN, Light ME, Gale PA: **Squaramides as potent transmembrane anion**

transporters. *Angew Chem, Int Ed* 2012, **51**:4426–4430, https://doi.org/10.1002/anie.201200729.

30. Ryder WG, Wu EG, Chen L, Fares M, McNaughton DA, Tran K, Yu C, Gale PA: **Furazan bis-ureas: a heterocyclic scaffold for anion binding and transport**. *Org Chem Front* 2024, **11**: 1290–1298, https://doi.org/10.1039/D3QO01956J.

31. Alonso-Carrillo D, Arias-Betancur A, Carreira-Barral I, Fontova P, Soto-Cerrato V, García-Valverde M, Pérez-Tomás R, Quesada R: **Small molecule anion carriers facilitate lactate transport in model liposomes and cells**. *iScience* 2023, **26**, 107898, https://doi.org/10.1016/j.isci.2023.107898.

32. Wang P, Fares M, Eladwy RA, Bhuyan DJ, Wu X, Lewis W, Loeb SJ, Macreadie LK, Gale PA: **Platinum-based metal complexes as chloride transporters that trigger apoptosis**. *Chem Sci* 2024, **15**:11584–11593, https://doi.org/10.1039/D4SC02115K.

33. ** Ryder WG GM, Levina A, Hawkins B, Hibbs DE, New EJ, Gale PA: **Subcellular Targeted Anion Transporters**. *Chem* 2025, https://doi.org/10.1016/j.chempr.2024.07.009.
Comparison of anionophores targeted to lysosomes, mitochondria, and endoplasmic reticulum show impact of subcellular localization on cytotoxicity.

34. Zhang T, Hong X-Q, Zhi H-T, Hu J, Chen W-H: **Synthesis and mechanism of biological action of morpholinyl-bearing arylsquaramides as small-molecule lysosomal pH modulators**. *RSC Adv* 2022, **12**:22748–22759, https://doi.org/10.1039/D2RA02146C.

35. Molero-Valenzuela A, Fontova P, Alonso-Carrillo D, Carreira-Barral I, Torres AA, García-Valverde M, Benítez-García C, Pérez-Tomás R, Quesada R, Soto-Cerrato V: **A novel late-stage autophagy inhibitor that efficiently targets lysosomes inducing potent cytotoxic and sensitizing effects in lung cancer**. *Cancers* 2022, **14**:3387, https://doi.org/10.3390/cancers14143387.

36. Pérez-Hernández M, Arias A, Martínez-García D, Pérez-Tomás R, Quesada R, Soto-Cerrato V: **Targeting autophagy for cancer treatment and tumor chemosensitization**. *Cancers* 2019, **11**:1599, https://doi.org/10.3390/cancers11101599.

37. Das CK, Mandal M, Kögel D: **Pro-survival autophagy and cancer cell resistance to therapy**. *Cancer Metastasis Rev* 2018, **37**:749–766, https://doi.org/10.1007/s10555-018-9727-z.

38. * Ahmad M, Roy NJ, Singh A, Mondal D, Mondal A, Vijayakanth T, Lahiri M, Talukdar P: **Photocontrolled activation of doubly o-nitrobenzyl-protected small molecule benzimidazoles leads to cancer cell death**. *Chem Sci* 2023, **14**:8897–8904, https://doi.org/10.1039/D3SC01786A.
One of the few studies of photo-modulated transport in cells; demonstrated photoinduced cytotoxicity of photocaged anionophores.

39. ** Chen Z, Xie X, Jia C, Zhong Q, Zhang Q, Luo D, Cao Y, Mu Y, Ren C: **Concentration-driven evolution of adaptive artificial ion channels or nanopores with specific anticancer activities**. *Angew Chem, Int Ed* 2024, **63**, e202318811, https://doi.org/10.1002/anie.202318811.
Self-assembled artificial channels which show an excellent selectivity for cancerous cells over normal cells, outperforming current chemotherapeutic agent doxorubicin.

40. Salunke SB, Save SN, Roy NJ, Naorem R, Sharma S, Talukdar P: **Bisindole-based small molecules as transmembrane anion transporters and potential anticancer agents**. *Org Biomol Chem* 2024, **22**:4987–4992, https://doi.org/10.1039/D4OB00554F.

41. Pradhan NP, Namdev KR, Srivastava A: **Molecular clips with spatially proximal urea residues for efficient transmembrane co-transport of H^+/Cl^- ions**. *Org Biomol Chem* 2024, **22**:74–79, https://doi.org/10.1039/D3OB01431B.

42. McCarron A, Parsons D, Donnelley M: **Animal and cell culture models for cystic fibrosis: which model is right for your application?** *Am J Pathol* 2021, **191**:228–242, https://doi.org/10.1016/j.ajpath.2020.10.017.