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The Strong Antibacterial Properties of Anion Transporters: A Result of Depolarization and Weakening of the Bacterial Membrane

*Claude R. Elie, Guillaume David and Andreea R. Schmitzer**

Département de Chimie, Université de Montréal, CP 6128 Succursale Centre Ville, H3C 3J7, Montreal, Quebec, Canada. Correspondence should be addressed to A.R.S (ar. schmitzer@umontreal.ca)

ABSTRACT. The development of low molecular weight anionophores is an emerging topic in chemistry as the need for these compounds increases with the continuous discovery of pathologies involving anomalies in anion transport processes. Development of new concepts to initiate anion imbalance in living cells while fighting multidrug-resistant bacteria is a paramount topic. In this study, three series of compounds including N,N'-diphenylethynylbenzyl benzimidazolium salts (**1-2),** 1,1'-(pyridine-2,6-diyl)bis(3-(4-(phenylethynyl)benzyl)-1H-benzo[d]imidazol-3-ium) salts (**3-5)** and 1,1'-(pyridine-2,6-diylbis(methylene))bis(3-(4-(phenyl ethynyl)benzyl)-1H-benzo[d]imidazol-3-ium) salts **(6-8)** displaying high antimicrobial activity and low toxicity against human cells were designed, synthesized and studied. The most potent compound displayed micromolar minimal inhibitory concentrations in different gram-negative

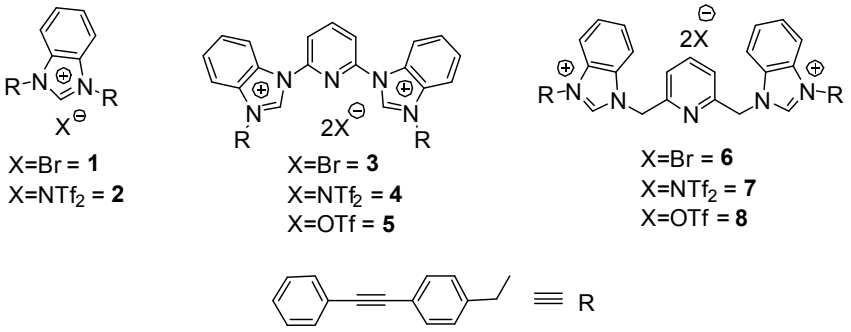
and gram-positive bacteria, while its hemolytic activity remained around 10% or less, even after a prolonged period of exposure. The mechanism of action of these benzimidazolium salts on bacterial membrane was assessed by bioanalytical techniques including assays in model membrane liposomes, membrane depolarization studies and scanning electron microscopy (SEM) in living bacteria.

Introduction

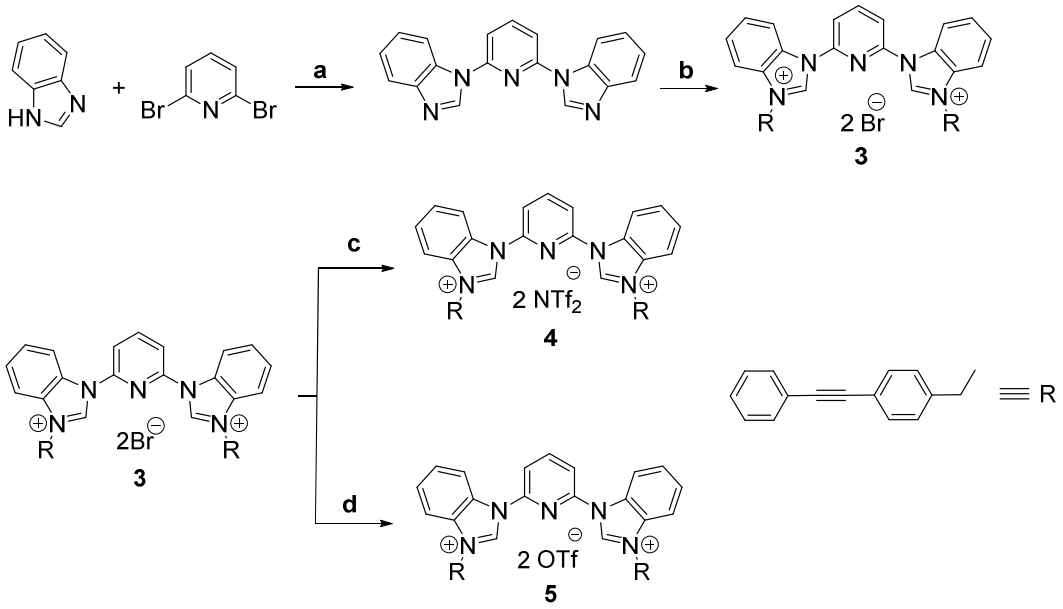
The study of synthetic anion transporters remains an important subject in medical research today. This interest comes from the considerable number of diseases originating in dysfunctions of natural anion channels.[1] In 1999, Sessler and Allen were the first to hypothesize that by restoring the efflux of chloride ions in cell membranes through a synthetic channel may be useful to treat cystic fibrosis.[2] This assumption marked the beginning of many breakthroughs in this field. Voyer et al. described later on a peptide-based chloride transporter, able to penetrate the cell membrane in a non-selective way.[3] In the same vein, Gale et al. developed prodigiosin analogs, natural amphiphilic products extracted from microorganisms such as *Streptomyces* and *Serratia*, that have showed anticancer activity, linked to a transport process of chloride ions across cell membranes.[4] More recently Quesada et al. also reported the *in vivo* cytotoxic effects of synthetic tambjamine analogs and they established a relationship between the cancer cell death and the membrane disruption, as a result of an electrolyte imbalance mechanism, involving nitrate and chloride anions.[5] The anionophoric properties of these natural compounds are obvious, but still poorly understood from a mechanistic, kinetic and thermodynamic perspective. Even if the main application of these synthetic transporters envisage the death of cancer cells, other types of pathogens, such as bacteria, could be targeted, knowing that 70% of nosocomial infections are becoming resistant to the commonly prescribed antibiotics in hospitals.[6] Several

synthetic amphiphilic transporters described in the literature are inspired by the natural antimicrobial peptides (AMPs), well known for their activity in bacteria, viruses, fungi and even cancer cells.[7] However, the synthesis of antimicrobial peptides often requires several synthetic steps and generally results in poor yields.[8] Moreover, their poor stability towards proteases is a clear concern. Besides, the major challenge to successfully mimic these antimicrobial peptides remains their low toxicity *in vivo*.[8a,b] For all these reasons we have developed synthetic anionophores based on benzimidazolium salts (Scheme 1), possesing the amphiphilic characteristics of AMPs, endowed with a powerful antibacterial activity and low toxicity for normal human cells. Since these salts do not contain peptidic bonds, they may be insensitive to the action of peptidases and therefore they should retain their biological activity under physiological media. Herein we demonstrate their anion transport properties and we provide evidence of their antibacterial action by membrane depolarization assays and SEM imaging of bacteria in the presence of these salts. Based on the amphiphilic scaffold of compounds **1** and **2** we had previously reported as potent anionophores,[9,10] new analogs were designed, synthesized and studied, all possessing the benzimidazolium unit and the phenylethylbenzyl substituents. Since others biscationic antimicrobials generally show a lower cytoxicity than the monocationic analogs,[8b,c] we decided to couple two benzimidazolium cations through a pyridyl or a lutidine unit. The pyridyl and lutidine units can act as protonation sites but could also participate in binding of different anions in aqueous medium. Details of the synthesis of these new compounds are provided in Schemes 2 and 3 and the Electronic Supporting Information (ESI).

Scheme 1. Benzimidazolium salts studied for transmembrane anionophoric properties

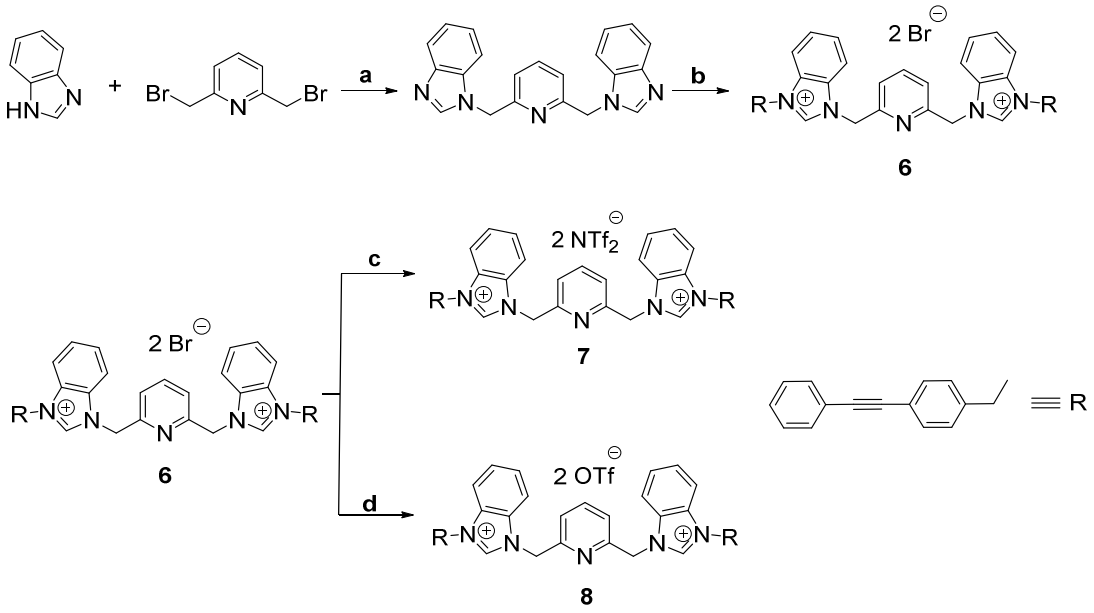


Scheme 2. Synthesis of pyridine-bridged bis-benzimidazolium salts 3-5*



*Reagents and conditions: (a) NaH, DMF (anh), 155 °C, 96 h. (b) R-Br, ACN, 85 °C, 72 h. (c) (1) $LiNTf_2$, MeOH/ACN, 85 °C, 12 h; (2) H_2O, 100 °C, 12 h. (d) LiOTf, MeOH/ACN, 85 °C, 12 h; (2) H_2O, 100 °C, 12 h.

Scheme 3. Synthesis of lutidine-bridged bis-benzimidazolium salts 6-7**



**Reagents and conditions: (a) KOH, TBAB, toluene/H_2O, 25 °C, 12 h. (b) R-Br, ACN, 85 °C, 72 h. (c) (1) $LiNTf_2$, MeOH/ACN, 85 °C, 12 h; (2) H_2O, 100 °C, 12 h. (d) LiOTf, MeOH/ACN, 85 °C, 12 h; (2) H_2O, 100 °C, 12 h.

Results and discussion

Anion transport assays

The chloride transport ability of all synthetic benzimidazolium salts was first evaluated in Egg yolk phosphatidylcholine (EYPC) liposomes. The transmembrane anion transport activity of the compounds was evaluated by fluorimetry, as previously reported.[8-14] EYPC vesicles containing 2 mM lucigenin, which fluorescence is inhibited by the presence of chloride anions, were loaded with a NaCl solution and suspended in a $NaNO_3$ buffer. The chloride efflux from the liposomes was followed over 300 seconds by monitoring lucigenin's fluorescence when the transporter, dissolved in MeOH, was added to the extravesicular medium. At the end of the assay, the liposomes were lysed with Triton-X, in order to normalize the 100% efflux of chloride. A dose-

dependent relationship was observed between the activity of the benzimidazolium salts and their concentration, thus making possible to calculate an EC_{50} reported in Table 1. Salts **6**, **7** and **8** possess better transport properties, with lower EC_{50} compared with their benzimidazolium salts analogs. In particular, compound **8** induces a 50% chloride ion efflux at a ratio of 0.46 mol% (with respect to the lipid). The presence of the methylene groups increases the flexibility of compounds **6**, **7** and **8**, which may allow them to adopt different conformations during the transport process when they penetrate the bilayer.

Table 1. Transport data determined by Hill analysis. EC_{50} represents the concentration (mol.% with respect to lipid) of ionophore required to mediate 50% chloride efflux after 250 sec.

Compounds	Counter-anion	$EC_{50,250s}$ (mol.%)
1	Br^-	14.8
2	NTf_2^-	5.8*
3	Br^-	24
4	NTf_2^-	6.9
5	OTf^-	12.0
6	Br^-	14
7	NTf_2^-	5.6
8	OTf^-	0.46

the value of 2.9% previously reported for **2 was calculated in different lipid concentration conditions*[15c]

We then investigated the transport mechanism of the more potent benzimidazolium salts **6-8**. First, the chloride ion efflux outside of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) liposomes was followed using the same lucigenin fluorescence assay, as previously reported in the literature.[10, 15] The DPPC liposomes were first loaded with a solution of NaCl containing lucigenin (2 mM), and then suspended in a nitrate solution. The benzimidazolium salt, diluted in methanol, was added to the extravesicular solution and the efflux of Cl^- from the liposomes at temperatures above and below DPPC's transition phase (41°C) was monitored. The 100% chloride release was calibrated by lysing the liposomes with Triton-X, after 300 seconds. The efficiency of a mobile anion transporter, limited by its diffusion through the membranes, is generally considerably reduced when the DPPC liposome bilayer is maintained in the gel-state (at temperatures inferior to 41 °C), whereas in the case of the formation of a transmembrane channel, no or only a small alteration of its efficiency is observed below the bilayer's gel-to-liquid crystalline phase transition temperature.[10,15]

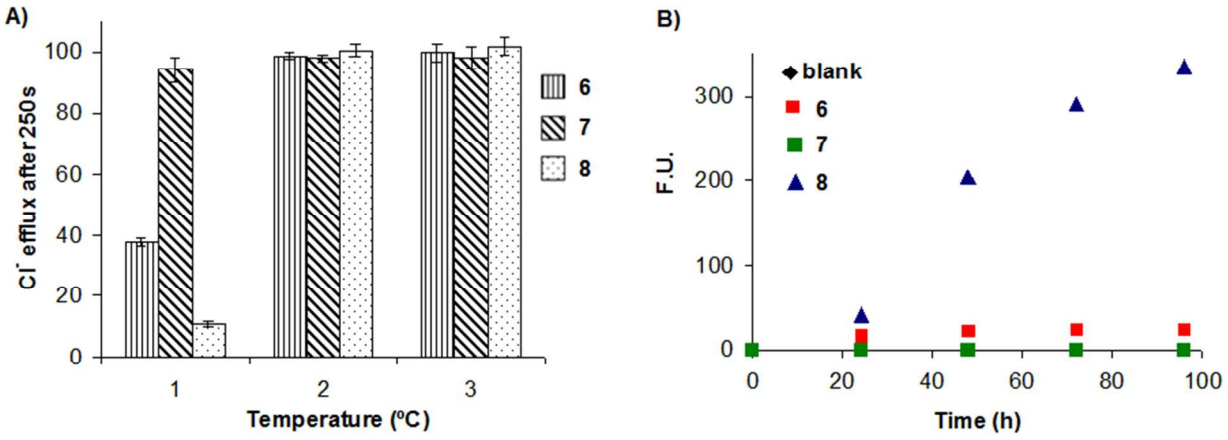


Figure 1. A) Chloride efflux in DPPC liposomes at 35°C, 40°C and 45°C. The data at each temperature are obtained by using 10 mol% of benzimidazolium salts **6-8** (relative to 6.25 mM DPPC concentration). The data at each temperature are the average of three series of

measurements. B) Increase of the lucigenin's fluorescence in U-tube tests in the presence of compounds **6**-**8** (a zoom is provided for **6**, **7** and the blank in the ESI).

According to our previous results[15a] and those shown in Figure 1A, compound **8** shows a behaviour characteristic to a mobile carrier. It is interesting to note that in this regard, the OTf salt (**8**) that shows a temperature dependent activity acts as a mobile carrier, while the NTf_2^- salt (**7**) for which the transport activity is only partially affected by the temperature, rather forms transmembrane channels for the same benzimidazolium cation. The bromide salt (**6**) appears meanwhile to obey an intermediate mechanism, suggesting that the nature of the counter-anion of these benzimidazolium salts alone may be sufficient to change the transport mechanism. Gokel et al. have also previously reported a switch from a transmembrane synthetic channel anion transport mechanism to mobile carriers over a certain concentration range of dipicolineamides.[16] At the same time, the change of the transport mechanism of these benzimidazolium salts could be due to chaotropic and chosmotropic factors modulated by the nature of the counter-anions, as different partition coefficients of the monomer, the aggregates and the association equilibrium in water and in the phospholipid bilayer. Nevertheless a broader range of counter-anions is under investigation in order to get a better insight of the influence of the nature of the counter-anion on the mechanism.

Among different other studies that can confirm the mechanism of transport,[17] we decided to perform additional U-tube tests for compounds **6**-**8**, as previously described by our group.[18] The transport of nitrate as lucigenin's counter-anion in a U-tube involves the translocation of the lucigenin across the organic phase that separates two aqueous phases. The U-tube experiments were performed with compounds **6**-**8** incorporated in the chloroform phase, by monitoring the increase of lucigenin's fluorescence in the receiving phase. As the formation of transmembrane

channels is impossible in the bulk organic chloroform phase, the translocation of lucigenin from one aqueous to the other aqueous phase is the result of a mobile transport mechanism of the nitrate anions, and thus of the lucigenin. As shown in Figure 1B, compound **8** actively transports nitrate anions, while a very slow transport mechanism and an intermediate process can be observed for compounds **7** and **6** respectively. These results allow us to affirm that **8** acts as an active mobile anion transporter.

Antibacterial studies

Liposomes are model membranes that historically have been indispensable for the development of our understanding of biological membranes. Nevertheless, bacterial cell membranes are structurally much more complex than liposomes, with lipid bilayers containing other components such as proteins, lipopolysaccharides and many other materials that can interfere with the activity of the studied compound. The antibacterial activity of the benzimidazolium salts was thus evaluated in the presence of gram-negative *E. coli* and gram-positive *B. thuringiensis* strain. The minimal inhibitory concentrations (MICs), presented in Table 2, have been determined as described in the ESI. At a first glance, the benzimidazolium salts **6-8** have a more pronounced antibacterial activity. In this respect, following the same pattern as the activity in liposomes, compound **8**, showed an improved antibacterial activity towards *E. coli*. The limited activity of the other benzimidazolium salts against *E.coli DH5a* could however come from the presence of an efflux pump protein (TolC) in this stain, which may limit the mass aggregation and accumulation of the ionophore in the bacteria.[19, 20] Indeed, it is well known that this transmembrane protein plays a role of protection, by limiting the accumulation of intruding molecules in the bacteria, including toxins and antimicrobial agents,

through their expulsion from the bacteria.[19, 20] In order to verify whether inhibiting this protective mechanism by disabling the TolC protein, it is possible to increase the activity of the benzimidazolium salts, we performed the same study in a mutant *E. coli* strain *SK037*, for which TolC has been deactivated.[21] As shown in Table 2, *E. coli SK037* presents an increased sensitivity to the benzimidazolium salts with MICs 2.5 to 10 times lower than those required to inhibit the growth of the wild type *E.coli DH5a* strain. Furthermore, the gram-positive strain *B. thuringiensis* presents even a greater sensitivity to all the benzimidazolium salts tested. The membrane of *B. thuringiensis* is devoid of an outer wall and efflux pumps, which seems to explain how, with a less robust and less protected membrane, this bacterial strain, can possess a higher sensitivity to the antibacterial effects of the studied ionophores.[22] Compounds **6**-**8** possess MICs in the micromolar range, compared to all the imidazolium-based dications reported in the literature with structural similarity which present antimicrobial properties, but in the milimolar ranges[8b].

Table 2. Antibacterial activity against gram negative and gram positive bacteria

Compounds	Minimal Inhibitory Concentrations (μM)		
	E.coli		*B. thuringiensis*
	DH5α	*SK037*	*HD73*
1	>100	>100	10
2	>100	25	10
3	>100	>100	10
4	>100	>100	10
5	>100	>100	10
6	100	10	10
7	50	10	10
8	25	10	2

Haemolytic assays

The antibacterial results are even more encouraging when they are associated with the very low cytotoxicity of these benzimidazolium salts to human cells. Haemolysis assays were performed over 1 hour and 24 hours (Figure 2). It is interesting to notice that compound **8**, which shows the best antibacterial potential in all the strains studied (MIC 2-25 μM) also has a haemolytic activity inferior to 10% after 1 hour and only 10 % after 24 hours on human red blood cells at a concentration of 25 μM. The toxicity of **8** on human cells therefore remains very low in its bacteriostatic concentrations, even after a prolonged exposure period of 24 hours.

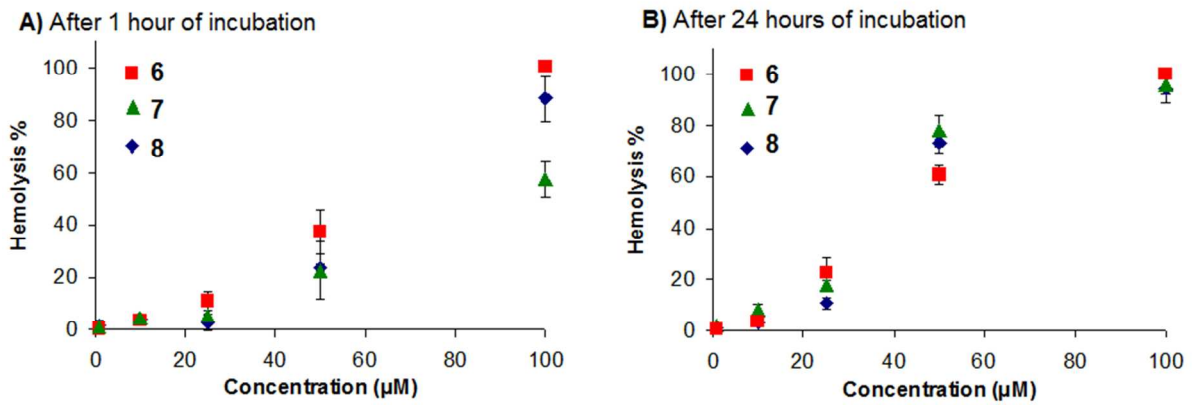


Figure 2. Haemolytic activities of compounds **6**, **7** and **8** after A) 1 hour of incubation and B) 24 hours of incubation. The points represent the average of six independent measurements.

Depolarization of the bacterial membrane

Having demonstrated the bacteriostatic capacity of these benzimidazolium salts, we decided to get better insights into the mechanism of the bacterial growth inhibition induced by these ionophores. The starting hypothesis was that an alteration of the permeability of the membrane of the bacteria causes an efflux of ions, such as observed in the liposomes. This ion imbalance should lead to a change in the membrane potential that provides the necessary energy and ensures the proper distribution, on both sides of the membrane, of the growth factors involved in the cell division of the bacteria.[20,23,24] Therefore, the depolarization of the cell membrane would be sufficient to seriously impair or inhibit the bacterial growth. Furthermore, given that the transmembrane potential of the bacteria is high (-120 to -200 mV) compared with the potential of eukaryotic cells (-60 to -80 mV) and erythrocytes (-8.4 mV), it may be possible to target bacterial growth selectively through their depolarization, without affecting dramatically the integrity of the eukaryotic cells and red blood cells[20,23,24]. Moreover, the transmembrane potential perturbation in bacteria is associated to the modification a vital mechanisms, including cell

division, emergence of resistance and energy formation.[20,23,24] In order to verify this hypothesis we incorporated a sensitive fluorescent dye ($diSC_2 5$) in the outer membrane of *E.coli (SK037)* bacteria. The $diSC_2 5$ is a fluorophore possessing the particularity of having a quenched fluorescence in the intact and polarized lipid membranes, and which becomes fluorescent upon returning to the aqueous extracellular medium, when the membrane is irreversibly damaged and depolarized.[23] In other words, while the decrease of fluorescence of $diSC_2 5$ signifies a membrane permeabilization, and hence the incorporation of the dye into the bilayer, the increase of the fluorescence signal is associated with a depolarization of the membrane and therefore the bacterial death.[20,26-28] The first step of this assay consisted of incubating a suspension of bacteria with $diSC_2 5$ in order to allow its incorporation in the outer membrane of the bacteria, until the fluorescence decreases to a stable baseline. Then, upon the addition of a solution of benzimidazolium salt in DMSO at different concentrations, the fluorescence of $diSC_2 5$ was followed over a period of ~2 hours. In figure 3, compounds **2** and **6** cause, even at a concentration corresponding to 40 times their MICs, only a slight decrease in the fluorescence of $diSC_2 5$. This result suggests that these benzimidazolium salts transiently increase the permeability of the outer membrane of the bacteria, allowing the incorporation of a small fraction of $diSC_2 5$. This increase in membrane permeability is however not associated with the depolarization of the membrane and thus does not lead to the death of the bacteria, even at high concentration of the anionophore after 2 hours. This result leads us to believe that **2** and **6** induce transient damage of the bacterial membrane, damage that the cell can quickly repair in order to prevent cytolysis. This hypothesis could allow the use these benzimidazolium salts and another antibacterial agents in synergy at a low dose, to temporarily weaken the bacteria, while acting concurrently on other targets, leading to the death of the microorganism. In the case of **7** and **8**,

compounds having shown the best EC_{50} and MIC, it is not surprising to observe a strong modification of the membrane during the depolarization tests. The fluorescence of diSC$_2$5 previously incubated with the *E.coli* increases upon addition of **7** and **8**, which suggests a sustained membrane permeabilization, also accompanied by the depolarization of the membrane and probably the cell death. The depolarization tests show that irreversible damage can be caused to the membrane of the pathogenic bacteria *E.coli*, especially in the presence of compound **8**.

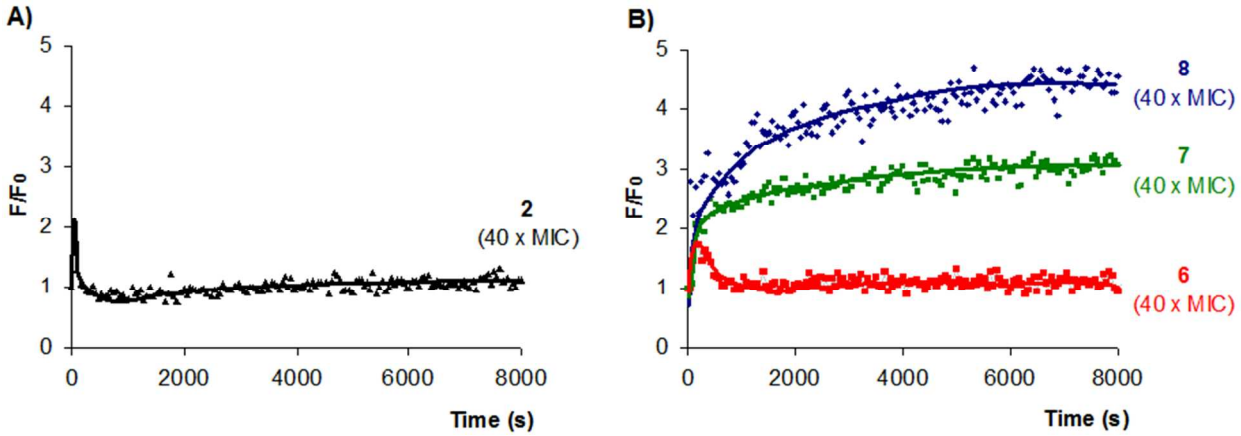


Figure 3. Effects of the antimicrobial benzimidazolium salts on the fluorescence intensity of diSC$_2$5 in the presence of the *E.coli (SK037)*. A) Benzimidazolium salts were added at 70 s, at a final concentration of 1 mM for **2** and B) 0.4 mM for **6**, **7** and **8** corresponding to 40 times their MIC with respect to *E.coli (SK037)*.

Modification of membrane morphology

The modification of *E. coli (SK037)* membrane in the presence of anionophore **8** was followed by SEM microscopy. As shown in Figure 4 (i, ii), untreated bacteria, which typically take the form of elongated cells, have a firm surface and a swollen appearance, and show an external membrane with a visible texture and surface, with multiple exocellular appendage forming a complex biofilm. In the presence of **8**, at a concentration corresponding to 0.5 time their MIC,

the appearance and distribution of bacteria after 12 and 24 hours change drastically (Figure 4 iii and iv). The size and the diameter of the bacteria are reduced, and concave collapses in the membrane can be observed while a large amount of debris, presumably the cell contents, are present around the few intact bacteria (Figure 4 iii, iv). This suggests that compound **8** provokes the leakage of the bacterial content to the extracellular area. It can also be observed that the surface of the bacteria is less well defined and a modification of the contour and the texture can be observed. The dose-dependent damage of the bacteria was confirmed with samples treated with higher concentrations of **8**, corresponding to the MIC (with respect to inoculums concentration), as more accentuated morphology changes were observed after 12 h and 24 h (Figure 4 v, vi).

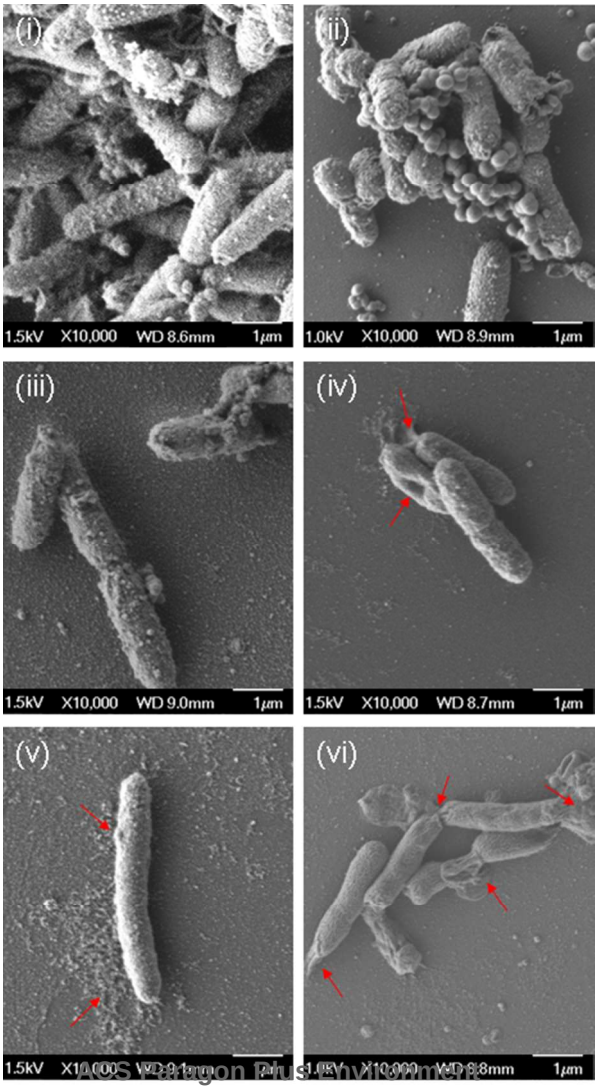


Figure 4. Change of the cell morphology and membrane damage by field emission scanning electron microscope. E.coli (SK037) after 12h (i) and 24 h(ii) (control). E.coli (SK037) cells treated with **8** at a 0.5xMIC concentration after 12 h (iii) and 24h (iv). E.coli (SK037) cells treated with **8** at the MIC concentration after 12 h (v) and 24h (vi).

Conclusion

In conclusion, we present here a class of small synthetic antimicrobial molecules, containing in their structure a benzimidazolium unit, and which are able to mimic the natural process of the anionic transport through both transmembrane channel and simultaneous mobile carrier mechanisms, depending on the nature of their counter-anion. Among the benzimidazolium salts studied here, we identified compound **8**, which presents interesting antibacterial properties as a result of its ability to induce an electrolytic imbalance and to disrupt the integrity and the potential of the bacterial membranes. At the same time this compound presents a low toxicity to human cells in bacteriostatic range concentrations, representing a new potential antimicrobial agent and an interesting paradigm that could be further optimized for therapeutic purposes.

EXPERIMENTAL SECTION

General Procedures. All chemicals were purchased from Aldrich Chemicals in their highest purity and used without further purification. Methanol-d_4, Dimethyl sulfoxide-d_6 or Chloroform-d_3 were also purchased from Aldrich Chemicals. All solvents were purchased from EDM and liquid reagents were degassed by bubbling nitrogen for 15 min before each use. NMR experiments were recorded on Bruker Avance spectrometers at 400 MHz or 100MHz. All NMR experiments were obtained by the use of the sequence commercially available on Brucker

spectrometer. Coupling constants are given in Hertz (Hz) and chemical shifts are given in ppm (δ) measured relative to residual solvent. Data are reported as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, quin = quintuplet, m = multiplet). Mass spectra analyses were obtained at the Université de Montréal Mass Spectrometry Facility and were recorded on a Mass spectrometer LC-LCQ Advantage (Ion trap) Thermo Scientific with ESI ionization source (direct injection), a mass spectrometer LC-TOF Agilent Technologies with ESI ionization source (direct injection) and on a mass spectrometer LC-MS Thermo Scientific. The purity of the compounds was determined by high performance liquid chromatography (HPLC). Purity of all final compounds was 95% or higher. The instrument was a Thermo Scientific system, on a Kinetex C8 (Phenomenex), 2.6 μm particle size (50 mm × 3.0 mm), eluent A: H_2O + 0.1% formic acid and eluent B: MeOH. The gradient started at 50% B and 95% B was reached in 7 minutes, kept for 1 minute at 95% B and go back to 50% within 0.5 min, for a total analysis time of 12 minutes. The flow rate was 0.35 mL/min and the detection performed at 300 nm. Fluorimetric studies were performed on a Varian Cary Eclipse Fluorescence spectrophotometer equipped with a temperature controller. The antimicrobial and haemolytic assays were performed on a Fluostar Optima plate reader. The Escherichia coli (DH5α and SK037) and Baccilus thuringiensis (HD73) strains were provided by prof. J. Pelletier, Chemistry Department, Universite de Montreal.

2,6-bis(1H-benzo[d]imidazol-1-yl)pyridine: Under nitrogen atmosphere, to a suspension of 1.5 g (13 mmoles) of benzimidazole and 0.43 g (18 mmoles) of NaH in DMF (anh), 1.5 g (6.3 mmoles) of dibromopyridine solution in DMF (anh) was added and the mixture was heated under reflux for 72 h. Then the mixture was cooled to room temperature and 100 mL of distilled water

were added to the solution. The residue obtained was filtered and washed with isopropanol. The crude product was dried in an oven to obtain 0.77 g (Yield 40%) of an orange solid. ESI-MS: m/z [M+H]+ (C19H6N5)+ calc.311.3, found 312.3. ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm = 8.99 (s, 2 H), 8.32 (t, J=8.2 Hz, 1 H), 8.28-8.26 (m, 2 H), 7.91 (d, J=8 Hz, 2 H), 7.63-7.61 (m, 2 H), 7.45-7.43 (m, 4 H).

1,1'-(pyridine-2,6-diyl)bis(3-(4-(phenylethynyl)benzyl)-1H-benzo[d]imidazol-3-ium) dibromide (**3**): 0.1935 g (0.623 mmoles) of 2,6-bis(1H-benzo[d]imidazol-1-yl)pyridine and 0.3551 g (1.31 mmoles) of (4-phenylethynyl)benzyl bromide are dissolved in 30 mL of ACN. The mixture is stirred under reflux for 72 h and the resulting precipitate is filtered, washed with 10 mL of ACN and dried in vacuuo. 0.32 g (61% Rdt) of a white powder was obtained. ESI-MS: m/z [M-H]+ (C49H34N5)+ calc. : 692.3, found : 692.3. ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm = 8.73 (t, *J*=8 Hz, 1 H), 8.52-8.50 (m, 2 H), 8.40 (d, *J*=8 Hz, 2 H), 8.05-8.04 (m, 2 H), 7.82-7.80 (m, 4 H), 7.66-7.65 (m, 8H), 7.51 (m, 4H), 7.40-7.39 (m, 6H), 6.02 (s, 4H). ^{13}C NMR (75 MHz, Dimethyl sulfoxide-d_6): δ ppm = 143.50, 138.25, 134.83, 132.40, 131.67, 131.86, 131.60, 129.46, 129.26, 129.19, 127.35, 123.17, 122.45, 122.08, 117.82, 114.57, 114.47, 113.56, 90.56, 89.14, 50.23.

1,1'-(pyridine-2,6-diyl)bis(3-(4-(phenylethynyl)benzyl)-1H-benzo[d]imidazol-3-ium) bis(trifluoromethane)sulfonimide (**4**): 100 mg (0.12 mmoles) of **3** are dissolved in a mixture of MeOH/ACN (50 :50). Then, 258 mg (0.90 mmoles) of $LiNTf_2$ are added to 10 mL of methanol before being poured onto the solution containing the imidazolium salt. The resulting mixture is then brought to 85°C and stirred with a magnetic stir bar during 12 hours. After evaporating the solvent under reduced pressure, 40 mL of distilled water are added to the powder obtained. The mixture is heated to 100ºC for 12 hours under magnetic stirring, and then filtered

on a fritted glass. The raw product is then dried in an oven, in order to obtain 96 mg of a white powder (95% yield). ESI-MS: m/z $[M]^-$ $(C_2F_6NO_4S_2)^-$ calculated : 280.1, founded : 280.0. ^{1}H NMR (400 MHz, Chloroform-d_3) δ ppm = 10.23 (s, 2 H), 8.55 (s, 1 H), 8.21 (q, *J*=8 Hz, 4 H), 7.77 (t, *J*=8 Hz, 2 H), 7.67-7.52 (m, 15 H), 7.37-7.36 (m, 5 H), 7.18 (s, 4 H), 5.81 (s, 4 H). ^{13}C NMR (75 MHz, Dimethyl sulfoxide-d_6): δ ppm = 146.89, 145.04, 145.00, 134.30, 132.35, 131.89, 130.29, 129.49, 129.38, 129.27, 128.54, 128.04, 126.33, 122.43, 122.07, 118.53, 117.80, 116.45, 113.53, 90.82, 89.12, 50.77.

1,1'-(pyridine-2,6-diyl)bis(3-(4-(phenylethynyl)benzyl)-1H-benzo[d]imidazol-3-ium) bis(trifluoromethane)sulfonate (**5**): 200 mg (0.23 mmoles) of **3** are dissolved in a mixture of MeOH/ACN (50 :50). Then, 270 mg (1.76 mmoles) of LiOTf were added to 10 mL of methanol before being poured onto the solution containing the imidazolium salt. The resulting mixture is then brought to 85°C and stirred with a magnetic stir bar during 12 hours. After evaporating the solvent under reduced pressure, 40 mL of distilled water are added to the powder obtained. The mixture is heated to 100ºC for 12 hours under magnetic stirring, and then filtered on a fritted glass. The raw product is then dried in an oven, in order to obtain 220 mg of a rosy brown powder (96% yield). ESI-MS: m/z $[M]^-$ $(CF_3O_3S)^-$ calculated : 149.6, found : 149.0. ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm = 8.72 (t, *J*=8 Hz, 1 H), 8.52 (s, 2 H), 8.39 (d, *J*=8 Hz, 2 H), 8.03 (s, 2 H), 7.80 (s, 4 H), 7.64 (s, 8H), 7.52 (s, 4H), 7.49 (m, 8H), 6.00 (s, 4H). ^{13}C NMR (75 MHz, Dimethyl sulfoxide-d_6) δ ppm = 146.88, 145.01, 143.94, 134.31, 132.36, 131.88, 131.69, 130.30, 129.50, 129.39, 129.28, 129.54, 128.05, 123.30, 122.43, 118.99, 118.54, 116.47, 114.87, 90.62, 89.15, 50.77.

2,6-bis((1H-benzo[d]imidazol-1-yl)methyl)pyridine: To a suspension of 0.44 g (3.76 mmoles) of benzimidazole and 0.84 g (15.0 mmoles) of KOH in water 0.0417 g (0.13 mmoles)

of TBAB solution in toluene was added and the mixture was stirred vigorously before adding 0.5 g (1.88 mmoles) of 2,6-dibromomethylpyridine. Then the mixture was abandoned to room temperature overnight before evaporate toluene. Then 100 mL of water was added to the residue before stirring 5 more minutes, then filtered and washed with water. The crude product was dried in an oven to obtain 0.905 g (71% yield) of an white solid. ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm = 8.35 (s, 2 H), 7.74 (t, J=8 Hz, 1 H), 7.66 (d, *J*=8 Hz, 2 H), 7.40 (d, *J*=8 Hz, 2 H), 7.20 - 7.11 (m, 6 H), 5.55 (s, 4 H).

1,1'-(pyridine-2,6-diylbis(methylene))bis(3-(4-(phenylethynyl)benzyl)-1H-benzo[d]imidazol-3-ium) dibromide (**6**) : 0.50 g (1.47 mmoles) of 2,6-bis(1H-benzo[d]imidazol-1-yl)pyridine and 0.879 g (3.24 mmoles) of (4-phenylethynyl)benzyl bromide are dissolved in 30 mL of ACN. The mixture is stirred under reflux for 72 h and the resulting precipitate is filtered, washed with 10 mL of ACN, and dried in vacuo. 0.91 g (86% Rdt) of a white powder was obtained. ESI-MS: m/z $[M-H]^+$ $(C_{51}H_{38}N_5)^+$ calc. 721.4, found 720.3. ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm 8.01 (t, *J*=8 Hz, 1 H), 7.86 (d, *J*=8 Hz, 2 H), 7.66 – 7.36 (m, 26 H), 5.82 (s, 4 H), 5.65 (s, 4 H). ^{13}C NMR (75 MHz, Dimethyl sulfoxide-d_6) δ ppm = 153.57, 143.55, 139.40, 134.95, 132.34, 131.86, 131.69, 130.89, 129.46, 129.27, 129.17, 129.17, 127.03, 123.12, 123.08, 122.41, 114.33, 114.16, 90.54, 89.13, 51.26, 50.00.

1,1'-(pyridine-2,6-diylbis(methylene))bis(3-(4-(phenylethynyl)benzyl)-1H-benzo[d]imidazol-3-ium) bis(trifluoromethane)sulfonimide (**7**) : 0.712 g (0.82 mmoles) of **6** are dissolved in a mixture of MeOH/ACN (50 :50). Then, 1.76 g (6.11 mmoles) of $LiNTf_2$ are added to 10 mL of methanol before being poured onto the solution containing the imidazolium salt. The resulting mixture is then brought to 85°C and stirred with a magnetic stir bar during 12 hours. After evaporating the solvent under reduced pressure, 40 mL of distilled water are added

to the powder obtained. The mixture is heated to 100ºC for 12 hours under magnetic stirring, and then filtered on a fritted glass. The raw product is then dried in an oven, in order to obtain 0.94 g of a white powder (90% yield). ESI-MS: m/z [M]-$(C_2F_6NO_4S_2)^-$ calc. 280.1, found 280.0. ^{1}H NMR (400 MHz, Methanol-d^4) δ ppm 8.01 (t, *J*=8 Hz, 1 H), 7.85 (d, *J*=8 Hz, 2 H), 7.64 – 7.38 (m, 28 H), 5.82 (s, 4 H), 5.65 (s, 4 H). ^{13}C NMR (75 MHz, Dimethyl sulfoxide-d_6): δ ppm = 153.61, 143.61, 139.43, 134.77, 132.39, 131.84, 130.98, 129.25, 129.04, 127.21, 127.05, 123.20, 123.01, 122.41, 122.08, 117.81, 114.24, 114.11, 113.55, 90.59, 89.05, 51.35, 50.08.

1,1'-(pyridine-2,6-diylbis(methylene))bis(3-(4-(phenylethynyl)benzyl)-1H benzo[d]imidazol-3-ium) bis(trifluoromethane)sulfonate (**8**) : 100 mg (0.12 mmoles) of **6** are dissolved in a mixture of MeOH/ACN (50 :50). Then, 140 mg (0.90 mmoles) of LiOTf are added to 10 mL of methanol before being poured onto the solution containing the imidazolium salt. The resulting mixture is then brought to 85°C and stirred with a magnetic stir bar during 12 hours. After evaporating the solvent under reduced pressure, 40 mL of distilled water are added to the powder obtained. The mixture is heated to 100ºC for 12 hours under magnetic stirring, and then filtered on a fritted glass. The raw product is then dried in an oven, in order to obtain 0.90 g of a white powder (76% yield). ESI-MS: m/z [M]- $(CF_3O_3S)^-$ calc. 149.6, found 149.0. ^{1}H NMR (400 MHz, Methanol-d_4) δ ppm = 8.02 (t, *J*=8 Hz, 1 H), 7.88-7.86 (m, 2H), 7.66-7.63 (m, 4 H), 7.50-7.46 (m, 10 H), 7.40-7.37 (m, 8 H), 5.82 (s, 4H), 5.67 (s, 4H). ^{13}C NMR (75 MHz, Dimethylsulfoxide-d_6): δ 153.60, 143.59, 139.45, 134.80, 132.40, 131.85, 131.77, 130.95, 129.27, 129.05, 127.22, 127.06, 123.27, 123.17, 123.02, 122.40, 119.00, 114.25, 114.12, 90.59, 89.07, 51.33, 50.06.

Preparation of EYPC large unilamellar vesicles (LUVs) for lucigenin based assays. A lipid film was formed by evaporating a chloroform solution containing 50 mg of EYPC under

reduced pressure at 25°C. The lipid film was then dried in vacuo at room temperature for at least 2 hours. The lipid film was then hydrated with 1 mL of a 2 mM lucigenin solution containing NaCl (100 mM), and sodium phosphate salt (10 mM, pH = 6.4). The obtained suspension was subjected to 20 freeze/thaw cycles (1 cycle = 1 minute at -20°C followed by 1 minute at 37°C). The mixture was vortexed for 1 minute after every cycle to help the hydration. The solution was then extruded through a 100 nm polycarbonate membrane 21 times until the solution was transparent and passed down a Sephadex G-25 column to remove extravesicular lucigenin dye. The eluant was a solution containing 100 mM of NaCl and 10 mM of sodium phosphate salt. 10.4 mL of liposome solution were isolated after gel filtration. The stock solution was 6.25 mM in lipid, assuming all EYPC was incorporated into the liposomes.

Preparation of DPPC large unilamellar vesicles (LUVs) for lucigenin based assays. A lipid film was formed by evaporating a chloroform solution containing 50 mg of DPPC under reduced pressure at 25°C. The lipid film was then dried under vacuum at 45ºC for at least 2 hours. The lipid film was then hydrated with 1 mL of a 2 mM lucigenin solution containing NaCl (100 mM), and sodium phosophate salt (10 mM, pH = 6.4). The obtained suspension was subjected to 15 freeze/thaw cycles (1 cycle = 1 minute at -4°C followed by 1 minute at 45ºC). The mixture was vortexed for 1 minute after every cycle to help the hydration. The solution was then extruded through a 100 nm polycarbonate membrane 21 times at temperature between 45-55ºC (fluid state lipid) until the solution was transparent and passed down a Sephadex G-25 column to remove extravesicular lucigenin dye. The eluant was a solution containing 100 mM of NaCl and 10 mM of sodium phosphate salt. 10.4 mL of liposome solution were isolated after gel filtration. The stock solution was 6.25 mM in lipid, assuming all DPPC was incorporated into the liposomes.

Chloride transport assays with DPPC and EYPC LUVs. A 20 μL aliquot of the stock solution of DPPC LUVs were added to a 2.5 mL gently stirred thermostated buffer solution containing 10 mM sodium phosphate salt (pH = 6.4), and 100 mM NaCl. The lucigenin fluorescence was monitored by excitation at λex = 372 nm and the emission was recorded at λem = 503 nm. At t = 50 s, 100 μL of solution of transporter in MeOH were added to give a solution of 10 mol.% (relative to lipids) in benzimidazolium. Finally at t = 300 s, 100 μL of a Triton-X 1% solution were added in order to lyse the liposomes. The temperature was set to 35°C, 40°C and 45°C. For assays in EYPC liposomes, at t = 50 s of the experiment, 100 μL of solution of transporter at different concentrations in MeOH were added. Finally at t = 300 s, 100 μL of a Triton-X 1% solution were added in order to lyse the liposomes. The temperature was set to 37°C. Experiments were repeated in triplicate and all traces reported are the average of the three trials.

U-tube experiment. In the U-shaped glass tube experiment the lipid bilayer is mimicked by chloroform, a dense hydrophobic solvent (Figure S32). We assumed that the formation of an ion channel is virtually impossible in such conditions. The organic phase consisted of 10 mL chlorofom and contained 1 mM of ionophore. A control experiment was performed with neat chloroform. 5 ml of aqueous lucigenin solution (0.1 mM) and water were placed in each arm of the U-tube, as donating and receiving aqueous phase respectively. Lucigenin (bis-*N*-methylacridinium nitrate) present initially in the donating phase should pass through the bulky hydrophobic chloroform phase paired to the nitrate anions, transported by a mobile carrier, in order to reach the receiving phase. The increase of lucigenin's concentration in the receiving phase was monitored by fluorimetry. The experiments were conducted at room temperature and

the lucigenin's fluorescence was monitored by excitation at λex = 372 nm and the emission was recorded at λem = 503 nm.

Minimal inhibitory concentration (MIC) determination. 5 mL of lysogeny broth (LB) medium were inoculated with *Escheria coli* (DH5α and SK037 strains) or *Bacillus thuringiensis* (HD73 strain). The precultures were grown overnight at 37°C under stirring, and resuspended in 75 mL of a fresh LB medium. The cultures were grown at 37°C until the OD600 = 0.5 and then rediluted in fresh LB medium until OD600 = 0.1. Assays were performed in 96-well culture plates. Each well was filled with 185 µL bacterial culture, 10 µL MiliQ water and 5 µL DMSO or compounds in DMSO solution, as the final volume in each well was 200 µL and the concentration in DMSO max 2.5 % (v/v). The plates were stirred in an thermostated incubator at 37°C and the OD600 was monitored at t = 0 h, 4 h, 8 h and 24 h. Every experiment was performed in triplicates in independent bacterial cultures. The MICs were determined as the minimal concentration at which no bacterial growth was detected.

Measurement of haemolytic activity. Haemolytic activity was tested against erythrocytes from human blood. Fresh human red blood cells (blood type O) were centrifuged for 10 minutes at 1000 rpm, then washed three times with PBS buffer and diluted to a concentration of 2% (v/v) in PBS buffer. 10 µL of two-fold serial dilutions of compounds **6**, **7** and **8** in DMSO were added to 96-well plates, after which 190 µL of erythrocyte suspension were added. After 1 or 24 h of incubation at 37°C with gentle shaking, the plates were centrifuged for 10 minutes at 2000g. 50 µL of supernatant of each well were transferred to a fresh plate and the release of hemoglobin was monitored by measuring the absorbance at 405 nm. The values for 0% and 100% hemolysis were determined by incubating erythrocytes with PBS or with 0.5% (v/v) Triton X-100. The hemolysis percentage is calculated using the following equation:

$$\text{Hemolysis (\%)} = \frac{A - A_0}{A_{100} - A_0} \times 100$$

where A is the absorbance of supernatant **6**, **7** and **8** solutions, A_0 is the absorbance of supernatant with PBS and A_{100} is the absorbance of supernatant with 0.5% Triton X-100. Data are the mean of three separate experiments.

Membrane depolarization with Gram-negative strain. Preparation of bacterial suspensions was the same as in the anti-bacterial activity assay for *E.coli* (SK037). Bacterial cultures were grown overnight in LB broth at 37°C. Cells were harvested by centrifugation three times at 1600 rpm and washed in a buffer containing 20 mM glucose and 5 mM HEPES (pH 7.3). Then, after the washing, pellets were resuspended in the same HEPES buffer. Fractions from each cell suspension were diluted into a cuvette to an optical density (A_{600}) of 0.15 along with the dye diSC$_2$5 at a final concentration of 1 μM in the buffer. The mixture was allowed to equilibrate for 1h to a stable baseline at 37°C. The diSC$_2$5 fluorescence was monitored by excitation at λex = 600 nm and the emission was recorded at λem = 660 nm. Samples were stirred during the measurement at a constant temperature of 37°C. At t = 50 s, 63 μL of solution of transporter in DMSO were added to a 2.5 mL of suspension to give a solution of 1 mM in **2** or 0.4 mM in **6**, **7** and **8**.

Field emission SEM. Preparation of bacterial suspensions was the same as in the anti-bacterial activity assay with *E.coli(SK037)*. Cell suspensions were centrifuged, washed three times and re-suspended in the same volume of 10 mM of PBS (pH 7.4). Fractions from each cell suspension were diluted to an optical density (A_{600}) of 1.20. Subsequently, 1 mL cell suspensions (9 x 10^8 CFU/mL) were transferred along with 10 μL of solution of transporter in DMSO to give solutions of 0.04 mM and 0.08 mM in **8** to poly-lysine-coated glass slides previously placed in

the wells of polystyrene culture dishes. The plates were kept at 37°C for 12h or 24 h to allow the adhesion of treated *E. coli* cells to the glass slides. After required time, slide-immobilized cells were washed with PBS three times, then fixed with 2.5% (v / v) glutaraldehyde in 10 mM PBS, washed three times with the same buffer and stained with 1% (v / v) OsO_4 in 10 mM PBS. Samples were then dehydrated with a graded ethanol series. After critical-point drying and carbon coating, the samples were observed with a JEOL JSM-7400F field emission microscope SEM (JEOL Ltd, Tokyo, Japan).

ASSOCIATED CONTENT

Supporting Information. Synthetic details, NMR and MS spectra for all compounds, anion transport assay, U-tube experiment, antibacterial assay, measurement of haemolytic activity, membrane depolarization assay and crystallographic data tables. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

* Email: ar. schmitzer@umontreal.ca

Author Contributions

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ABBREVIATIONS

ACN, acetonitrile; AMP, antimicrobial peptide; anh, anhydrous; di$SC_2$5, 3,3′-diethylthiadicarbocyanine iodide; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; EYPC, egg yolk phosphatidylcholine; LC-LCQ, liquid chromatography quadrupole; LC-TOF, liquid chromatography-time of flight; MeOH, methanol; mol. %, mole percentage; mV, milivolt; NTf_2, bis(trifluoromethanesulfonimide); OTf, trifluoromethanesulfonate

REFERENCES

1. a) Busschaert, N.; Gale, P. A. Small-molecule lipid-bilayer anion transporters for biological applications. *Angew. Chem. Int. Ed.* **2013**, 52, 1374-1382. b) Haynes, C. J. E.; Gale, P. A. Transmembrane anion transport by synthetic systems. *Chem. Comm.* **2011**, 47, 8203-8209. c) Davis, J. T.; Okunola, O.; Quesada, R. Recent advances in the transmembrane transport of anions. *Chem. Soc. Rev.* **2010**, 39, 3843-3862. d) Davis, A. P.; Sheppard, D. N.; Smith, B. D. Development of synthetic membrane transporters for anions. *Chem. Soc. Rev.* **2007**, 36, 348-357.

e) Gale, P. A.; Pérez-Tomás, R.; Quesada, R. Anion transporters and biological systems. *Acc. Chem. Res.* **2013**, 46, 2801-2813.

2. Sessler, J. L.; Allen, W. E. Anion carriers: new tools for crossing membranes. *CHEMTECH* **1999**, 9, 16-24

3. Boudreault, P.-L.; Arseneault, M.; Otis, F.; Voyer, N. Nanoscale tools to selectively destroy cancer cells. *Chem. Comm.* **2008**, 2118-2120.

4. Davis, J. T.; Gale, P. A.; Okunola, O. A.; Prados, P.; Iglesias-Sánchez, J. C.; Torroba, T.; Quesada, R. Using small molecules to facilitate exchange of bicarbonate and chloride anions across liposomal membranes. *Nat. Chem.* **2009**, 1, 138-144.

5. Hernandez, P. I.; Moreno, D.; Javier, A. A.; Torroba, T.; Perez-Tomas, R.; Quesada, R. Tambjamine alkaloids and related synthetic analogs: efficient transmembrane anion transporters. *Chem. Comm.* **2012**, 48, 1556-1558.

6. LeDell, K.; Muto, C. A.; Jarvis, W. R.; Farr, B. M. SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of staphylococcus aureus and enterococcus. *Infect. Contr. Hosp. Epidemiol.* **2003**, 24, 639-641.

7. a) Boman, H. G. Peptide antibiotics and their role in innate immunity. *Annu. Rev. Immunol.* **1995**, 13, 61-92. b) Zasloff, M. Antimicrobial peptides of multicellular organisms. *Nature* **2002**, 415, 389-395.

8. a) Brogden, N. K.; Brogden, K. A. Will new generations of modified antimicrobial peptides improve their potential as pharmaceuticals*? Int. J. Antimicrob. Agents* **2011**, 38, 217-225. b) Gindri, I. M.; Siddiqui, D. A.; Bhardwaj, P.; Rodriguez, L. C.; Palmer, K. L.; Frizzo, C. P.;

Martins, M. A. P.; Rodrigues, D. C. D. Dicationic imidazolium-based ionic liquids: a new strategy for non-toxic and antimicrobial materials. *RSC Adv.* **2014**, 4, 62594-62602. c) Garcia-Lorenzo, A.; Tojo, E.; Tojo, J.; Teijeira, M.; Rodriguez-Berrocal, F. J.; Gonzalez, P.; Martinez-Zorzano, V. S. Cytotoxicity of selected imidazolium-derived ionic liquids in the human Caco-2 cell line. Sub-structural toxicological interpretation through a QSAR study. *Green Chem*, **2008**, 10, 508-516.

9. Elie, C.-R.; Noujeim, N.; Pardin, C.; Schmitzer, A. R. Uncovering new properties of imidazolium salts: Cl^- transport and supramolecular regulation of their transmembrane activity. *Chem. Comm.* **2011**, 47, 1788-1790.

10. Elie, C.-R.; Charbonneau, M.; Schmitzer, A. R. An anion structure-activity relationship of imidazolium-based synthetic transporters. *Med. Chem. Comm.* **2012**, 3, 1231-1234.

11. Davis, A. P.; Sheppard, D. N.; Smith, B. D. Development of synthetic membrane transporters for anions. *Chem. Soc. Rev.* **2007**, 36, 348-357.

12. Sidorov, V.; Kotch, F. W.; Kuebler, J. L.; Lam, Y.-F.; Davis, J. T. Chloride transport across lipid bilayers and transmembrane potential induction by an oligophenoxyacetamide. *J. Am. Chem. Soc.* **2003**, 125, 2840-2841.

13. Harrell, J. W. A.; Bergmeyer, M. L.; Zavalij, P. Y.; Davis, J. T. Ceramide-mediated transport of chloride and bicarbonate across phospholipid membranes. *Chem. Comm.* **2010**, 46, 3950-3952.

14. McNally, B. A.; Koulov, A. V.; Smith, B. D.; Joos, J.-B.; Davis, A. P. A fluorescent assay for chloride transport; identification of a synthetic anionophore with improved activity. *Chem. Comm.* **2005**, 1087-1089.

15. a) Koulov, A. V.; Lambert, T. N.; Shukla, R.; Jain, M.; Boon, J. M.; Smith, B. D.; Li, H.; Sheppard, D. N.; Joos, J.-B.; Clare, J. P.; Davis, A. P. Chloride transport across vesicle and cell membranes by steroid-based receptors. *Angew. Chem. Int. Ed.* **2003**, 42, 4931-4933. b) Krasne, S.; Eisenman, G.; Szabo, G. Freezing and melting of lipid bilayers and the mode of action of nonactin, valinomycin, and gramicidin. *Science* **1971**, 174, 412-415. c) Elie, C.-R.; Hebert, A.; Charbonneau, M.; Haiun, A.; Schmitzer, A. R. Benzimidazolium-based synthetic chloride and calcium transporters in bacterial membranes. *Org. Biomol. Chem.* **2013**, 11, 923-928.

16. Yamnitz, C. R.; Negin, S.; Carasel, I. A.; Winter, R. K.; Gokel, G. W. Dianilides of dipicolinic acid function as synthetic chloride channels. *Chem. Comm.* **2010**, 46, 2838-2840.

17. a) Litvinchuk, S.; Bollot, G.; Mareda, J.; Som, A.; Ronan, D.; Shah, M. R.; Perrottet, P.; Sakai, N.; Matile, S. Thermodynamic and kinetic stability of synthetic multifunctional rigid-rod β-barrel pores: evidence for supramolecular catalysis. *J. Am. Chem. Soc.* **2004**, 126, 10067-10075. b) Bhosale, S.; Matile, S. A simple method to identify supramolecules in action: hill coefficients for exergonic self-assembly. *Chirality* **2006**, 18, 849-856. c) Mora, F.; Tran, D.-H.; Oudry, N.; Hopfgartner, G.; Jeannerat, D.; Sakai, N.; Matile, S. Interface engineering of synthetic pores: towards hypersensitive biosensors. *Chem. Eur. J.* **2008**, 14, 1947-1953. d) Hennig, A.; Fischer, L.; Guichard, G.; Matile, S. Anion−macrodipole interactions: self-assembling oligourea/amide macrocycles as anion transporters that respond to membrane polarization. *J. Am. Chem. Soc.* **2009**, 131, 16889-16895. e) Butterfield, S. M.; Hennig, A.;

Matile, S. Colorful methods to detect ion channels and pores: intravesicular chromogenic probes that respond to pH, pM and covalent capture. *Org. Biomol. Chem.* **2009**, 7, 1784-1792. f) Rhee, J. S.; Ebihara, S.; Akaike, N. Gramicidin perforated patch-clamp technique reveals glycine-gated outward chloride current in dissociated nucleus solitarii neurons of the rat. *J. Neurophysiol.* **1994**, 72, 1103-1108.

18. Gravel, J.; Schmitzer, A. R. Transmembrane anion transport mediated by adamantyl-functionalised imidazolium salts. *Supramol. Chem.* **2014**, DOI: 10.1080/10610278.2014.969265

19. Aono, R.; Tsukagoshi, N.; Yamamoto, M. Involvement of outer membrane protein TolC, a possible member of the marsox regulon, in maintenance and improvement of organic solvent tolerance of escherichia coli K-12. *J. Bacteriol.* **1998**, 180, 938-944.

20. Goldberg, K.; Sarig, H.; Zaknoon, F.; Epand, R. F.; Epand, R. M.; Mor, A. Sensitization of gram-negative bacteria by targeting the membrane potential. *FASEB J.* **2013**, 27, 3818-3826.

21. Vidal, M.; Elie, C.-R.; Campbell, S.; Claing, A.; Schmitzer, A. R. Biologically active binaphthol-scaffolded imidazolium salts. *Med. Chem. Comm.* **2014**, 5, 436-440.

22. Heritage, J.; Evans, E.G.V.; Killington, R.A. Microbial Structure and Mode of Life., in Introductory Microbiology, Cambridge university press Eds, Cambridge, **1996**

23. Martinac, B.; Saimi, Y.; Kung, C. Ion channels in microbes. *Physiol. Rev.* **2008**, 88, 1449-1490.

24. a) Strahl, H.; Hamoen, L. W. Membrane potential is important for bacterial cell division. *Proc. Nat. Ac. Sci. U.S.A* **2010**, 107, 12281-12286. b) Cheng, K.; Haspel, H. C.; Vallano, M. L..; Osotimehin, B.; Sonenberg, M. Measurement of membrane potentials (psi) of erythrocytes and

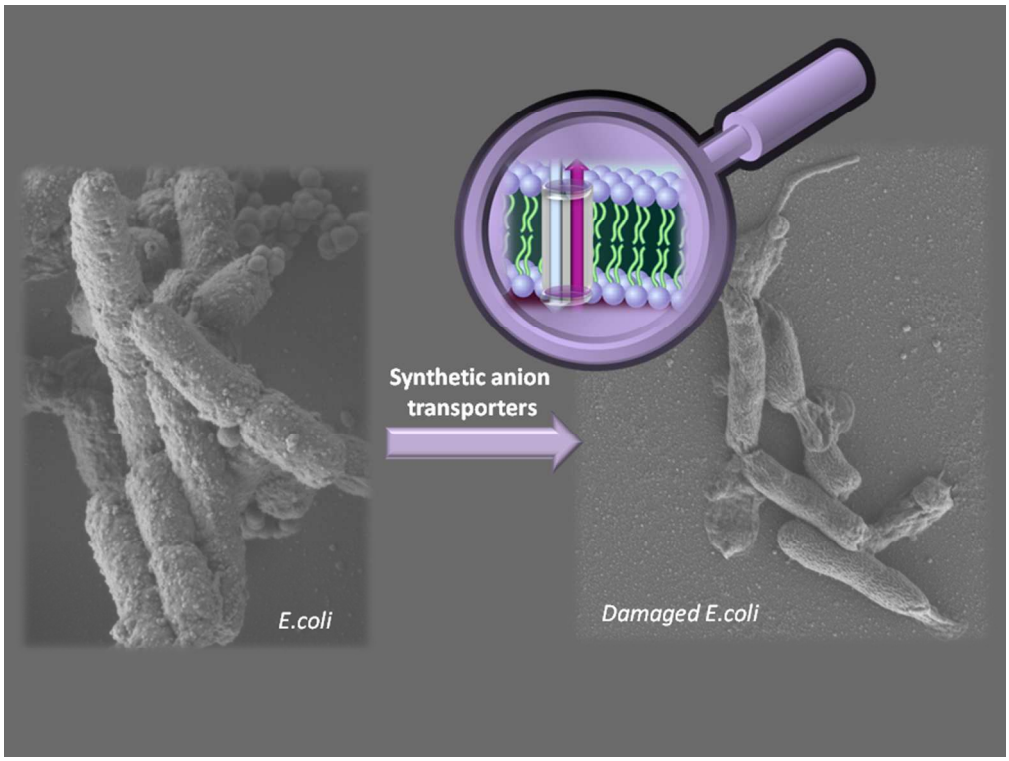
white adipocytes by the accumulation of triphenylmethylphosphonium cation. *J. Membr. Biol.* **1980***,* 56, 191-201.

25. Epand, R. F.; Pollard, J. E.; Wright, J. O.; Savage, P. B.; Epand, R. M. Depolarization, bacterial membrane composition, and the antimicrobial action of ceragenins. *Antimicrob. Agents Chemother.* **2010**, 54, 3708-3713.

26. Silverman, J. A.; Perlmutter, N. G.; Shapiro, H. M. Correlation of daptomycin bactericidal activity and membrane depolarization in staphylococcus aureus. *Antimicrob. Agents Chemother.* **2003**, 47, 2538-2544.

27. Livne, L.; Kovachi, T.; Sarig, H.; Epand, R. F.; Zaknoon, F.; Epand, R. M.; Mor, A. Design and characterization of a broad-spectrum bactericidal acyl-lysyl oligomer. *Chem. Biol.* **2009***,* 16, 1250-1258.

28. Qian, C.-D.; Wu, X.-C.; Teng, Y.; Zhao, W.-P.; Li, O.; Fang, S.-G.; Huang, Z.-H.; Gao, H.-C. Battacin (octapeptin B5), a new cyclic lipopeptide antibiotic from paenibacillus tianmuensis active against multidrug-Resistant gram-negative bacteria. *Antimicrob. Agents Chemother.* **2012**, 56, 1458-1465.



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