

# Carbene formation as a mechanism for efficient intracellular uptake of cationic antimicrobial carbon acid polymers

Received: 9 August 2023

Accepted: 30 June 2025

Published online: 12 July 2025

 Check for updates

Chong Hui Koh<sup>1,2,14</sup>, Mallikharjuna Rao Lambu<sup>1,2,14</sup>, Chongyun Tan<sup>1,2,14</sup>, Guangmin Wei<sup>1,2,14</sup>, Zhi Yuan Kok<sup>1,2</sup>, Kaixi Zhang<sup>1,2,3</sup>, Quang Huy Nhat Vu<sup>1,2</sup>, Muthuvel Panneerselvam<sup>1,2</sup>, Ying Jie Ooi<sup>1,2</sup>, Shiow Han Tan<sup>1,2</sup>, Zheng Wang<sup>1,2</sup>, Madhu Babu Tatina<sup>1,2</sup>, Justin Tze Yang Ng<sup>4</sup>, Aoxin Guo<sup>4</sup>, Panyawut Tonanon<sup>1</sup>, Tram T. Dang<sup>1</sup>, Yunn-Hwen Gan<sup>5</sup>, Yuguang Mu<sup>4</sup>, Paula T. Hammond<sup>3,6</sup>, Yonggui Robin Chi<sup>1</sup>, Richard D. Webster<sup>1,7</sup>, Sumod A. Pullarkat<sup>1</sup>, Qingjie Li<sup>8</sup>, E. Peter Greenberg<sup>9</sup>, Angelika Gründling<sup>10</sup>, Kevin Pethe<sup>2,11,12,13</sup>✉ & Mary B. Chan-Park<sup>1,2,11</sup>✉

Cationic polymers have emerged as promising next-generation antimicrobial agents, albeit with inherent limitations such as low potency and limited biocompatibility. Classical cationic polymers kill bacteria via physical membrane disruption. We propose a non-classical mechanism of crossing the bacterial plasma membrane barrier, a step required for subsequent inhibition of intracellular targets, by cationic polymers which are carbon acids. Oligoimidazolium (OIM) carbon acids, instead of lysing bacteria, transiently deprotonate in water to form hydrophobic N-heterocyclic carbenes (NHCs) and exhibit efficient plasma membrane translocation. Only OIMs that are carbon acids have potent antibacterial activities against even colistin- and multidrug-resistant bacteria. OIM amide derivatives exhibit excellent antibacterial efficacy in murine sepsis and thigh infection models, while a polymeric version acts as a prophylactic agent against bovine mastitis, which is a global agricultural problem. This study unveils a promising path for the development of an alternative class of potent antimicrobial agents.

Antibiotics have long served as essential therapeutic and prophylactic tools for biomedical, as well as agricultural, applications, underpinning numerous modern biomedical interventions, including chemotherapy and surgical procedures. However, the escalating global crisis of bacterial resistance to virtually all classes of antibiotics has cast a shadow over these medical advances. This crisis is further compounded by the sluggish pace of development of next-generation antimicrobial agents, a pressing challenge that has persisted for several decades<sup>1–3</sup>. Notably, the approval of new antibiotic classes for the treatment of Gram-negative bacterial infections has remained elusive for over six decades<sup>4,5</sup>. Conventional antibiotics typically act by targeting specific

enzymes involved in conserved metabolic processes, inevitably leading to the emergence of resistant strains.

In this context, the bacterial membrane represents one of the last frontiers in the quest for alternative antibacterial drugs. Membrane-targeting agents hold promise, as they are usually less susceptible to resistance development. Conventionally, antimicrobial polymers and peptides (AMPs) depend on disrupting the integrity of bacterial cytoplasmic membranes to induce cell death. However, the advancement of classical cationic polymer and peptide agents that target bacterial membrane permeability has been hindered by concerns regarding their toxicity and limited metabolic stability<sup>6–8</sup>. Furthermore, our

A full list of affiliations appears at the end of the paper. ✉ e-mail: [kevin.pethe@ntu.edu.sg](mailto:kevin.pethe@ntu.edu.sg); [mbechan@ntu.edu.sg](mailto:mbechan@ntu.edu.sg)

understanding of the intricate interactions between cationic agents and bacterial membrane remains incomplete. Exploring non-classical mechanisms of action for cationic polymers and peptides, distinct from the conventional physical membrane disruption associated with classical cationic agents, could broaden the array of available treatment options.

Previously, we discovered a non-classical class of cationic polymers, known as main chain cationic polyimidazoliums (PIMs), that exhibit a remarkable antibiotic-like potency coupled with minimal cytotoxicity<sup>9</sup> when compared to conventional cationic polymers. Notably, unlike classical cationic polymers, PIMs do not exert their antimicrobial action through membrane disruption; instead, they target intracellular processes<sup>9,10</sup>. However, the precise mechanism of action underlying the excellent potency of imidazolium-derived polymers remains unexplained. Additionally, it remains unaddressed whether short imidazolium-containing polymeric chains with low cationic charge density would exhibit a similar antibiotic-like potency.

Separately, it has been reported by Amyes et al. that cationic imidazolium monomers, possessing unsubstituted hydrogens at the C2-carbon (hereafter denoted as C(2)-H), function as carbon acids, which undergo deprotonation in physiological pH aqueous solutions to form stable yet short-lived N-heterocyclic carbenes (NHCs)<sup>11</sup>. They have demonstrated that imidazolium monomers spontaneously form NHC in neutral pH water, and the resulting imidazol-2-yl carbenes, although highly susceptible to protonation by solvent water, exhibit some degree of stability due to the limited reorganization of solvent water molecules around the NHC moiety. Building upon this insight, we hypothesize that the transient presence of NHCs in water of imidazolium-containing polymers, which act as Brønsted carbon acids (Fig. 1A), facilitates and ultimately contributes to their exceptional antibacterial potency. The transformation of some (but not all) of the cationic repeats of imidazolium-containing polymers, at any single moment, to uncharged hydrophobic NHCs would convert the initially cationic hydrophilic homopolymer to an amphiphilic copolymer (Fig. 1A) that enhances copolymer translocation across bacterial membrane at low threshold concentrations, a step preceding the cytosol entry necessary to reach its intracellular target (Fig. 1B). This distinct mode of entry, facilitated initially by the electrostatic attraction of the cationic polymer to anionic bacterial membrane, and subsequently by the bacterial membrane potential, stands in contrast to the physical disruption approach employed by classical cationic polymers, as the latter likely requires higher charge concentration threshold that results in lower potency and selectivity.

Herein, we seek to prove the facile entry of short oligoimidazoliums (OIMs) is assisted by NHC formation, which contributes to the superior potency of this class of antibiotics. Building upon this, an engineered secondary diamide oligoimidazolium (OIM) derivative demonstrates good broad-spectrum efficacy in murine models of sepsis and thigh infections, notably against methicillin-resistant *Staphylococcus aureus* (MRSA) LAC strain and multi-drug-resistant (MDR) Gram-negative strains of *Acinetobacter baumannii* and *Klebsiella pneumoniae*. We also extend our findings to the agriculture domain, where the polymeric imidazolium (i.e., polyimidazolium, PIM) acts as an effective prophylactic agent against dairy mastitis, a pervasive global issue in the dairy industry.

## Results

### Synthesis and antibacterial properties of the precision main chain imidazolium oligomers

We synthesized a series of OIMs with precisely controlled molecular weights amenable to rigorous characterizations required for mechanistic studies. This series, designated as OIM1-6, comprises eight derivatives (1-8), each featuring six imidazolium rings (Fig. 2A). The syntheses of these compounds, including the parent OIM1-6-CH (1), were achieved via a step-by-step synthetic strategy. Detailed

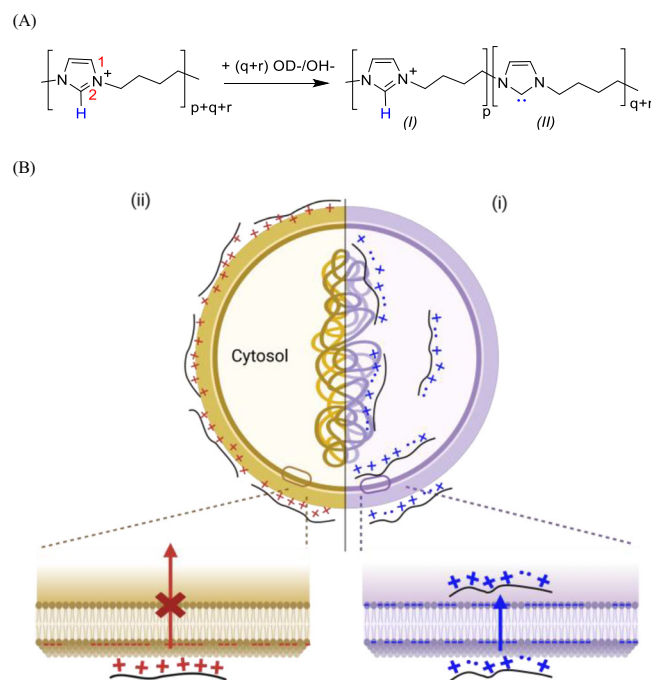
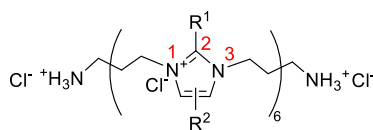


**Fig. 1 | N-heterocyclic carbene (NHC) formation of oligoimidazolium (OIM) carbon acid and its contribution to bacterial membrane translocation.** **A** OIMs with free C2-hydrogens are carbon acids that deprotonate in neutral water to form NHCs (II) that are uncharged and hydrophobic. **B** Non-classical mechanism of translocation: (i) Right side: OIM carbon acid converts to an amphiphilic copolymer (made of hydrophilic cation and hydrophobic NHC repeats) to efficiently translocate across the plasma membrane into bacterial cytosol at lower polymer threshold concentration, as compared with (ii) Left side: a classical cationic polymer that forms physical pores. The OIMs bind with their intracellular target (DNA as previously studied) in bacterial cytosol, resulting in bacterial death (represented in purple), as opposed to classical cationic polymer, which is ineffective in killing the bacterium (live bacterium is represented in brown). Created in BioRender. Chan, M. (2025) <https://BioRender.com/zhts02o>.

information regarding the synthesis methods and chemical characterizations of the OIMs are described in Supplementary Figs. S1A and S1.1–S1.52. To assess their antibacterial efficacy, we subjected the OIM1-6 series compounds (1-8) to a battery of tests against a diverse spectrum of Gram-positive and Gram-negative bacteria (Table 1). Notably, OIM1-6-CH (1), characterized by its carbon acidity with the presence of a C(2)-H, exhibited substantial potency with a low geometric mean of minimum inhibitory concentration (Geo-MIC) of 4.0 µg/mL against a panel of ESKAPE pathogens that includes *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter cloacae*.

The derivatives (2-4), devoid of carbon acid characteristic as their C(2)-H atoms were replaced by various substituents that encompass both weakly electron-donating (methyl- (-CH<sub>3</sub>)), and electron-withdrawing (chloro- (-Cl) and trifluoro- (-CF<sub>3</sub>)) groups, exhibited markedly diminished potency when compared to the parent OIM1-6-CH (1). Specifically, the three non-carbon acid compounds (2-4) possessing C2-substituted groups displayed Geo-MIC values ranging from 118.5 µg/mL to 344.6 µg/mL, which were significantly higher than the Geo-MIC value of 4.0 µg/mL observed for compound (1). In parallel, we synthesized analogous derivatives (5-8) with substituents introduced at the C4-position instead, preserving the carbon acidity with the retention of C(2)-H atoms. Strikingly, the C4-substituted derivatives (5-8) maintained their potent antibacterial activity, with Geo-MIC of 4.4–23.8 µg/mL. These values were notably lower than those recorded for their isomers (2-4), underscoring the

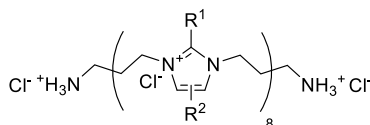
(A)



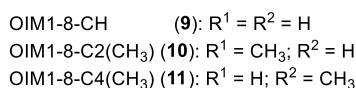
OIM1-6 series (1-8)



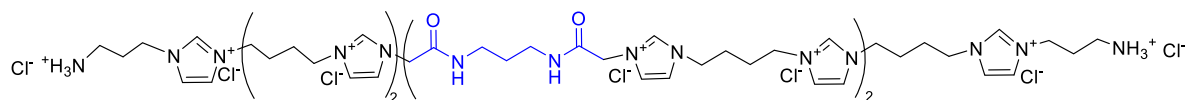
(B)



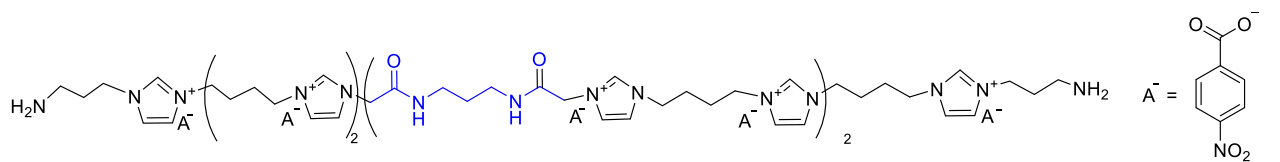
OIM1-8 series (9-11)



(C)



OIM1-8-2D (12)



OIM1-8-2D-4NBZate (13)

4-nitrobenzoate

**Fig. 2 | Chemical structures of OIM derivatives synthesized and evaluated. A** OIM1-6 series, **B** OIM1-8 series, and **C** secondary diamide OIM series.

pivotal role of the carbon acid characteristic in preserving anti-bacterial potency.

To investigate the influence of chain length on OIM potency, we synthesized oligomers containing eight repeat units, specifically OIM1-8-CH (9) and its methyl-substituted derivatives (10, 11) (Fig. 2B and Supplementary Figs. S1B, S1.53–S1.61). The parent OIM1-8-CH (9) characterized by the presence of a C(2)-H functionality has Geo-MIC of 3.4  $\mu\text{g/mL}$ , which was one order of magnitude more potent than OIM1-8-C2(CH<sub>3</sub>) (10), which is not a carbon acid since it lacks the C(2)-H atom (Geo-MIC = 43.1  $\mu\text{g/mL}$ ). Furthermore, OIM1-8-C4(CH<sub>3</sub>) (11), possessing a C(2)-H, displayed potency identical to (9), with a Geo-MIC of 3.4  $\mu\text{g/mL}$ . Two OIM1-8 carbon acid derivatives which have unsubstituted C(2)-H, having main chain secondary diamide linkages with different counterions, specifically chloride (same counterion as 1-11) and an organic acid (4-nitrobenzoate) respectively (12-13), were also tested (Fig. 2C and Supplementary Figs. S1C, S1.62–S1.69). The counterion exchange to 4-nitrobenzoate for (13) was evaluated by <sup>1</sup>H and <sup>13</sup>C NMR and the absence of bromide or chloride in XPS analysis. These carbon acids retained robust antibacterial potency against various bacterial strains, displaying Geo-MIC of 4.9  $\mu\text{g/mL}$  and 7.3  $\mu\text{g/mL}$ ,

respectively (Table 1). The OIM1-8 series further confirms the importance of carbon acidity in achieving antibacterial potency.

We also conducted biocompatibility tests for the OIM compounds across various eukaryotic cell lines. Both the parent OIM1-6-CH (1) and OIM1-8-CH (9) carbon acids exhibited low short-term toxicity (i.e., Geo-IC<sub>50,24</sub>  $\geq 1024 \mu\text{g/mL}$  and 385.1  $\mu\text{g/mL}$ , respectively, after a 24-h incubation period). However, they showed elevated longer-term toxicity (i.e., Geo-IC<sub>50,72</sub> = 57.7  $\mu\text{g/mL}$  and 31.3  $\mu\text{g/mL}$  respectively at the 72-h time point) against all tested cell lines (3T3 fibroblast cells, human embryonic kidney (HEK) cells, and liver hepatocellular carcinoma (HepG2) cells) (Table 1 and Supplementary Table S1 and Supplementary Fig. S2). However, several substituted carbon acid derivatives, such as the C4-methyl (5, 11), C4-chloro (6) compounds, as well as the secondary diamide OIM derivatives (12, 13), displayed improved selectivity indices (SI > 60 at the 72-h time point, in which SI = Geo-IC<sub>50,72</sub>/Geo-MIC) compared to their respective parent molecules, i.e., OIM1-6-CH (1) and OIM1-8-CH (9) (SI = 14.4 and 9.2 respectively at 72-h time point).

We selected three key compounds for further mechanistic investigations into the relevance of carbon acidity, namely, the parent

Table 1 | Antibacterial and cytotoxicity properties of OIM compounds

| No. | Oligomer                    | Minimum inhibitory concentration, MIC (μg/mL) |                                     |                                        |                                       |                                  | Geometric mean of IC <sub>50</sub> <sup>a</sup><br>(μg/mL) |                                     |                             |                                     |                                     | Selectivity index, SI <sup>b</sup>   |                                      |
|-----|-----------------------------|-----------------------------------------------|-------------------------------------|----------------------------------------|---------------------------------------|----------------------------------|------------------------------------------------------------|-------------------------------------|-----------------------------|-------------------------------------|-------------------------------------|--------------------------------------|--------------------------------------|
|     |                             | <i>S. aureus</i><br>ATCC 29213 (+)            | <i>E. faecium</i><br>ATCC 19434 (+) | <i>K. pneumoniae</i><br>ATCC 13883 (-) | <i>A. baumannii</i><br>ATCC 19606 (-) | <i>P. aeruginosa</i><br>PAO1 (-) | <i>E. coli</i><br>MG1655 (-)                               | <i>E. cloacae</i><br>ATCC 13047 (-) | Geometric mean<br>(Geo-MIC) | 24-h (Geo-<br>IC <sub>50,24</sub> ) | 72-h (Geo-<br>IC <sub>50,72</sub> ) | Geo-IC <sub>50,24</sub> /<br>Geo-MIC | Geo-IC <sub>50,72</sub> /<br>Geo-MIC |
| 1   | OIM1-6-CH                   | 1–2                                           | 2                                   | 4                                      | 4                                     | 2–4                              | 4                                                          | 16–32                               | 4.0                         | >1024                               | 57.7                                | >256.0                               | 14.4                                 |
| 2   | OIM1-6-C2(CH <sub>3</sub> ) | 256                                           | >512                                | 256                                    | 128                                   | 128                              | 512                                                        | >512                                | 344.6                       | 1002.2                              | >1024                               | 2.9                                  | >3.0                                 |
| 3   | OIM1-6-C2(Cl)               | 16                                            | >512                                | 32                                     | 32                                    | 256                              | 64–128                                                     | 256–512                             | 118.5                       | 748.6                               | 479.7                               | 6.3                                  | 4.0                                  |
| 4   | OIM1-6-C2(CF <sub>3</sub> ) | 32                                            | >512                                | 64                                     | 64                                    | 128                              | 128                                                        | 256                                 | 128.0                       | 693.4                               | 727.0                               | 5.4                                  | 5.7                                  |
| 5   | OIM1-6-C4(CH <sub>3</sub> ) | 2–4                                           | 8                                   | 16                                     | 16                                    | 8–16                             | 8                                                          | 64                                  | 10.1                        | >1024                               | 675.3                               | >101.4                               | 66.9                                 |
| 6   | OIM1-6-C4(Cl)               | 2                                             | 4                                   | 4                                      | 8                                     | 4                                | 4                                                          | 8                                   | 4.4                         | >1024                               | 267.5                               | >232.7                               | 60.8                                 |
| 7   | OIM1-6-C4(F)                | 2                                             | 32                                  | 16                                     | 4                                     | 32                               | 32                                                         | 64                                  | 16.0                        | 365.1                               | 66.9                                | 22.8                                 | 4.2                                  |
| 8   | OIM1-6-(BZ)                 | 8                                             | 64                                  | 8                                      | 64                                    | 64                               | 8                                                          | 32                                  | 23.8                        | 39.5                                | 135.8                               | 1.7                                  | 5.7                                  |
| 9   | OIM1-8-CH                   | 2                                             | 8                                   | 4                                      | 2–4                                   | 2                                | 4                                                          | 4                                   | 3.4                         | 385.1                               | 31.3                                | 113.3                                | 9.2                                  |
| 10  | OIM1-8-C2(CH <sub>3</sub> ) | 8                                             | >512                                | 32                                     | 16                                    | 32                               | 32                                                         | 64                                  | 43.1                        | 835.5                               | 781.6                               | 19.4                                 | 18.1                                 |
| 11  | OIM1-8-C4(CH <sub>3</sub> ) | 2                                             | 4–8                                 | 4                                      | 2–4                                   | 2                                | 4                                                          | 4                                   | 3.4                         | >1024                               | 387.2                               | >301.2                               | 113.9                                |
| 12  | OIM1-8-2D                   | 2                                             | 4                                   | 2                                      | 4                                     | 16                               | 8                                                          | 8                                   | 4.9                         | 758.9                               | 419.5                               | 154.9                                | 85.6                                 |
| 13  | OIM1-8-2D-4NBzate           | 4                                             | 16                                  | 4                                      | 4                                     | 8                                | 8                                                          | 16                                  | 7.3                         | 1000.5                              | 720.0                               | 137.0                                | 98.6                                 |
| 14  | Gentamicin                  | ≤1                                            | 64                                  | ≤1                                     | 8                                     | ≤1                               | ≤1                                                         | ≤1                                  | 2.4                         | >1024                               | >1024                               | >426.7                               | >426.7                               |
| 15  | Colistin                    | 128                                           | >512                                | 8                                      | 2                                     | 2                                | 2                                                          | 512                                 | 23.8                        | 791.4                               | 630.5                               | 33.3                                 | 26.5                                 |
| 16  | Ciprofloxacin               | 1–2                                           | 4                                   | ≤1                                     | ≤1                                    | ≤1                               | ≤1                                                         | ≤1                                  | 1.3                         | 187.2                               | 202.2                               | 144.0                                | 155.6                                |

<sup>a</sup>Geometric mean of half-maximal inhibitory concentration (IC<sub>50</sub>) is calculated from the IC<sub>50</sub> of 3 cell lines: 3T3 fibroblast cells, human embryonic kidney (HEK) cells and liver hepatocellular carcinoma (HepG2) cells. The complete IC<sub>50</sub> data are shown in Supporting Table S1, and the raw cytotoxicity data are shown in Supporting Fig. S2.

<sup>b</sup>Selectivity index (SI) is the ratio of Geo-IC<sub>50</sub> of a specific timepoint to Geo-MIC.

**Table 2 | Antibacterial potency of OIM1-6 series compounds compared to the activity of antibiotics against a panel of multi-drug-resistant (MDR) bacteria**

| Bacteria (Gram-positive)                  | MIC of oligomers/antibiotics (µg/mL) |                                  |                                  |                |                        |          |            |           |            |
|-------------------------------------------|--------------------------------------|----------------------------------|----------------------------------|----------------|------------------------|----------|------------|-----------|------------|
|                                           | OIM1-6-CH (1)                        | OIM1-6-C2 (CH <sub>3</sub> ) (2) | OIM1-6-C4 (CH <sub>3</sub> ) (5) | OIM1-8-2D (12) | OIM1-8-2D-4NBZate (13) | Imipenem | Vancomycin | Colistin  | Gentamicin |
| <i>S. aureus</i> USA300 (MRSA)            | 2                                    | 512                              | 4–8                              | 2              | 4                      | 0.25–0.5 | 1–2        | 256       | 1          |
| <i>S. aureus</i> (LAC)                    | 2                                    | 128                              | 4                                | 2              | 4                      | ≤0.25    | 1          | 64        | ≤1         |
| <i>S. aureus</i> (LAC*)                   | 2                                    | 64                               | 4                                | 8              | 4                      | ≤0.25    | 0.5–1      | >512      | ≤1         |
| <i>S. aureus</i> MRSA BAA 39              | 2                                    | 512                              | 4                                | 2              | 4                      | ≤0.25    | 1          | 128       | 256        |
| <i>E. faecalis</i> 583 (VRE) <sup>a</sup> | 32–64/2                              | >512/>512                        | 128/8                            | 16/4           | 8/4                    | 1        | 8/16       | >512/>512 | >512/>512  |
| <i>S. epidermidis</i> (MRSE 700563)       | 1                                    | 32                               | 1                                | 4              | 2                      | 1        | 2          | 128       | ≤0.5       |
| Bacteria (Gram-negative)                  |                                      |                                  |                                  |                |                        |          |            |           |            |
| <i>A. baumannii</i> BAA 2803              | 2                                    | 32                               | 8                                | 2              | 4–8                    | 32–64    | 64         | 4         | >512       |
| <i>A. baumannii</i> AB-1 (MDR)            | 2                                    | 128                              | 8                                | 4              | 8                      | 32–64    | 128–256    | 2         | >512       |
| <i>B. thailandensis</i> 700388            | 8                                    | >512                             | >512                             | 16             | 64–128                 | ≤0.25    | >256       | >512      | 64         |
| <i>E. coli</i> NMT 1833                   | 2                                    | 256                              | 8                                | 16             | 16                     | 16-32    | 256        | 4         | 1          |
| <i>E. coli</i> 958 (MDR)                  | 4                                    | 1024                             | 8                                | 16             | 16                     | ≤0.25    | 256        | 2         | 2          |
| <i>E. coli</i> BAA 2774                   | 4                                    | 128                              | 2                                | 8              | 16                     | ≤0.25    | 256        | 2         | 32         |
| <i>E. coli</i> UTI89                      | 4                                    | ≥512                             | 16                               | 16             | 16                     | ≤0.25    | 128–256    | 2         | 0.5        |
| <i>E. cloacae</i> 13047-MDR               | 4                                    | >512                             | 32                               | 8              | 16                     | 1        | >256       | 128       | ≤1         |
| <i>K. pneumoniae</i> (KPNR)               | 2                                    | 256                              | 2                                | 4              | 8                      | 8        | >256       | 2         | >512       |
| <i>K. pneumoniae</i> SGH 10               | 2                                    | 32                               | 4                                | 2              | 8                      | 0.25–0.5 | >256       | 2         | 2          |
| <i>P. aeruginosa</i> BAA 2797             | 4                                    | >512                             | 32                               | 16             | 32                     | 256      | >256       | 2         | >512       |
| <i>P. aeruginosa</i> PAER                 | 4                                    | 64                               | 16                               | 16             | 32                     | >256     | >256       | 2         | 128        |
| <i>S. marcescens</i> ATCC 13880           | 4                                    | >512                             | 32                               | 16             | 32                     | 0.5–1    | >256       | 256       | 2          |
| Geometric mean (Geo-MIC)                  | 3.5                                  | 265.0                            | 10.1                             | 7.4            | 10.6                   | 2.3      | 39.5       | 26.9      | 20.4       |

<sup>a</sup>The first MIC values were tested in MHB, and the second MIC values (after the “/”) were tested in MHB supplemented with hemin.

compound (**1**) and the two CH<sub>3</sub>-substituted derivatives (compounds **2** and **5**). These compounds were subjected to comprehensive testing against an expanded panel of clinically relevant MDR bacteria. Interestingly, while both the parent OIM1-6-CH (**1**) and C4-methyl substituted derivative (**5**) exhibited potent antimicrobial activity, the C2-methyl substituted derivative (**2**) did not (Table 2), reinforcing the notion that their potent antimicrobial activities are correlated with their carbon acid characteristic. We conducted time-kill kinetics studies for these three OIMs (**1**, **2**, and **5**) against MRSA LAC strain and *Pseudomonas aeruginosa* PAO1 (Supplementary Fig. S3A–F). Notably, both (**1**) and (**5**) were bactericidal and displayed fast killing kinetics with complete eradication of the bacteria within 1–2 h at concentrations 4–8 times their MICs. In contrast, compound (**2**) displayed reduced potency, failing to eradicate the bacteria even with prolonged exposure (20 h) at 8 times MIC. Subsequently, we conducted a comprehensive series of mechanistic studies to investigate the entry of the OIM1-6 parent compound (**1**) and its methyl-substituted derivatives (**2** and **5**) into bacterial membrane-mimicking liposomes and giant unilamellar vesicles (GUV), and live MRSA bacteria to elucidate the specific role of carbon acidity and the resulting deprotonated form of NHC in the OIM entry process.

**OIM carbon acids form deprotonated NHCs in aqueous solution and persist in the membrane of bacteria-mimicking liposomes** Using DiSC<sub>3</sub>(5) and propidium iodide (PI) assays, we assessed the impact of these compounds on bacterial membrane potential and physical integrity in MRSA. Surprisingly, the three compounds induced minimal perturbation to the bacterial membrane potential and to the

physical membrane integrity (Supplementary Fig. S3G, H). The electrostatic adsorption of OIM1-6-CH (**1**) to the bacterial surface was confirmed by zeta-potential measurements, while SEM and TEM images indicate that OIM1-6-CH (**1**) did not induce significant membrane disruption (Supplementary Fig. S4). These findings indicate that the antibacterial effects of these compounds differ significantly from those of classical cationic polymers, which typically cause physical pores or holes in membranes.

Prior research from Amyes et al. established that monomeric imidazolium rings form transient NHCs in water (H<sub>2</sub>O/D<sub>2</sub>O) at physiological pH<sup>11</sup>. Building on this knowledge, we hypothesized that for OIM carbon acids, i.e., compounds (**1**) and (**5**), a fraction of the imidazolium repeats with dissociable C(2)-H could convert to NHCs (*II*, Fig. 1A) in buffered aqueous solution at physiological pH and temperature. To detect the NHC formation of OIMs, we employed <sup>1</sup>H NMR spectroscopy in D<sub>2</sub>O solvent to measure the hydrogen-to-deuterium (H-D) exchange at the C(2)-position of imidazolium cations<sup>11</sup> (Fig. 3A). For compound (**1**), the C(2)-H <sup>1</sup>H NMR resonance signal rapidly decreased at pH 7.16 and pH 8.21 (Fig. 3B), indicative of a fast H-D exchange rate. Conversely, at more acidic values (pH 6.81 and pH 6.63), the H-D exchange rate slowed due to lower deuteride (base) concentration (Fig. 3B). These findings corroborated the notion that compound (**1**) functions as a carbon polyacid with imidazolium repeats that readily deprotonate at physiological pH, releasing protons to form the residual NHC (*II*) repeats which are uncharged and hydrophobic. We hypothesize that these hydrophobic repeat units of OIM mediate the efficient penetration of the transiently formed copolymer of cation and NHC into the anionic

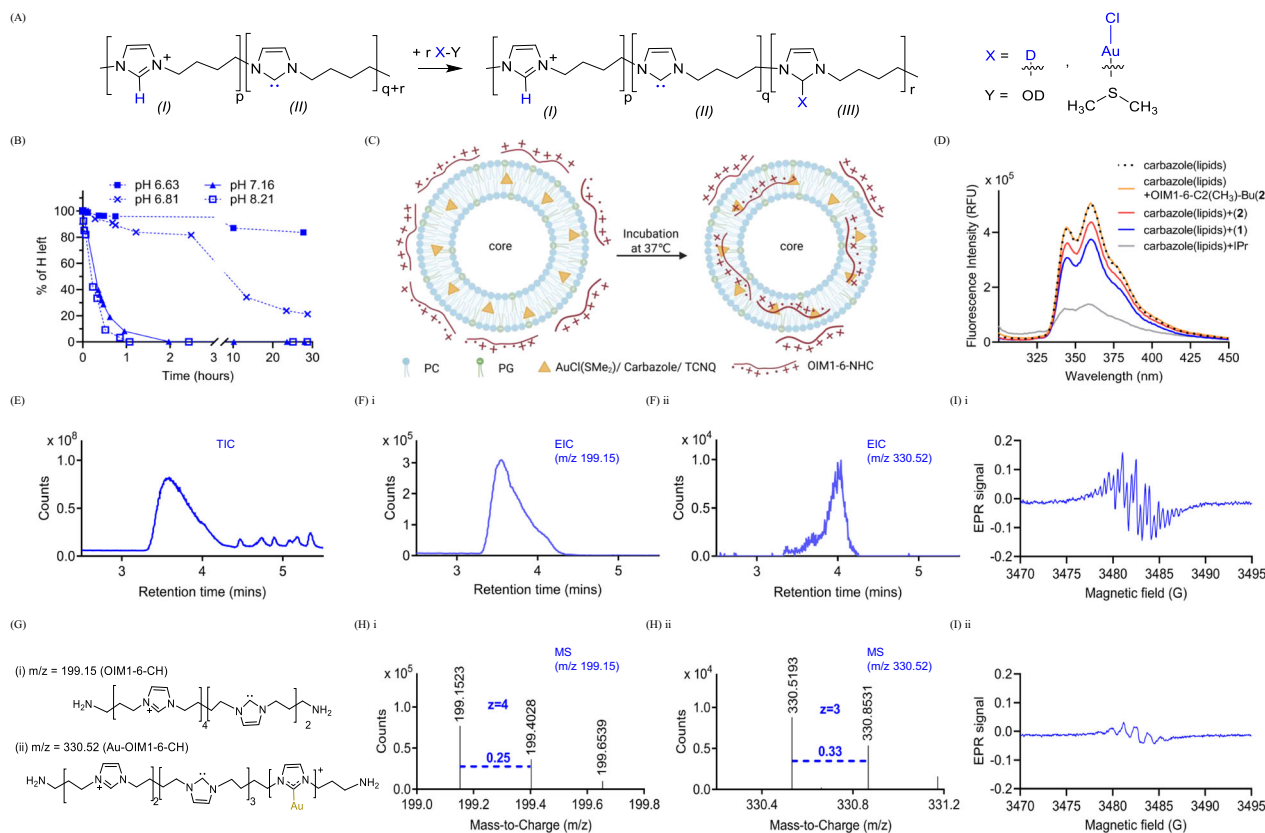


**Fig. 3 | NHC formation studies with OIM1-6-CH (1).** **A** Detection of NHC by the reaction of some carbene (**I**) monomers with D<sub>2</sub>O or AuCl(SMe<sub>2</sub>). **B** Hydrogen-deuterium exchange in <sup>1</sup>H NMR of OIM1-6-CH (**1**) in aqueous solution as a function of time at pH 6.63, pH 6.81, pH 7.16, and pH 8.21 in D<sub>2</sub>O. **C** Schematic of interaction of phosphatidylcholines/phosphatidylglycerol (PC/PG) liposome containing NHC probes (carbazole dye or AuCl(SMe<sub>2</sub>) or TCNQ) with OIM1-6-CH (**1**). Created in BioRender. Chan, M. (2025) <https://BioRender.com/src3b3b3>. **D** Fluorescence of carbazole embedded in liposome bilayer with OIMs or stable carbene (IPr) in solution. **E–H** Proof of NHC formation by OIM (**1**) using AuCl(SMe<sub>2</sub>) embedded in liposome bilayer and LC-MS/MS: **E** Total ion chromatogram (TIC) of Au(lipids)-

OIM1-6-CH (**1**) obtained by LC-ESI-MS (+). **F** Extracted ion chromatogram (EIC) of (i) OIM1-6-CH (*m/z* 199.15), and (ii) Au-OIM1-6-CH containing one Au atom (*m/z* 330.52) (both at pH 7.4). **G** Assigned chemical structures of (i) *m/z* = 199.15 to be OIM1-6-CH (**1**) containing 4 cations and 2 NHCs, (ii) *m/z* = 330.52 to be 1Au-OIM1-6-CH containing 1Au-NHC cation, 2 imidazolium cations (without Au), and 3 NHCs. **H** Mass spectrum (MS) of (i) *m/z* = 199.15 (retention time 3.55 min) with *m/z* difference of 0.25 Da, indicating *z* = 4, and total ion mass = 796.60, and (ii) *m/z* 330.52 (retention time 4.04 min) with *m/z* difference of 0.33 Da, indicating *z* = 3, and total ion mass = 991.56. **I** Electron paramagnetic resonance (EPR) signals of OIM1-6-CH (**1**) added to TCNQ(lipids) in PBS at (i) pH 7.4 and at (ii) pH 6.6.

bacterial plasma membrane, which is otherwise generally impermeable to the initial hydrophilic polycation.

To prove the presence of NHC in bacteria-mimetic membranes, we endeavored to capture OIM-NHCs in the bilayer membrane of anionic liposomes using three NHC probes: carbazole, chloro(dimethylsulfide) gold(I) (AuCl(SMe<sub>2</sub>)), and tetracyanoquinodimethane (TCNQ) (Fig. 3C). We constructed negatively charged liposomes that mimic bacterial plasma membranes and embedded the NHC probes within the bilayer. This composite of probe within lipids is denoted as probe(lipids), such as carbazole(lipids), Au(lipids), and TCNQ(lipids). It has been reported that when a stable NHC, such as 1,3-Bis(2,6-diisopropylphenyl)-1,3-dihydro-2H-imidazol-2-ylidene (IPr), binds with carbazole which is fluorescent in the free state (such as in an organic solvent), the resulting NHC-carbazole adduct (which is formed from the hydrogen bonding of the carbene carbon that has lone pair electrons, with R-NH of carbazole) leads to photoluminescence quenching<sup>12</sup>. Our experiments demonstrated that the addition of IPr (control) to the carbazole(lipids) aqueous solution significantly reduced carbazole emission by ~70%, supporting the formation of the [carbazole+IPr NHC] complex (Fig. 3D). When OIM1-6-CH (**1**) was introduced into the carbazole(lipids) solution, a 30% decrease in carbazole emission was observed, which is higher than the 12% reduction from (**2**). The reduction observed with (**2**) was attributed to the carbazole interaction with the NH<sub>2</sub> terminal, and not with the NHC, as confirmed with the 0% fluorescence reduction using another

control (**2'**) (derivative of (**2**) without NH<sub>2</sub> terminal: synthesis and characterization are described in Supplementary Information Section 1.5 and Supplementary Fig. S1.70). This corroborates the presence of OIM-NHC in the membrane that leads to [carbazole+OIM-NHC] complex within the lipid bilayer (Fig. 3C).

Furthermore, previous research has indicated that AuCl(SMe<sub>2</sub>) can react with NHC under mildly basic ambient conditions, resulting in the formation of the covalently linked [AuCl(NHC)] complex<sup>13</sup> (Fig. 3A). The hydrophobic AuCl(SMe<sub>2</sub>) was placed in the liposome bilayer to attempt to capture NHCs generated from OIM. Then, OIM1-6-CH (**1**) aqueous solution was added to the Au(lipids) suspension. Following incubation and lipid removal, we analyzed the reaction product using liquid chromatography coupled with electrospray ionization mass spectrometry in positive ion mode (LC-ESI-MS(+)). A major total ion chromatogram (TIC) peak at an elution time of 3.81 min, as well as two extracted ion chromatogram (EIC) peaks with observed *m/z* values of 199.15 and 330.52 at 3.55 min and 4.04 min, respectively (Fig. 3E, F i–ii) were observed. The EIC peak of *m/z* = 199.15 was attributed to unreacted (excess) OIM1-6-CH (**1**) (Fig. 3G i) based on a *m/z* difference of 0.25 Da between isotope peaks (Fig. 3H i). The EIC peak with *m/z* = 330.52 was identified as Au-OIM1-6-CH (Fig. 3G ii) due to its MS (Fig. 3H ii) showing a *m/z* difference of 0.33 Da between isotope peaks. The two structural assignments were further confirmed through H-D exchange of the C(2)-H and structural analysis of fragments, both

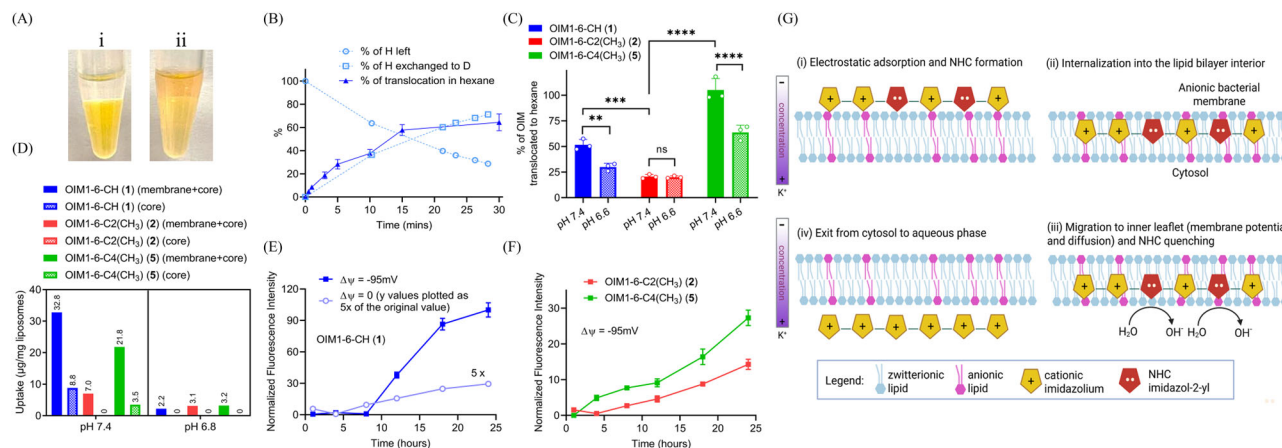


**Fig. 4 | NHC-assisted translocation across bacterial membrane mimics.**

**A–C** Studies with hexane–water model system ( $n = 3$ ): **A** 2-phase system to study translocation of FITC-OIM1-6-CH into bacterial bilayer interior mimic (bottom phase: PBS; top phase: hexane with (i) PC, (ii) PG). Using **A(ii)**, **B** comparison of H-D exchange of OIM1-6-CH (**1**) and translocation percent of FITC-OIM1-6-CH to hexane. **C** FITC-OIMs translocation percent to hexane at pH 7.4 and pH 6.6. Two-way Anova: \*\*\*\* $p < 0.0001$ , \*\* $p < 0.0021$ , \* $p < 0.0331$ . ns (not significant)  $p > 0.05$ . Exact  $p$  values

are provided in Source Data. Uptake of OIMs into vesicles (PC:PG = 8:2): **D** liposome (membrane + core) or core only; and **E**, **F** giant unilamellar vesicles (GUVs) in the absence or presence of membrane potential of  $-95$  mV ( $n = 30$ ). **G** Proposed mechanism of NHC-assisted translocation across bacterial membrane. Created in BioRender. Chan, M. (2025) <https://BioRender.com/tlibxig>. Data are presented as the mean of independent replicates  $\pm$  standard deviation.

analyzed with LC-MS/MS (Supplementary Fig. S5.1). We show that the NHCs formed persist long enough in the aprotic liposome bilayer to react with the embedded AuCl(SMe<sub>2</sub>) probe to produce the stable Au-OIM (**1**) within the membrane.

We also explored the possibility of NHC formation in the two methyl-substituted derivatives, OIM1-6-C2(CH<sub>3</sub>) (**2**) and OIM1-6-C4(CH<sub>3</sub>) (**5**). The compound (**5**), but not (**2**), demonstrated NHC formation and the Au-OIM (**5**) was also isolable in the membrane bilayer, albeit less readily than (**1**) (Supplementary Fig. S5.2). The methyl group on (**5**) exerts an electron-donating effect, making its C(2)-H less susceptible to deprotonation and thereby weakening its NHC formation as compared to (**1**).

To provide further evidence of NHC existence in the liposome bilayer, electron paramagnetic resonance (EPR) spectroscopy was used to detect TCNQ<sup>•−</sup> radicals generated from TCNQ (which was pre-embedded in the liposome bilayer) via an one-electron reduction by NHC<sup>14</sup>. TCNQ was chosen as a tracker since NHC itself is in the singlet state (with a stable lone pair of electrons) that could not be detected by EPR. To verify the method, NHC was generated from (**1**) in an organic solvent (DMSO) by controlled potential electrolysis (CPE) in an electrochemical two-compartment cell. A strong EPR signal was detected for (**1**)-NHC after the addition of TCNQ (Supplementary Fig. S5.3A i), as NHC reduced the TCNQ to TCNQ<sup>•−</sup> via the one-electron transfer reaction. No EPR signal was observed for (**1**)-NHC generated by CPE in the absence of TCNQ (Supplementary Fig. S5.3A ii). Further, the negative controls of single components, i.e., TCNQ in DMSO (Supplementary Fig. S5.3A iii) or TCNQ(lipids) in PBS alone (Supplementary Fig. S5.3B i), showed small EPR absorbances due to trace amounts of TCNQ<sup>•−</sup> radicals being generated via reduction of the starting TCNQ under atmospheric conditions, which can be regarded as baseline signals. Blank liposomes (without TCNQ) (Supplementary Fig. S5.3B ii) also did not show any absorbances. Strong EPR signals were also observed when (**1**) was incubated with TCNQ(lipids) in PBS at pH 7.4 (Fig. 3I i), corroborating the formation of (**1**)-NHC, which caused the generation of TCNQ<sup>•−</sup> radicals via the one-electron reduction in the liposome bilayer. At pH 6.6 (in PBS), no obvious EPR peak was observed when (**1**) was added to TCNQ(lipid) (Fig. 3I ii), which corroborates low NHC generation at acidic pH. Further, lower intensity EPR signals were observed when (**5**) was added to TCNQ(lipids) (Supplementary Fig. S5.3C i), as compared to when (**1**) was added to

TCNQ(lipids) (Fig. 3I i), while no EPR signal was observed when (**2**) was added to TCNQ(lipids) (Supplementary Fig. S5.3C ii). Taken together, the EPR measurements provide further evidence to corroborate that (**1**) generates NHCs that translocate into the liposome bilayer. The NHCs generated persist long enough in the aprotic liposome bilayer so as to generate TCNQ<sup>•−</sup> radicals by reacting with the pre-embedded TCNQ, as well as interact/react with the embedded carbazole, or AuCl(SMe<sub>2</sub>), corroborating that (**1**) form NHCs in PBS, which penetrate the membrane bilayer.

In summary, our investigations have demonstrated that the acidic hydrogen located at the C2-carbon of imidazolium rings of the carbon acids (**1**) and (**5**) can dissociate at physiological pH, giving rise to the formation of NHC. This phenomenon was detected by NMR in water, and NHC was detected in the liposome membrane bilayer by the embedded carbazole, AuCl(SMe<sub>2</sub>), and TCNQ probes. Compound (**1**) exhibited a greater propensity for NHC formation compared to (**5**) and had a lower  $pK_a$  of 21.32 than 22.15 (Supplementary Table S2). Conversely, the methyl-substitution at the C2-carbon on compound (**2**) prevents carbene formation of the non-carbon acid.

### NHC-mediated efficient translocation across bacterial membrane mimics

We proceeded to further understand the translocation of NHC-forming OIM carbon acids into bacteria-mimicking membrane bilayer. We modeled the OIM translocation from the aqueous phase to the bacterial lipid bilayer with a 2-phase hexane–water mixture model system<sup>15</sup>. The hexane and water solvents mimic the lipid bilayer interior and external aqueous phase, respectively. The three OIM derivatives (**1**, **2**, and **5**) were labeled with fluorescein isothiocyanate (FITC) fluorochrome (Supplementary Figs. S1.71–S1.73). With only zwitterionic lipids (phosphatidylglycerol (PC)) added to the hexane phase, and without any anionic lipids (Fig. 4A i), (**1**) did not translocate to the top hexane phase even after vortexing and standing, thus the hexane phase remained clear and colorless. As bacterial plasma membrane generally contains anionic lipids, anionic phosphatidylglycerol (PG) lipids were added to hexane (Fig. 4A ii). With PG, (**1**) was able to translocate to the top hexane phase after vortexing/standing, resulting in the yellowish top hexane phase due to the color of FITC-OIM (**1**). The translocation of (**1**) into the PG/hexane phase (Fig. 4B) reached over 60% after 30 min of vigorous vortexing. Further, the dynamic NHC

formation curve (as measured by the H-D  $^1\text{H}$  NMR exchange) closely followed the percent translocation curve (Fig. 4B), corroborating that NHC aids the translocation. Further, LC-MS was used to track the translocation of (1), (2), and (5) to the hexane phase at different pHs. The translocation to hexane of (1) and (5) decreased greatly from 51% and 99% to 29% and 64% respectively with more acidic pH values (7.4 for the former versus 6.6 for the latter) (Fig. 4C), whereas that of (2) which does not form NHC, maintained the same low value (of around 20%) at different pHs, corroborating that NHC formation assists OIM translocation to the hydrophobic anionic hexane phase which mimics bacterial membrane interior.

We then compared (1), (2) and (5) for their uptake into PC:PG liposomes, specifically (i) the total OIM uptake into the liposomes (which includes the liposome membrane and core), and (ii) OIM uptake into the liposome core only (Fig. 4D and Supplementary Fig. S6.1). The latter is indicative of the OIM's susceptibility to bacterial cytosol uptake to achieve intracellular targeting. Our results showed that at physiological pH (7.4), both (1) and (5) exhibited significantly higher total uptake compared to (2), with only (1) and (5), but not (2), entering the liposome core. At the acidic pH of 6.8, at which NHC formation was inhibited, the total uptake of all three compounds was greatly reduced, with core uptake becoming negligible. Consequently, the extent of core uptake for these three compounds is correlated with the ease of NHC formation. At physiological pH, (1) which forms NHC most readily among the three compounds, displayed the highest core uptake. In contrast, (2) failed to enter the core, as it could not penetrate the bilayer membrane "likely" due to its inability to form the hydrophobic NHCs (Supplementary Fig. S5.2A). The compound (5), while forming NHCs but to a lesser extent than (1), exhibited intermediate core uptake among the three compounds.

We also conducted computer simulations to analyze the interaction of the purely cationic versus the purely NHC forms of OIM1-6-CH (1) (denoted as OIM and OIM-NHC respectively) with *S. aureus* mimetic membrane. The modeled OIM had six repeats of cationic rings while OIM-NHC had 6 repeats of NHC. After placement outside of the surface of the constructed membrane, the OIM-NHC formed numerous stable contacts with the membrane, whereas the cationic OIM had limited membrane interaction (Supplementary Fig. S6.2A, B). Notably, the cationic OIM preferred binding towards the surface of the membrane (Supplementary Fig. S6.2C), while OIM-NHC immediately penetrated and remained within the hydrophobic interior of the membrane bilayer (Supplementary Fig. S6.2D). These results support the idea that NHC formation enhances the hydrophobicity of OIM, facilitating its insertion into the bacterial membrane bilayer.

To further understand the NHC-enhanced translocation into bacteria, we extended our uptake studies with GUV. By encapsulating Sulforhodamine B (SRB) dye into the GUV core, we did not observe any dye leakage upon the addition of (1) which contrasted with nisin (Supplementary Fig. S6.3A). Hence, (1) translocates through the bilayer without forming pores<sup>16,17</sup>. As we have reported the contribution of proton motive force (PMF) for the bacterial uptake of (1)<sup>10</sup>, we then studied the effect of membrane potential ( $\Delta\psi$ , a major constituent of PMF) on the OIM uptake into the GUV core. When FITC-OIM (1) was added to GUV (now without SRB) without membrane potential ( $\Delta\psi=0$ ), an increase of fluorescence from the GUV interiors was observed with time (Fig. 4E), indicating that (1) is able to translocate across the GUV membrane, albeit the amount is not high, though finite (corroborating the results with liposomes, Fig. 4D). We then created a membrane potential of  $-95$  mV across the GUV membrane according to a previous protocol<sup>18</sup>. The translocation of FITC-OIM (1) at  $\Delta\psi$  of  $-95$  mV was significantly higher than its translocation in the absence of membrane potential (Fig. 4E and Supplementary Fig. S6.3B i), corroborating that bacterial membrane potential is important to OIM bacterial translocation. Further, (5) was able to translocate across the membrane with membrane potential more effectively than (2) (Fig. 4F

and Supplementary Fig. S6.3B ii-iii), corroborating that NHC enhances OIMs translocation across bacterial membrane.

In summary, the translocation into the anionic hexane phase (in the hexane-water system) or the anionic liposome/GUV core is much higher for (1) and (5) than for (2). The entry into/through the anionic membrane bilayer interior (for these bacteria-mimicking models) by OIM carbon acids ((1) and (5)) is higher than by (2), which is not a carbon acid. Further, compared to acidic pHs, physiological pH which results in higher carbene formation also results in higher uptakes of (1) and (5). Hence, NHC formation "hydrophobizes" OIMs (1) and (5) to aid the oligomer entry into the membrane bilayer, an essential step preceding the internalization into the liposome/GUV core. Conversely, the inability of (2) to form NHC impedes the entry of the charged hydrophilic OIM into both the bilayer and core of the liposome/GUV. The enhanced translocation due to NHC in the presence of anionic lipids, as well as membrane potential, corroborates the OIM selectivity of bacteria over mammalian cells.

### Intracellular accumulation of OIM derivatives in bacteria correlates with their carbon acidity and ease of NHC formation

We then investigated the uptake of the three OIMs (1, 2 and 5) into bacteria, specifically the parental MRSA LAC strain and its respiration-deficient mutants which have low PMF. FITC-OIMs were used, and their uptake was quantified using flow cytometry (Supplementary Fig. S7). Given the higher cell density required for flow cytometry experiments ( $10^6$  CFU/mL), we determined the MIC\* values at the higher cell density. The average MIC\* values against the parental *S. aureus* strain for the three compounds were as follows:  $11\text{ }\mu\text{g/mL}$  (1),  $>4096\text{ }\mu\text{g/mL}$  (2), and  $43\text{ }\mu\text{g/mL}$  (5) (Fig. 5A), which align with the MIC values presented in Table 1. We assessed the percentage of LAC cell count with (i) total uptake (into both the membrane and the cytosol) and (ii) cytosolic uptake. For (1), the percentages of *S. aureus* strain count exhibiting cytosolic uptake, as well as total uptake, were significant at all tested concentrations (Fig. 5B), indicating that a significant portion of bacteria had taken up (1) into their cytosol. In the case of (5), the percentages of bacterial count with cytosolic and total uptake were low at low OIM concentrations but increased substantially at  $32\text{--}64\text{ }\mu\text{g/mL}$  (at its MIC\* range). Conversely, for (2), the percentages of bacterial count with cytosolic uptake remained low at all tested concentrations. (In the small cell populations with OIM (2) uptake, the amount of OIM (2)-FITC uptake into the cytosol per cell is high, resulting in high mean fluorescence intensity (MFI) (Supplementary Fig. S7H)). When correlating the results presented (Fig. 5B) with the MIC values of (1), (2), and (5) (Table 1), it became apparent that there was an inverse correlation between the compound's MIC and the percentage of LAC cell count with cytosolic uptake. Specifically, (1) and (5), which have low MIC values and are carbon acids, displayed high cytosolic uptake at the MIC values. Conversely, (2), which has a high MIC value and is a non-carbon acid, displayed low cytosolic uptake.

However, aside from the contribution of NHC formation, the enhanced uptake of (1) and (5) into bacteria may also involve the proton motive force (PMF), a mechanism we previously proposed for the uptake of the polymeric versions that have longer chains<sup>9,10</sup>. To better understand the relative contribution of these two factors (NHC formation versus PMF) to compound uptake into the bacterial cytosol, we conducted additional tests using respiration-deficient mutants with low PMF.

When comparing the MRSA LAC parental strain to the respiration-deficient mutants lacking PMF, specifically LAC  $\Delta menD$  and LAC  $\Delta hemB$ , at pH 7.2 (Fig. 5C i versus ii-iii), we observed an increased MIC\* of (1) by a factor of 10–16 when PMF was absent (while NHC was still present). This result confirms that PMF is an important factor in antibacterial efficacy. When the pH was decreased to acidic pHs of 6.8 and 6.6, the MIC\* against the parental and mutant strains increased (Fig. 5C i-iii), corroborating that NHC formation is important. The use of

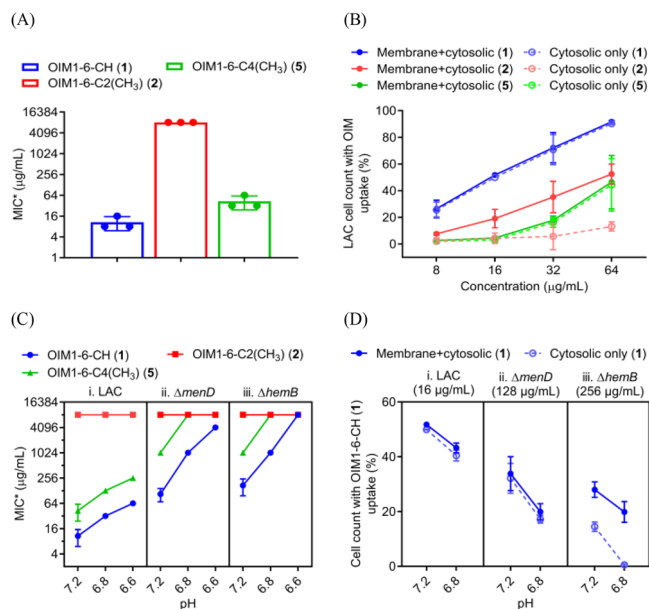


**Fig. 5 | Effect of NHC formation on OIM potency and uptake into bacteria.**

**A** MIC\* of OIMs against *S. aureus* parental strain. MIC\* is tested up to 4,096  $\mu\text{g/mL}$ , MIC\* >4096  $\mu\text{g/mL}$  is indicated as 8192  $\mu\text{g/mL}$ . **B** The percentage of LAC cell count with OIM uptake treated in TSB for 1 h. **C** MIC\* of OIMs against (i) wildtype LAC, (ii) LAC  $\Delta\text{menD}$  and (iii) LAC  $\Delta\text{hemD}$  at different pH. MIC\* is tested up to 4096  $\mu\text{g/mL}$ , MIC\* > 4096  $\mu\text{g/mL}$  is indicated as 8192  $\mu\text{g/mL}$ . **D** The cell count percentage of (i) wildtype LAC, (ii) LAC  $\Delta\text{menD}$ , and (iii) LAC  $\Delta\text{hemD}$  with OIM1-6-CH (1) uptake tested at pH 7.2 and pH 6.8 in TSB for 1 h, at their respective 1x MIC\* at pH 7.2 (wildtype LAC at 16  $\mu\text{g/mL}$ , LAC  $\Delta\text{menD}$  at 128  $\mu\text{g/mL}$ , and LAC  $\Delta\text{hemB}$  at 256  $\mu\text{g/mL}$ ). Data are presented as the mean of three biological replicates  $\pm$  standard deviation.

mutants rules out the impact of pH acting exclusively on bacterial PMF. Additionally, the percentages of bacterial count with cytosolic and total uptake were lower at pH 6.8 than at pH 7.2, which correlates with their increased MIC\* values (Fig. 5D i–iii). Therefore, inhibiting NHC formation impeded the intracellular uptake of OIM, resulting in poor killing. This test at acidic pH reinforced the importance of NHC formation for the internalization of (1).

In summary, these data indicate that the NHC formation of (1) carbon acid aids efficient bacterial cytosolic uptake. The suppression of NHC formation at acidic pH resulted in a reduction of antibacterial efficacy and reduced cytosolic uptake. The inability of (2), a non-carbon acid, to form NHC impeded its ability to reach the bacterial cytosol, greatly impairing its antibacterial efficacy. OIM (5), characterized by a weaker ability to form NHC due to its reduced carbon acidity (Supplementary Fig. S5.2), has higher MIC and lower cytosolic uptake. Taken together, these findings support the hypothesis that carbene formation by carbon acids (1), and to some extent by (5), promotes their entry into bacterial cytosol, resulting in their potent MIC values.

### OIM is efficacious in a murine systemic infection model

For murine studies, we prioritized OIM1-8-2D (12) due to its good potency and selectivity index (Table 1). We further improved this compound by exchanging the chloride counterion with 4-nitrobenzoate (4NBZate) to form compound (13) labeled as OIM1-8-2D-4NBZate (13) (Fig. 2C and Supplementary Fig. S1C), which shows improved biocompatibility while retaining high potency (Tables 1 and 2).

To assess the efficacy of our lead compound (13), we initiated experiments using a murine intraperitoneal (IP) model. In this model, mice were infected with MRSA through IP injection. Subsequently, they were treated with a single dose of 2 mg/kg OIM1-8-2D-4NBZate

(13), or 2 mg/kg vancomycin (control), or PBS via IP injection at 2 h post-infection (Fig. 6A). The untreated mice succumbed to the infection. The administration of a single dose of (13) successfully rescued all the mice and achieved CFU reduction comparable to vancomycin treatment (Fig. 6B, C and Supplementary Fig. S8.1A–C). A single IP treatment of (13) at 7 mg/kg also successfully rescued all the mice infected with carbapenem-resistant *A. baumannii* and significantly reduced the bacterial load in major organs, which was comparable to colistin (Supplementary Fig. S8.1D, E). In contrast, untreated mice treated with PBS or imipenem succumbed to the infection within 36 h. By determining the bacterial load in the liver at 26-h post-infection and comparing it to the initial load at 2-h post-infection (Fig. 6C and Supplementary Fig. S8.1E), (13) was found to exhibit bacteriostatic activity against MRSA but bactericidal activity against carbapenem-resistant *A. baumannii*. To evaluate the in vivo safety of the OIM, we subjected the mice intraperitoneally to a single dose of (13) at 16 mg/kg (Fig. 6D–F and Supplementary Fig. S8.1F, G). The weight loss, biomarkers, and histology measurements remained within the normal ranges, indicating the absence of significant toxicity at that dose. However, the administration of a single dose of 20 mg/kg exhibited some signs of nephrotoxicity (Supplementary Fig. S8.1H). For repetitive dosing of (13), IP administration of seven consecutive daily doses at 4 mg/kg showed negligible toxicity, whereas higher daily repetitive doses resulted in varying levels of inflammation (Supplementary Fig. S8.2). Further optimizations with degradable linker or formulation (for example, with enzyme-responsive carrier<sup>19</sup>) are ongoing to increase the therapeutic window of the compounds.

We then evaluated the efficacy of (13) against both Gram-positive and Gram-negative MDR strains (MRSA LAC and hypervirulent *K. pneumoniae* SGH10 respectively) in a thigh infection model (Fig. 6G). Two subcutaneous injections of (13) at 15 mg/kg, given at 2- and 4-h post-infection, resulted in 4.1 log<sub>10</sub> CFU reduction for MRSA, as compared to 2.3 log<sub>10</sub> reduction achieved with vancomycin treatment (Fig. 6H). Furthermore, compound (13) resulted in 3.9 log<sub>10</sub> CFU reduction against the MDR *K. pneumoniae* SGH10 as compared to 1.1 log<sub>10</sub> achieved with imipenem treatment using the same dosing protocol (Fig. 6I). (13) demonstrated bacteriostatic activity against both MRSA and *K. pneumoniae* SGH10 strains, as indicated by the similar CFU count at 26-h post-infection relative to the CFU count before treatment, i.e., at 2-h post-infection (Fig. 6H, I). The safety profile of (13) at a single dose of 30 mg/kg administered subcutaneously was assessed, and no significant toxicity was observed (Fig. 6J–L and Supplementary Fig. S8.3). These findings show the excellent broad-spectrum ability of (13) to eradicate bacteria in murine thigh infection models, coupled with its good biocompatibility. To correlate in vivo efficacy with drug exposure, we conducted a pharmacokinetic study using FITC-tagged OIM (13) as shown in Supplementary Fig. S8.4. Following a single-dose subcutaneous injection of 15 mg/kg, the drug exhibited a plasma elimination half-life ( $T_{1/2}$ ) of 1.2 h and reached a plasma maximum concentration ( $C_{\text{max}}$ ) of 14.4  $\mu\text{g/mL}$ . Administration of a second dose 2 h after the first sustained plasma concentrations above the MIC for ~3.3 h (from 7.5th min to 1.5th h and from 2.1th h to 4.0th h), with a brief dip slightly below the MIC for about 36 min (from 1.5th h to 2.1th h). These findings confirm that drug exposure aligns with in vivo efficacy. This is the first report demonstrating the efficacy of a cationic polymer in the thigh model against pathogenic Gram-negative MDR strains.

### OIM derivative (12) eradicates bacteria to prevent dairy mastitis in animal trial

We extended our investigations to explore the potential application of imidazolium-containing oligomer/polymer in preventing dairy mastitis, a significant global problem affecting dairy herds<sup>30</sup>. We first tested the in vitro bactericidal activity of our compounds in milk against

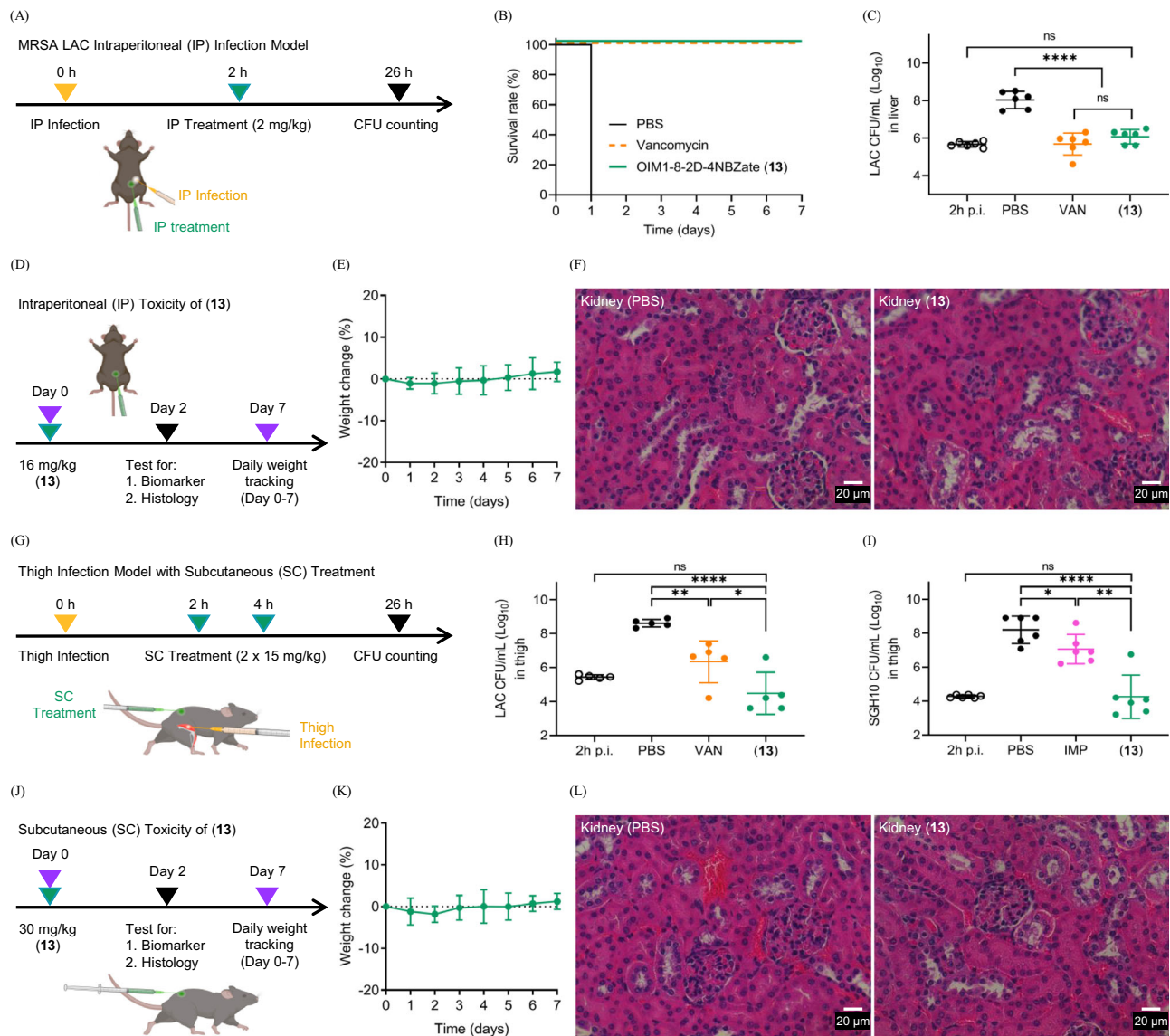


**Fig. 6 | Murine studies of secondary diamide OIMs. A** Procedure overview of intraperitoneal (IP) infection model. Mice ( $n = 6$  per group) were infected with MRSA LAC via IP injection, and treated with a single dose of PBS, or 2 mg/kg of vancomycin (VAN), or 2 mg/kg of (13) intraperitoneally at 2 h post-infection. Bacterial burdens in organs were quantified at 2 h (baseline without treatment) and 26 h post-infection, respectively. **B** Survival rate and **C** bacterial log reduction in liver in MRSA IP infection model. **D** Biocompatibility evaluation of (13) administered via IP route. 16 mg/kg of (13) was injected into mice via IP route. The weight of the mice ( $n = 6$ ) was recorded for 7 days. On day 2 post-administration, clinical biomarkers ( $n = 6$  per group) and histology ( $n = 3$  per group) of kidney and liver of the treated mice were assessed. **E** Weight loss and **F** kidney histology of mice treated with 16 mg/kg of (13) intraperitoneally. **G** Procedure overview of thigh infection model. Mice were infected intramuscularly at their left thighs. Mice were

treated with PBS, or 15 mg/kg of antibiotic control (VAN for MRSA LAC and imipenem (IMP) for *K. pneumoniae*), or 15 mg/kg of (13) administered at 2- and 4-h post-infection via subcutaneous (SC) route. Bacterial counts in thighs were quantified at 2 h (baseline) and 26 h post-infection, respectively. Bacterial load of **H** MRSA LAC ( $n = 5$  per group) and **I** *K. pneumoniae* ( $n = 6$  per group) in the thigh muscle. **J** Biocompatibility evaluation of (13) given via SC route. 30 mg/kg of (13) was injected into mice via SC route. The biocompatibility tests were assessed as described in **(D)**. **K** Weight loss and **L** kidney histology of mice treated with 30 mg/kg of (13) subcutaneously. Data are presented as mean  $\pm$  standard deviation. Unpaired two-sided *t*-test, \*\*\*\* $p < 0.0001$ , \*\* $p < 0.0021$ , \* $p < 0.0331$ . ns (not significant)  $p > 0.05$ . Exact *p* values are provided in Source Data. For **F** and **L**, white line represents scale bar. Illustrations were created in BioRender. Chan, M. (2025) <https://BioRender.com/vgp81qe>.

mastitis-causing pathogens, specifically *E. coli* 10536, *S. aureus* 6538, and *S. uberis* 19436, following the industrial standard BS EN 1656<sup>21</sup>. Effective test compounds are required to achieve a minimum of 5 log<sub>10</sub> reduction of viable bacteria within 30 min or less. The control drug chlorhexidine exhibited poor performance, failing to achieve the required 5 log<sub>10</sub> reduction for the two Gram-positive bacteria (Supplementary Fig. S9.1A), while (12) fell just short with a 4.55 log<sub>10</sub> reduction for *S. uberis* (Supplementary Fig. S9.1B). We then explored the longer polymer version of (12), called PIMID, a compound we had previously reported<sup>9</sup>. PIMID met the 5 log<sub>10</sub> reduction requirement for

all three bacteria (Supplementary Fig. S9.1C). Moving from laboratory testing to practical application, a farm trial was conducted using PIMID as a post-milking teat dip antibacterial agent to prevent dairy mastitis infection (Fig. 7A and Supplementary Fig. S9.2). Prior to this, we assessed the safety of PIMID-based teat dip and confirmed that it did not cause adverse effects on the cows (Supplementary Information Section 9.3 and Supplementary Fig. S9.3). In the mastitis challenge trial, we determined the onset of intramammary infection (IMI) based on bacteria count ( $>500$  CFU/mL) and somatic cell count (SCC,  $>200,000$ /mL) in milk samples<sup>22</sup>. According to both indicators, PIMID-

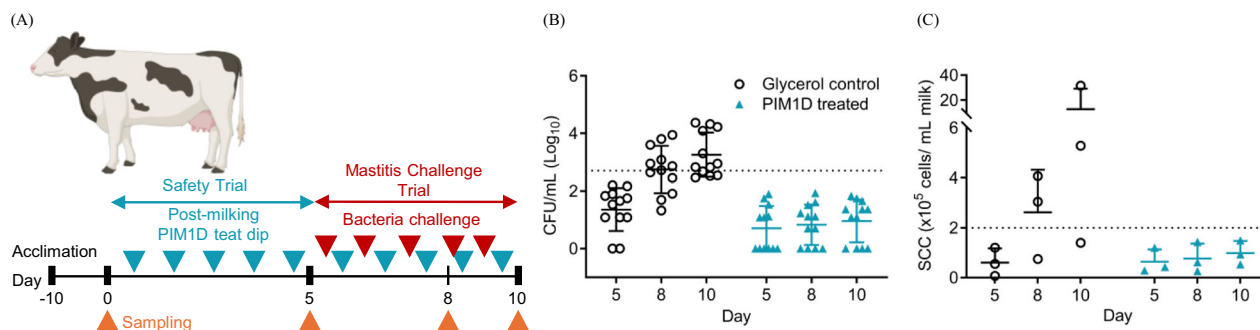


**Fig. 7 | In vivo dairy mastitis testing.** **A** Timeline and experimental setup of in vivo farm trial. **B** Bacterial count in milk treated with glycerol control or PIM1D over time upon bacteria challenge. Each symbol ( $n = 12$  per group) represents one milk sample from one teat (also known as one quarter). The dotted line indicates the threshold CFU count (500 CFU/mL) for mastitis infection. **C** Somatic cell count

(SCC) in milk samples ( $n = 3$  per group) treated with glycerol control or PIM1D upon bacteria challenge. Each symbol represents the sample from one cow. Data are presented as mean  $\pm$  standard deviation. Illustrations were created in BioRender. Chan, M. (2025) <https://BioRender.com/vgp81qe>.

based teat dip successfully prevented mastitis infection upon repeated exposure to *S. aureus*, whereas cows treated with glycerol control developed mastitis over time (Fig. 7B, C). Importantly, the PIM1D-based teat dip did not affect the milk composition (Supplementary Fig. S9.4).

## Discussion

Our study underscores the pivotal role of carbon acidity in the antibacterial properties of OIMs, particularly those containing deprotonable C(2)-H groups (**1**, **5-9**, and **11-13**). The carbon acids (**1**, **5-9**, and **11-13**) exhibit robust antibacterial efficacy, while non-carbon acids lacking C(2)-H (**2-4**, and **10**) show considerably reduced effectiveness. Compared with classical advanced cationic polymers that we and others have invented, the OIM carbon acids have MICs that can be much lower (about one order of magnitude lower) and/or have broad spectrum potency with good toxicity profile<sup>6,7,23</sup>. We propose an NHC-mediated translocation mechanism (Fig. 4G) that aids the efficient entry of OIM carbon acids into bacterial cytosol so that they can efficiently reach their intracellular DNA target<sup>10</sup>. The model attempts to mimic the details (i.e., anionic lipids and membrane potential) of bacterial membranes.

NHCs are formed by carbon acids in physiological pH water (Fig. 4G i). The disappearance/deprotonation of the C(2)-H of (**1**) and (**5**) in neutral aqueous water was shown by <sup>1</sup>H NMR, corroborating their carbon acidity. The NHCs formed from OIM carbon acid (**1**) are detectable within the aprotic membrane bilayer with the AuCl(SMe<sub>2</sub>) and TCNQ probes as well as carbazole dye, corroborating that the carbon acids efficiently penetrate bilayer membranes with NHC formation (Fig. 4G ii). Compound (**1**) exhibited a greater propensity for NHC formation compared to (**5**) and had a lower  $pK_a$  of 21.32 than 22.15 (Supplementary Table S2). The higher NHC formation ability of (**1**) than (**5**) is corroborated by the higher amount of NHC detected with these probes.

Further, the hexane-water system (Fig. 4A–C) corroborated that OIM carbon acids selectively translocate across bacterial membrane, and not mammalian membrane, due to the anionic lipid components in bacterial membrane. The imidazolium charge groups form ion pairs effectively with anionic lipids and the ion pair appears to be needed for the OIM effective translocation into the hydrophobic interior of the bacterial bilayer. However, NHC formation of (**1**) and (**5**) enhances the ion-pair translocation into the lipid bilayer, as the translocation of (**2**) is much less (Fig. 4C).

The classical cationic polymers containing ammonium charge group cannot enter the bacterial plasma membrane as the charged polymers are hydrophilic. Above certain critical cationic charge concentrations, the cationic polymers form pores or holes in the

membranes which then lead to cell death<sup>24</sup>. The threshold concentrations for pore formation are typically higher, so the MIC values are usually tens of  $\mu\text{g/mL}$ . With the unique NHC-mediated entry into the membrane, no pores or holes are needed for the entry into the membrane. The OIM carbon acids (**1** and **5**) enter the vesicle core/bacterial cytosol at lower cationic charge concentrations than (**2**). The OIM (**2**) cannot enter the bacterial cytosol or liposome/GUV cores (Figs. 4D, F and 5B respectively) as compared with (**5**) or (**1**) and has much higher MIC as it cannot form NHC that increases its hydrophobicity. The OIM (**1**), though likely to be more hydrophilic than the methyl-substituted derivatives (**2**) and (**5**), can enter the membrane and vesicle core/bacterial cytosol more effectively, as it effectively forms NHC to enter the membrane bilayer through membrane translocation. The OIM transforms via NHC formation from an initially hydrophilic to an amphiphilic copolymer which is composed of the hydrophilic imidazolium cation repeats and also the hydrophobic imidazol-2-yl carbene repeats, to achieve effective membrane entry (Fig. 1B). The positively charged nature of the cationic OIMs causes their electrostatic attraction to the negatively charged membrane surface of bacteria-mimicking vesicles, while the hydrophobic NHC repeats aid the insertion of OIMs into the vesicle membrane, as supported by our simulation data (Supplementary Fig. 6.2).

After the OIMs enter into the lipid bilayer, some then exit it and enter the vesicle core/bacterial cytosol (Figs. 4D–F and 5B). The NHC is short-lived and is probably easily quenched, particularly with a favorable concentration gradient and membrane potential, as it migrates to the inner leaflet of the bilayer due to thermal diffusion or membrane potential (Fig. 4G iii). At the inner leaflet, it may regain a proton from water. Alternatively, it may also gain a proton from the electron transport chain. After the NHC quenching, the OIM will regain its hydrophilicity and will exit the hydrophobic membrane into the bacterial cytosol (Fig. 4G iv). We showed that the entry of (**1**) into the liposome cores at physiological pH was much higher than at acidic pH (Fig. 4D), corroborating the NHC importance for internalization. Further, the uptake of (**2**) into the GUV core even with membrane potential is much less compared with (**1**) and (**5**) (14 versus 100 and 28 at the 24th h respectively) (Fig. 4E, F), further corroborating the importance of NHC for the OIM uptake into the GUV core, as well as bacterial cytosol. Figure 4G proposes an efficient mechanism for membrane entry of cationic polymers that are also carbon acids, through NHC formation.

Regarding the translocation of OIMs from the membrane into the liposome core (Fig. 4G iv), it is worth noting that the quantity of (**1**) and (**5**) entering the liposome core is smaller in comparison to their presence in the membranes (Fig. 4D). In live bacteria which have a functional PMF, we show that the membrane potential across the

membrane favors the translocation of OIM-NHC into the cytosol. Alternatively, the acidic protons of cycling reduced quinone shuttlers of redox-active electron transport chain (ETC) might induce the quenching of OIM carbene into cationic OIM within the bacterial membrane, rendering it hydrophilic again. This shift towards increased hydrophilicity could drive OIMs to exit the hydrophobic membrane and enter the cytosol. Regardless of the precise transport mechanism from the membrane to the cytosol, our research demonstrates that carbon acids, which form NHC, successfully infiltrate the bilayer membrane and then exit it to achieve high cytosolic uptake, ultimately reaching their intracellular targets to result in rapid bacterial killing<sup>10</sup>. Our mechanism proposes that the facile NHC formation of (**1**) facilitates its internalization into the bacterial cytoplasm, even under conditions of low PMF, as evidenced by experiments with respiration-deficient mutants.

Moreover, our optimized secondary diamide compounds (**12**) and (**13**) exhibit low minimum inhibitory concentrations (MICs) against multi-drug-resistant bacteria. In murine infection, sepsis, and thigh infection models, the OIM (**13**) demonstrated substantial efficacy against multi-drug-resistant MRSA LAC, *A. baumannii*, and *K. pneumoniae* bacteria. Additionally, in a field trial, the polymeric version of (**12**), PIM1D, was successfully employed to prevent dairy mastitis infection, with no adverse effects observed in the cows.

This class of carbene-forming cationic oligomers with potent antimicrobial activities which is facilitated by efficient membrane translocation, holds immense promise, given the various possibilities for modifying the ring and linker structures. These compounds can be further developed and optimized for biomedical and agricultural applications to combat multi-drug-resistant bacteria, adding to their versatility.

## Methods

### Bacterial culture

All bacterial strains annotated with “ATCC” were purchased from American Type Culture Collection (ATCC). *Pseudomonas aeruginosa* PAO1, *Enterococcus faecalis* 583 (VRE), and *E. coli* 958 (MDR) were obtained from Singapore Center for Environmental and Life Sciences (SCELSE). Clinical isolates, PAER (multi-drug-resistant *P. aeruginosa*), AB-1 (multi-drug-resistant *A. baumannii*), and KPNR (carbapenem-resistant *K. pneumoniae*) were obtained from Tan Tock Seng Hospital (TTSH) Singapore. *K. pneumoniae* SGH10 was provided by National University of Singapore. MRSA LAC, LAC\*, LAC  $\Delta$ menD, and LAC  $\Delta$ hemB have been described previously<sup>25</sup> and were provided by Angelika Gründling, Imperial College London. *B. thailandensis* 700388 was provided by Samuel I. Miller, University of Washington. All broth or agar media used here were purchased from Becton Dickinson Company. The bacteria strains were stored in 15% glycerol at  $-80^{\circ}\text{C}$ .

### Minimum inhibitory concentration (MIC)

MICs were determined according to standard broth microdilution method with slight modifications<sup>26</sup>. Briefly, a single colony was picked and inoculated in MHB to obtain an overnight culture. A subculture was prepared the next day and grown to exponential phase. A two-fold serial dilution of the test compound in 50  $\mu\text{L}$  MHB was prepared in a 96-well plate, followed by the addition of 50  $\mu\text{L}$  exponential phase bacteria at a concentration of  $5 \times 10^5$  CFU/mL. The solution in the plate was thoroughly mixed by shaking the plate vigorously for 20 s, and the plate was subsequently incubated statically at  $37^{\circ}\text{C}$  for 18 h. OD<sub>600</sub> readings were taken and the minimum concentration that inhibited bacterial growth by 90% (MIC) was determined. For the MIC determination of *E. faecalis* 583 (VRE), the overnight culture and subculture were prepared as described above but in TSB, and the MIC was tested without and with supplementation of 8  $\mu\text{g/mL}$  hemin. The data are representative of three independent experiments.

### Cell cytotoxicity MTT assay

In vitro biocompatibility was investigated using mouse fibroblast cells (3T3), human embryonic kidney (HEK293) cells, and human hepatocellular carcinoma (HepG2) cells purchased directly from ATCC. Briefly, Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin/streptomycin) was used to culture 3T3 and HepG2 cells. To culture HEK, 15% FBS supplementation was used. All cells were incubated at  $37^{\circ}\text{C}$  in a  $\text{CO}_2$  incubator. When 80% confluence was observed under microscopy, cells were treated with trypsin, concentrated, and counted using a hemocytometer.  $1 \times 10^4$  cells/well were then seeded into a 96-well plate. Cells were grown for 24 h and subsequently challenged for 24, 48, and 72 h with varying concentrations of compounds (total volume, inclusive of drug, was 200  $\mu\text{L}$ /well). Cell health condition was then qualitatively checked by microscopy and quantified using an MTT (3-[4, 5-dimethylthiazoyl-2-yl]-2, 5-diphenyl tetrazolium bromide) assay. The cell viability was calculated by comparing the absorbance of formazan formed by the cells in the challenge wells and in the untreated wells. The compound concentration which caused 50% reduction of cell growth compared to untreated control (IC<sub>50</sub>), was determined. The data are representative of three independent experiments.

### Time-kill assay

The killing kinetics of OIMs were determined via time-kill assay. Exponential phase bacteria were diluted to  $5 \times 10^5$  CFU/mL and treated with varying concentrations of OIMs with constant shaking at  $37^{\circ}\text{C}$ . Aliquots of bacterial samples were collected at specific time intervals (30 min, 1 h, 2 h, 3 h, 4 h, 6 h, and 24 h) and serially diluted with PBS. The serially diluted samples were then spotted onto agar plates, and the number of colonies was counted after overnight incubation. The data are representative of three independent experiments.

### Bacterial cytoplasmic membrane depolarization and membrane integrity assay

The membrane potential-sensitive dye 3,3'-Dipropylthiadicarbocyanine Iodide (DiSC<sub>3</sub>(5)) was used to determine the membrane depolarization activity of OIMs. The assay was performed following previously described protocols with minor modifications<sup>27,28</sup>. Exponential phase MRSA LAC was pelleted, washed twice with 5 mM HEPES containing 5 mM glucose, and resuspended to  $4 \times 10^7$  CFU/mL. DiSC<sub>3</sub>(5) was added at a final concentration of 0.5  $\mu\text{M}$ , and 175  $\mu\text{L}$  of bacterial suspension was aliquoted into a white 96 well plate. The fluorescence was monitored using a Spark 10 M plate reader (Tecan, Switzerland) at an excitation/emission wavelength (Ex/Em) of 622 nm/670 nm every 2 min with continuous shaking. DiSC<sub>3</sub>(5) accumulates in the bacterial membrane, resulting in the quenching of the fluorescence signal. Once a stable baseline reading was obtained, OIMs were added at varying concentrations in 25  $\mu\text{L}$ , and the fluorescence reading was recorded for 1 h. Gramicidin was used as a positive control. For the membrane integrity assay, LAC was prepared as described above, and propidium iodide (PI) was added at 1  $\mu\text{M}$ . The PI fluorescence signal was measured before the addition of OIMs and nisin (positive control) to establish a baseline and then recorded for 2 h post-treatment at Ex/Em of 535 nm/620 nm using the Tecan plate reader. The data are representative of three independent experiments.

### Hydrogen-deuterium exchange of OIMs measured by $^1\text{H}$ NMR

PBS buffers at desired pHs were prepared by adjusting the ratio of  $\text{K}_2\text{DPO}_4$  (0.1 M stock concentration) and  $\text{KD}_2\text{PO}_4$  (0.1 M stock concentration) according to previous study<sup>29</sup>. All reactions were carried out in  $\text{D}_2\text{O}$  at ionic strength = 1.0 (KCl). Briefly, OIMs were dissolved in PBS at 10 mM (concentration of oligomer repeat units) with 10 mM TMA (tetramethylammonium hydroxide) as internal standard.

Deuterium exchange was measured by  $^1\text{H}$  NMR spectroscopy<sup>11</sup> with a Bruker 400 MHz NMR at 25 °C.

### NHC capture by carbazole probe

**(i) Carbazole(lipids) preparation.** Stock solutions of phosphatidylglycerol (PG; 10 mg/mL,  $1.3 \times 10^{-5}$  mol/mL), phosphatidylcholine (PC; 10 mg/mL,  $1.3 \times 10^{-5}$  mol/mL), and carbazole (2.2 mg/mL,  $1.3 \times 10^{-5}$  mol/mL) were prepared in chloroform. A mixture of PC/PG/carbazole at molar ratio of 8: 2: 1 was prepared by mixing 0.8 mL of PC, 0.2 mL of PG, and 0.1 mL of carbazole stock solutions in a round-bottom flask (25 mL). The chloroform was removed by rotary evaporation at 50 mbar, 20 °C for 20 min. The lipid film was hydrated with 1 mL of PBS buffer (pH 7.4). The suspension was then vortexed at top speed for 1.5 min, followed by 2 min of sonication in ice bath. This vortex-sonication process was performed three times, and the solution was then extruded 19 times through a 200 nm polycarbonate membrane with an Avanti mini-extruder. The resulting liposome solution was dialyzed against water with MWCO 2000 tube for 36 h to remove free carbazole. The size distribution and zeta-potential of the resulting liposomes were checked with a Malvern Nano Series Nano-ZS instrument (data not shown). All prepared liposome solutions were stored at 4 °C prior to use. **(ii) Fluorescence measurement of carbazole(lipids)-OIM.** OIM stock solutions (1, 2, and 5) were prepared in  $\text{H}_2\text{O}$  at 10 mg/mL. The solution was adjusted to pH 7.4 with 1 M NaOH. Stock solution of IPr (50 mg/mL in DMF) was prepared. Carbazole(lipids)-OIM was prepared by mixing 10  $\mu\text{L}$  of carbazole(lipids), 100  $\mu\text{L}$  of OIM stock, and 890  $\mu\text{L}$  of PBS (pH 7.4). Carbazole(lipids)-IPr, used as a positive control, was prepared by mixing 10  $\mu\text{L}$  of carbazole(lipids), 10  $\mu\text{L}$  of IPr stock, and 980  $\mu\text{L}$  of PBS (pH 7.4). All the mixtures were incubated at 37 °C for 36 h prior to fluorescence measurement. Emission fluorescence spectra were obtained at an excitation wavelength of 295 nm.

### NHC capture by AuCl(SMe<sub>2</sub>) probe

**(i) Au(lipids) preparation.** Au(lipids) was prepared by the same protocol used for carbazole(lipids), replacing carbazole solution with the AuCl(SMe<sub>2</sub>) stock solution (3.82 mg/mL,  $1.3 \times 10^{-5}$  mol/mL), with PC/PG/AuCl(SMe<sub>2</sub>) molar ratio (8: 2: 1). After vortex-sonication, no extrusion was performed because of the sensitivity of AuCl(SMe<sub>2</sub>). Hydrophobic AuCl(SMe<sub>2</sub>) that was not trapped within liposome bilayer was removed by centrifugation at  $4000 \times g$  for 20 min at 10 °C. The resulting pellets were washed with water once. It was very important to remove untrapped AuCl(SMe<sub>2</sub>) immediately after Au(lipids) was prepared, since free AuCl(SMe<sub>2</sub>) molecules would react with glycerol groups of PG to form Au nanoparticles, leading to a purple solution that was not suitable for further study. **(ii) Formation of Au-OIM in liposome.** Au(lipids) pellets obtained after washing were dispersed in 10 mL of 0.1× phosphate buffer (pH 7.4), followed by adding OIM stock solution at final concentration of 75  $\mu\text{g/mL}$ . The mixture was incubated at 37 °C in the dark for 48 h. Then the mixture was freeze-dried. The dried powder was dissolved in 1 mL of methanol. The addition of methanol would break the liposome structure, thus releasing Au-OIM into the solution. The solution was centrifuged at  $8000 \times g$  for 30 min at 10 °C to remove any Au nanoparticles. The resulting solution was subjected to chloroform-methanol-water extraction to remove lipids<sup>30</sup> and AuCl(SMe<sub>2</sub>) before LC-MS measurement. **(iii) LC-MS characterization.** Mass spectra were recorded by an Agilent Q-TOF/MS (6550 iFunnel) machine. Electrospray ionization (ESI) mass spectroscopy (MS) positive ion mode was used. C18 column (50 mm  $\times$  2.1 mm i.d., 1.8  $\mu\text{m}$ , Agilent) was used at 35 °C for all the analyses. The mobile phase consisted of a linear gradient system of (A) water (0.1% formic acid) and (B) acetonitrile (0.1% formic acid). The gradient conditions of the mobile phase were as follows: 0–2 min, 95% A; 2–3.5 min, 95–30% A; 6.5–8.5 min, 30–95% A, 8.5–13.5 min, 95% A. The flow rate was 0.2 mL/min. The injection volume was 5  $\mu\text{L}$ . **(iv) Structural confirmation of**

**Au-OIM with deuterium-hydrogen exchange.** The sample was prepared as stated above ((iii) LC-MS characterization), a drop of  $\text{D}_2\text{O}$  was added to replace the active hydrogen with deuterium, and then the sample was analyzed by LC-MS in the same conditions. **(v) Structural confirmation of Au-OIM (1) with MS-MS.** The target ion  $m/z$  330.52 (Au-OIM-(1)) in the previous sample prepared as stated above ((iii) LC-MS characterization) was targeted with collision energy (collision-induced dissociation, 10 V) by LC-MS to get the fragments from the target ion.

### NHC Capture by TCNQ probe

**(i) Generation of positive control of NHC from CPE.** All Controlled Potential Electrolysis (CPE) experiments were conducted using a Metrohm Autolab PGSTAT302N potentiostat, with control provided by NOVA<sup>31</sup>. The experiments were performed at a potential of  $-2.4\text{ V}$  with respect to the Ag wire pseudoreference electrode in 0.1 M of *n*-Bu<sub>4</sub>NPF<sub>6</sub> solution in DMSO to facilitate the reduction of imidazolium to carbene. The electrolyte solution consisted of 0.1 M Tetrabutylammonium hexafluorophosphate in DMSO. Glassy carbon electrodes served as both the working and counter electrodes, while an Ag wire was employed as the reference electrode. **(ii) Determination of EPR signals from OIM-NHC with TCNQ(lipids).** The preparation of TCNQ(lipids) followed a previously published protocol with slight modifications<sup>32</sup>. Specifically, 132  $\mu\text{L}$  TCNQ (0.3 mg/mL in acetonitrile) was added to 800  $\mu\text{L}$  liposome solution (640  $\mu\text{L}$  PC, and 160  $\mu\text{L}$  PG, both lipids were dissolved in chloroform at 10 mg/mL). A 50:1 molar ratio between lipids and TCNQ was maintained to preserve the bilayer integrity. Then, the solution was dried by rotary evaporation for 20 min at 40 °C and rehydrated using 1 mL of PBS buffer (pH 7.4). The TCNQ(lipids) stock solution was then vortexed for 2 min and sonicated for 2 min, with this process repeated three times to ensure homogeneity. Additionally, a 200  $\mu\text{M}$  solution of OIM in PBS buffer at pH 7.4 was prepared. All stock solutions were stored at 4 °C for future use. Subsequently, 150  $\mu\text{L}$  of OIM solution or PBS buffer was added to 150  $\mu\text{L}$  of TCNQ(lipids) or blank liposome stock solution. The samples were incubated at 37 °C for 30 min, followed by immediate measurement of electron paramagnetic resonance (EPR) signals by a Bruker ELEXSYS E500 EPR spectrometer.

### Translocation of OIM into hexane/water model system

**(i) Measurement via UV-VIS of FITC-OIM.** Thirty microliters of PG (2.5 mg/mL in chloroform) was added into 270  $\mu\text{L}$  of hexane in a 1.5 mL centrifuge tube to mimic the lipid bilayer (interior). Three hundred microliters of FITC-OIM (0.1 mg/mL in PBS, pH 7.4) was added to the tube to mimic the external water phase<sup>33</sup>. After vigorous vortexing and standing for 15 min, the water phase solution was diluted 2 times with PBS, and the UV-VIS spectra were measured by a SHIMADZU UV-1800 UV spectrophotometer. The calibration curves of each FITC-OIM were measured with different concentrations of FITC-OIM in PBS. **(ii) Measurement via LC-MS.** 30  $\mu\text{L}$  of PG (2.5 mg/mL in chloroform) was added into 270  $\mu\text{L}$  of hexane, followed by 300  $\mu\text{L}$  of OIM (0.1 mg/mL in PBS, pH 7.4) in a 1.5 mL centrifuge tube<sup>33</sup>. After vigorous vortexing and standing for 15 min, the water phase solution was diluted 2 times with PBS and measured with LC-MS (Agilent Q-TOF/MS (6550 iFunnel)). The calibration curves of each OIM were measured by varying OIM concentrations of 2, 4, and 6  $\mu\text{g/mL}$  in PBS.

### OIM uptake in liposomes

**(i) Uptake assay in liposome membrane and core.** Liposomes were prepared by the same protocol used for carbazole(lipids) with the exception that no carbazole was added. Liposome stock (10 mg/mL), OIM stock (10 mg/mL), and water were mixed to make 1 mL solution with final liposome and OIM concentration at 100 ppm and 5000 ppm, respectively. After incubation at 37 °C for 20 h, free OIM was removed by Tangential Flow Filtration (TFF) (MidiKros® Hollow Fiber Module,

MWCO 3 kDa) with syringes through 30 cycles of extrusion with  $0.2 \times$  concentration of PBS. The resulting solution (around 1 mL) was split into two equal portions. One portion was freeze-dried to measure total uptake of OIM in the membrane and the core. The freeze-dried powder was dissolved in methanol and lipids were removed by chloroform-methanol-water extraction<sup>30</sup>. The top fraction (water/methanol phase) was collected for LC-MS measurement. The other portion (around 0.5 mL) was added into 0.17 mL ethanol (final ethanol concentration 25% v/v) and incubated at room temperature for 2 h to damage the membrane's integrity, thus releasing OIM in the core. OIM released from the core and OIM trapped in the bilayer were separated by Vivaspinn (MWCO 30 kDa) with centrifugation at  $4000 \times g$  at 25 °C for 30 min, as OIM released from core would pass through the Vivaspinn membrane while OIM trapped in the liposome bilayer would be retained at the membrane due to the size of the liposomes. **(ii) Calibration curves of OIMs measured by LC-MS.** To obtain an accurate calibration curve, we prepared lipid matrix with the same procedures used in the uptake experiments. Specifically, 200  $\mu$ L of liposome stock, containing 0.4 mg PG and 1.6 mg PC, was freeze-dried. After that, 16 mL methanol was added to dissolve the liposomes, and then 14.4 mL DI water, 16 mL chloroform, and 46.4  $\mu$ L formic acid (final concentration 0.1% v/v) were added to remove lipids to avoid any damage to the C18 column. After centrifugation, the supernatant (top phase) was collected as the lipid matrix. OIM1-6-CH (**1**), OIM1-6-C2(CH<sub>3</sub>) (**2**), and OIM1-6-C4(CH<sub>3</sub>) (**5**) were prepared with the lipid matrix at 0, 1, 2, 3, and 4 ppm to build calibration curves. These standard samples were then tested with an Agilent Q-TOF/MS (6550 iFunnel) machine. Results are shown in Supplementary Fig. S6.1.

### Molecular dynamics simulation of the interaction of cationic versus NHC forms of OIM1-6-CH with model *S. aureus* membrane

**(i) Membrane construction.** A 286-lipid membrane bilayer representative of the *S. aureus* membrane composition, comprising 58% phosphatidylglycerol (PG) and 42% Cardiolipin (CL)<sup>34</sup>, was constructed using the CHARMM-GUI<sup>35</sup> membrane builder tool. DMPG (14:0/14:0) and TMCL2 (14:0,14:0/14:0,14:0) forms of PG and CL were used, respectively. The distribution of PG and CL lipids on the upper and lower leaflets was symmetrically constructed. Menaquinone-8 (MQ8) molecules were constructed on the membrane model. Parameters of membrane lipids were based on the CHARMM36 force field and parameters of the MQs and OIM1-6-CH (**1**) were based on the CHARMM General Force Field<sup>36,37</sup>. Topologies of MQ8 molecules were generated using the CHARMM-GUI ligand reader and modeler<sup>38</sup> tools, with adopted ratio of total lipid: total MQ8 = 10:1. An equal number of MQ molecules were manually inserted randomly into the upper and lower leaflet of the constructed *S. aureus* membrane. Manual adjustments of coordinates and energy minimization steps were subsequently performed to remove structural clashes between atoms. The membranes were solvated with TIP3P<sup>39</sup> water molecules and counterions were added to neutralize the system. The constructed *S. aureus* membranes were subjected to a 100 ns molecular dynamics (MD) simulation using GROMACS<sup>40</sup> 5.1.2 software. The LINCS<sup>41</sup> algorithm was used to constrain bonds between heavy atoms and hydrogen to enable a timestep of 2 fs. A 1.2 nm cutoff was used for Van der Waals interaction and short-range electrostatic interactions calculations, and Particle Mesh Ewald method was implemented for long-range electrostatic calculations. Simulation temperature was maintained at 310 K using a V-rescale thermostat<sup>42</sup> and 1 bar pressure using Parrinello-Rahman<sup>43</sup> barostat. **(ii) Simulation of OIM1-6-CH (**1**) on *S. aureus* membranes.** Coordinates of the cationic and NHC forms of the OIM1-6-CH (**1**) were constructed using Discovery Studio 4.1<sup>44</sup>. Topologies of (**1**) were obtained from the CHARMM-GUI ligand reader and modeler<sup>38</sup>. Partial charges of the NHC carbene forms were calculated from Gaussian09<sup>45</sup> in the triplet state at the level of HF 6-31 G\* and RESP<sup>46</sup> fitting. The last simulation frame from the previous 100 ns molecular dynamics

simulation run of *S. aureus* membranes was used for the simulation of the OIM on the membrane. Eight simulation systems were set up of the cationic OIM or OIM-NHC on *S. aureus* membrane. A single oligomer molecule was placed 0.8 nm above the center of the upper leaflet of the membrane bilayer. Each system was subjected to classical molecular dynamics simulations for 200 ns. MD simulations were performed with settings similar to those described in the previous section. **(iii) Analysis of simulation.** The number of contacts between the OIM and the membrane as a function of simulation time was calculated to analyze the behavior of interactions between the oligomer and membrane. Contact number calculations were performed using the GROMACS<sup>40</sup> gmx mindist tool. A contact was defined if the minimum distance between the OIM atom and membrane atom was less than 0.4 nm. To further visualize the binding between OIM and the membrane, the last frame of each simulation system was visualized using pymol.

### Translocation of OIM into GUV

**(i) Induced leakage assay.** GUV containing Sulforhodamine B (SRB) was prepared according to previous studies<sup>47,48</sup>. Briefly, a 10% (w/v) solution of polyvinyl alcohol (PVA,  $M_w$  = 146 kDa–186 kDa, 99+% hydrolyzed, Sigma-Aldrich, USA) was prepared by vortexing PVA in water while heating at 90 °C. After PVA was fully dissolved in water, 100  $\mu$ L of PVA solution was spread on a clean microscope coverslip. The excess of PVA solution was removed from the coverslip to form a thin film, and the thin film was dried for 30 min on a hotplate at 50 °C. 10  $\mu$ L of PC/PG (1 mg/mL in chloroform) with molar ratio of 4/1<sup>17</sup> was spread on the dried PVA film and placed under vacuum for 30 min to remove the solvent completely. The dried lipid film was incubated with an aqueous solution of 200 mM sucrose containing 0.1 mM SRB in a homemade chamber for 3 h at room temperature. The GUVs were harvested by pipetting and kept in 4 °C prior to use. **(ii) OIM translocation in the presence of membrane potential.** GUVs were prepared by following the above procedure except incubating the dried lipid film with KPBS buffer (pH 7.4) containing 100 mM sucrose. GUVs were washed twice ( $300 \times g$ , 5 min) with NaPBS buffer containing 100 mM glucose. KPBS buffer and NaPBS buffer were prepared according to a previous study<sup>49</sup>. 8  $\mu$ L of GUV solutions, 8  $\mu$ L of NaPBS buffer containing 100 mM glucose, 2  $\mu$ L of valinomycin (0.14  $\mu$ g/mL in NaPBS buffer), 2  $\mu$ L of FITC-OIMs (20 ppm in NaPBS buffer) were added into a small centrifuge tube (0.2 mL), then the mixture was incubated in the dark at 37 °C for predetermined times (1, 4, 8, 12, 18, and 24 h). The final concentration of valinomycin and FITC-OIMs was at  $1/10^4$  molar ratio (mol/mol lipid) and 2 ppm, respectively. After incubation, the GUVs were washed with 180  $\mu$ L of NaPBS buffer containing 100 mM glucose 4 times ( $300 \times g$ , 5 min) to remove the free FITC-OIMs. The resulting GUVs were observed with a fluorescence microscope. The fluorescence images were edited by subtracting the background with MetaMorph Microscopy Automation and Image Analysis Software. The fluorescence intensity inside of the GUVs was averaged by 30 GUVs.

### OIM-FITC uptake assay

The OIM-FITC uptake experiment was conducted as described previously<sup>50–52</sup>. Exponential phase bacteria were prepared and diluted to  $10^6$  CFU/mL in TSB, then incubated with FITC-conjugated OIM (7.5% molar ratio of OIM-FITC) at desired concentrations for 1 h in the dark. The cultures were centrifuged ( $10,000 \times g$ , 10 min) and washed twice with filter-sterilized PBS. Bacterial pellets were resuspended in 800  $\mu$ L PBS. The fluorescence of 150  $\mu$ L of each sample was directly measured using a flow cytometer (BD FACSVerse Flow Cytometer (3) Laser) via the FITC channel, and this data represents the unquenched uptake (Supplementary Fig. S7). A 300  $\mu$ L aliquot of each remaining sample was quenched with 0.01% Trypan blue (TB) dye before measurement; the resulting fluorescence data reflects the total OIM uptake (i.e.,

bacterial membrane and cytosol). Another 300  $\mu\text{L}$  aliquot of the remaining sample was treated with 0.04% Triton X-100 (TX) for 15–20 min followed by TB quenching; the resulting data reflects the uptake in the membrane fraction. Histogram subtraction of the total uptake minus the uptake in the membrane fraction provides the cytosolic uptake fraction. Detailed experimental methods and the flow cytometry histograms are provided in Supplementary Fig. S7. Bacteria without OIM-FITC treatment served as negative control.

### Murine in vivo studies

C57BL/6J female mice at 9–10 weeks old, obtained from Jackson Laboratories (purchased through InVivos Pte Ltd), were used for all experiments. Mice were housed under specific pathogen-free conditions on a 12-h light/dark cycle with controlled temperature and humidity, and had ad libitum access to food and water. **Murine intraperitoneal (IP) infection model.** The animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Nanyang Technological University (AUP A20029). For IP infection, exponential phase cultures of MRSA LAC or *A. baumannii* ATCC BAA 2803 were washed twice with PBS and resuspended to  $10^7$  CFU/mL and  $10^5$  CFU/mL respectively in 5% mucin saline solution. Mice ( $n=6$  for each experimental group) were injected with 300  $\mu\text{L}$  of bacterial suspension via IP route, and the bacterial counts in peritoneal fluid, livers, kidneys, and spleens at 2 h post-infection were determined. For treatment of MRSA LAC infection, a single dose of 2 mg/kg OIMI-8-2D-4NBZate (**13**), or 2 mg/kg vancomycin, or PBS was injected via IP route at 2 h post-infection. For treatment of *A. baumannii* infection, a single dose of 7 mg/kg OIMI-8-2D-4NBZate (**13**), or 7 mg/kg colistin, or 7 mg/kg imipenem, or PBS (untreated control) was administered to the mice via IP injection. For the quantitative antibacterial efficacy study, mice were euthanized at 26 h post-infection, and the bacterial counts in the peritoneal fluid, livers, kidneys, and spleens were determined. For the survival test, mice were monitored over 7 days post-infection. The toxicity of OIMI-8-2D-4NBZate (**13**) was assessed via the IP route, with daily measurement of animal weight ( $n=6$ ) (7 days for single dosing and 14 days for 7 daily repetitive doses). The clinical biomarkers ( $n=6$  per group) and histology analyses of kidney and liver ( $n=3$  per group) were performed 2 days after the final administration of (**13**), with PBS as negative control. **Murine thigh infection model with subcutaneous (SC) treatment.** The experiments were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Nanyang Technological University (AUP A22038). Exponential phase bacteria were washed twice with PBS and resuspended in PBS to the desired CFU/mL. The mice were infected intramuscularly in the left thigh ( $n=5$  or 6 for each experimental group) with either Gram-positive or Gram-negative strains. For Gram-positive MRSA LAC strain, 60  $\mu\text{L}$  of  $10^5$  CFU/mL LAC was injected into the left thighs, and the bacterial load in the thighs at 2 h post-infection was determined. The mice were treated subcutaneously with 15 mg/kg of OIMI-8-2D-4NBZate (**13**), or 15 mg/kg of vancomycin, or PBS at 2- and 4-h post-infection respectively. For Gram-negative *K. pneumoniae* SGH10 strain, 100  $\mu\text{L}$  of  $10^4$  CFU/mL of SGH10 was injected into the thighs to initiate infection, and the bacterial load in the thighs at 2 h post-infection was determined. The mice were then treated subcutaneously with 15 mg/kg OIMI-8-2D-4NBZate (**13**), or 15 mg/kg imipenem, or PBS at 2- and 4-h post-infection, respectively. Mice were euthanized at 26 h post-infection, and the bacterial counts in the thighs were determined. Cyclophosphamide was injected at 150 mg/kg on Day -4 and 100 mg/kg on Day -1 prior to infection for LAC but was not required for *K. pneumoniae* SGH10. The toxicity of OIMI-8-2D-4NBZate (**13**) was evaluated via SC administration of a single dose of (**13**) at 30 mg/kg, with daily measurement of animal weight ( $n=6$ ) for 7 days. At 2 days post-administration, clinical biomarkers ( $n=6$  per group) were measured and histology analyses of kidney and liver ( $n=3$  per group) were performed, with PBS as negative control.

### In vitro mastitis test (BS EN 1656)

*S. aureus* ATCC 6538, *S. uberis* ATCC 19436 and *E. coli* ATCC 10536 were used for the tests, as recommended by BS EN 1656 standard<sup>21</sup>. Bacteria of 2nd subculture were streaked out from TSA plates and inoculated into Tryptone NaCl diluent solution (0.1% tryptone and 0.85% NaCl) at  $1.5$  to  $5 \times 10^8$  CFU/mL. Test compounds were dissolved in hard water (0.119 g  $\text{MgCl}_2$ , 0.277 g  $\text{CaCl}_2$ , 0.28 g  $\text{NaHCO}_3$  in 1 L water) at desired concentration. 20  $\mu\text{L}$  skimmed milk (10 g/L) was added into a 96 well plate, followed by the addition of 10  $\mu\text{L}$  bacteria test suspension. The plate was mixed and incubated at 30  $^\circ\text{C}$  for 2 min. Subsequently, 80  $\mu\text{L}$  of test compound was added and mixed well, and then incubated at 30  $^\circ\text{C}$ . At the desired time points, 20  $\mu\text{L}$  of the product/milk/bacteria mixture was transferred to a new 96-well plate containing 160  $\mu\text{L}$  neutralizer (Lecithin 3%, Tween 80 10% (w/v), Sodium Thiosulphate 0.3%) and 20  $\mu\text{L}$  Milli-Q water, mixed well and incubated at room temperature for 5 min to fully neutralize the compound. The mixture was then serially diluted 10-fold in Tryptone NaCl diluent solution and plated on TSA plates. Colonies were counted after 24 h incubation at 37  $^\circ\text{C}$ . The data are representative of three independent experiments.

### In vivo mastitis farm trial

**(i) Farm condition.** The mastitis protocol was approved by Changchun University of Chinese Medicine (Ethics Protocol No. 202/205) to Principal Investigator Professor Li Qingjie. The trial was conducted in a dairy farm (using Holstein female cattle) located at Jilin Province, Changchun city, Jiutai district, Longjia town, Xiaochengzi village. Detailed experimental methods are described in Supplementary Information (Section 9.2). **(ii) Experimental setup.** The experiment procedures were designed in accordance with recommended protocols<sup>22</sup>. A 10-day acclimation period (Day -10) was implemented prior to the start of the experiment. Safety trial started on Day 0. The first sampling was done before teat dip application ( $t=\text{Day } 0$ ) to establish baseline. Teat dip filled in a conventional foam dip cup was applied to the distal 25 mm of teats immediately after milking. Teat dip was applied daily for 5 consecutive days. The second sampling was done on Day 5. Samples collected: milk sample for quality check and residual check; teat surface sample for residual check. The experimental results are described in Supplementary Information Section 9.3 and Supplementary Fig. S9.3. The challenge trial started on Day 5 immediately after milking. The first sampling of bacterial CFU count in milk was done before bacteria exposure to establish the baseline ( $t=\text{Day } 5$ ). *S. aureus* ATCC 49525<sup>53</sup> ( $5 \times 10^7$  CFU/mL in TSB) was applied to the teats at a depth of ~25 mm in a conventional foam dip cup right after each milking. Teat dip was applied immediately after exposure to the bacteria suspension. The challenge was carried out daily for 5 consecutive days. 2nd and 3rd samplings were done on Day 8 and Day 10, respectively. Samples collected: milk sample for quality check and bacteria CFU count. **(iii) Sampling procedures in mastitis challenge trial.** **(a) Milk sampling.** All milk samples were collected immediately prior to regular automated milking. Briefly, three or four streams of foremilk (procedure stated in pre-milking udder preparation in Supplementary Information Section 9.2) were discarded from each quarter before sanitizing teat ends with cotton swabs and collecting samples. Approximately 10 mL of milk samples from each teat were collected daily starting from the onset of the experiment. To determine the milk quality (e.g., somatic cell count), milk samples were passed and tested by qualified testing labs within 24 h. **(b) Bacteria CFU count.** The milk samples were collected according to the procedures stated above. The number of microbes in milk was counted using standard protocol. **(c) Criteria for diagnosing infections.** A new intramammary infection in a quarter was diagnosed when the same bacterial species was isolated from (i) two consecutive samples during the trial ( $>500$  CFU/mL); or (ii) a single sample from a quarter with clinical mastitis ( $>100$  CFU/mL); or (iii) three consecutive samples during the trial ( $>100$  CFU/mL).

## Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

## Data availability

Data supporting the findings of this study are available in this article and its Supplementary Information file. All data underlying this study are available from the corresponding author upon request. Source data are provided with this paper.

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## Acknowledgements

This research is supported by the Ministry of Education, Singapore, under its MOE AcRF Tier 3 Awards of MOE2018-T3-1-003, and MOE-MOET32023-0004, and Tier 1 RG3/23 (M.B.C.). We also thank SMART for the funding support to P.T. Hammond (P.T.H.) through grant ING-000685BIO. Chong Hui Koh acknowledges the support of a NTU Ph.D. scholarship. We also thank NTU for the funding support and of the NTU Centre for Aquaculture Research, Innovation and Education (CARIE) through grant 020797-00001 (M.B.C.). This research was also supported by the Wellcome Trust through grant 210671/Z/18/Z to Angelika Gründling (A. Gründling). The authors thank Dr. Cui Liang (SMART) for his technical contribution to the LC-MS experiment. The authors also thank Ms. Jamal Shubi for her contribution to MIC testing. The authors extend their appreciation to Thang Loi Pham and Dennis Bao Rong Wang for their assistance with the syntheses and characterization of the OIM compounds. The authors also express their gratitude to the reviewers for their valuable feedback and insightful suggestions. Schematic illustrations are drawn using Biorender.com.

## Author contributions

M.B.C. supervised and guided the overall research; C.H.K., M.R.L., G.W., C.T., and M.B.C. conceived the project. Z.Y.K., M.R.L., M.P., Z.W., and M.B.T. synthesized the oligomers. G.W., Q.H.N.V., and C.T. carried out the carbene testing. Y.R.C. and S.A.P. supervised the carbene formation experiment. P.T. and R.D.W. supervised the EPR experiment. C.H.K., Y.J.O., and S.H.T. conducted the in vitro biological tests. J.T.Y.N., A.Guo, and Y.M. carried out and analyzed the simulation data. C.H.K. carried out the mice experiments. T.T.D. analyzed the histology study for the murine toxicity testing. C.H.K. and S.H.T. carried out in vitro mastitis testing. K.Z.

carried out the mastitis field trial with the help of P.T.H. and Q.L. Q.L. provided the laboratory and farm facilities for the mastitis field trial. Y.G., E.P.G., K.P., and A. Gründling supervised the biological testing and interpretation. C.H.K., M.R.L., C.T., G.W., Z.Y.K., K.P., A. Gründling, and M.B.C. wrote the manuscript with contributions from the other authors.

## Competing interests

The Authors declare no other financial and non-financial competing interests except the following: 1. Published patent PCT/SG2024/050366 titled Main Chain Cationic Oligo(Imidazolium) Forms N-Heterocyclic Carbene For Effective Bacterial Killing In Complex Environment is associated with this work. The applicants are NTU and Imperial College Innovations Limited, and the inventors include M.B.C., K.P., A. Gründling, C.H.K., M.R.L., Z.Y.K., G.W., and M.B.T. The patent covers the novel compound composition (**1-13**): their syntheses, characterizations (including NHC formation of **1-2** and **5**), in vitro studies, and mastitis studies, but not the mice data and not some of the NHC-assisted model translocation data. 2. Unpublished patent (Singapore provisional patent application number 10202500296 V) titled OIM1-6-1X is associated with this work. The applicant is NTU and the inventors include M.B.C., M.R.L., Z.W., C.T., Q.H.N.V., G.W., S.H.T., and C.H.K. The patent covers the novel compound composition (**12**): their syntheses, and in vitro MIC and toxicity characterizations. 3. Published patent PCT/SG2023/050007 titled Polyimidazolium-based Cationic Antimicrobial Polymers for Novel Mastitis Prophylactic Solutions is relevant to this manuscript. The applicants are MIT and NTU, and the inventors include M.B.C., P.H.T., Z.K., and Ferguson, Michelle, and Elizabeth. The patent covers mastitis studies.

## Additional information

**Supplementary information** The online version contains supplementary material available at <https://doi.org/10.1038/s41467-025-61724-y>.

**Correspondence** and requests for materials should be addressed to Kevin Pethe or Mary B. Chan-Park.

**Peer review information** *Nature Communications* thanks Mark Blaszkovich, Mark Broenstrup, and the other, anonymous, reviewers for their contribution to the peer review of this work. A peer review file is available.

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<sup>1</sup>School of Chemistry, Chemical Engineering and Biotechnology, Nanyang Technological University (NTU), Singapore, Singapore. <sup>2</sup>Centre for Antimicrobial Bioengineering, NTU, Singapore, Singapore. <sup>3</sup>Singapore-MIT Alliance for Research and Technology (SMART), Singapore, Singapore. <sup>4</sup>School of Biological Sciences, NTU, Singapore, Singapore. <sup>5</sup>Infectious Diseases Translational Research Program, Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore (NUS), Singapore, Singapore. <sup>6</sup>Massachusetts Institute of Technology (MIT), Cambridge, MA, USA. <sup>7</sup>Environmental Chemistry and Materials Centre, Nanyang Environment and Water Research Institute (NEWRI), Nanyang Technological University, Singapore, Singapore. <sup>8</sup>Research Center of Traditional Chinese Medicine, The Affiliated Hospital to Changchun University of Chinese Medicine, Changchun, China. <sup>9</sup>Department of Microbiology, University of Washington School of Medicine, Seattle, WA, USA. <sup>10</sup>Department of Infectious Disease, Section of Molecular Microbiology and Centre for Bacterial Resistance Biology, Imperial College London, London, UK. <sup>11</sup>Lee Kong Chian School of Medicine (NTU), Singapore, Singapore. <sup>12</sup>Singapore Centre for Environmental Life Sciences and Engineering (SCELSE), Singapore, Singapore. <sup>13</sup>National Centre for Infectious Diseases, Singapore, Singapore. <sup>14</sup>These authors contributed equally: Chong Hui Koh, Mallikharjuna Rao Lambu, Chongyun Tan and Guangmin Wei. ✉ e-mail: [kevin.pethe@ntu.edu.sg](mailto:kevin.pethe@ntu.edu.sg); [mbechan@ntu.edu.sg](mailto:mbechan@ntu.edu.sg)