### Title

Amide Moieties Modulate the Antimicrobial Activities of Conjugated Oligoelectrolytes against Gram-negative Bacteria




### Journal

ChemistryOpen, 11(2)

### ISSN

2191-1363



### Authors

Limwongyut, Jakkarin
Moreland, Alex S
Nie, Chenyao
et al.



### Publication Date

2022-02-01

### DOI

10.1002/open.202100260

Peer reviewed

ChemistryOpen

Research Article
doi.org/10.1002/open.202100260

Chemistry Europe
European Chemical Societies Publishing

www.chemistryopen.org

# Amide Moieties Modulate the Antimicrobial Activities of Conjugated Oligoelectrolytes against Gram-negative Bacteria

Jakkarin Limwongyut,[a] Alex S. Moreland,[a] Chenyao Nie,[b] Javier Read de Alaniz,[a] and Guillermo C. Bazan*[a, b]

Cationic conjugated oligoelectrolytes (COEs) are a class of compounds that can be tailored to achieve relevant in vitro antimicrobial properties with relatively low cytotoxicity against mammalian cells. Three distyrylbenzene-based COEs were designed containing amide functional groups on the side chains. Their properties were compared to two representative COEs with only quaternary ammonium groups. The optimal compound, **COE2**–**3C**–**C3-Apropyl**, has an antimicrobial efficacy against *Escherichia coli* with an MIC = 2 μg mL$^{-1}$, even in the presence of human serum albumin low cytotoxicity ($IC_{50}$ = 740 μg mL$^{-1}$) and minimal hemolytic activity. Moreover, we find that amide groups increase interactions between COEs and a bacterial lipid mimic based on calcein leakage assay and allow COEs to readily permeabilize the cytoplasmic membrane of *E. coli*. These findings suggest that hydrogen bond forming moieties can be further applied in the molecular design of antimicrobial COEs to further improve their selectivity towards bacteria.

## Introduction

Failure to combat drug-resistant bacteria is anticipated to result in a sharp increase in lethal infections[1,2] and in the risk of acquiring difficult to treat infections from hospitals.[3] Moreover, a significant increase in antibiotic use against secondary infections during the COVID-19 pandemic is likely to aggravate the prevalence of antibiotic-resistant bacteria.[4–6] Despite the alarming crisis, few new antibiotic classes have been introduced due to factors, such as a long development processes and poor investment incentives.[7,8] Developing novel classes of antimicrobial compounds is, therefore, greatly warranted.

Amphiphilic cationic molecules have emerged as novel antimicrobial agents.[9–11] This class of compounds acts against bacteria by disrupting their membranes and compromising cell integrity and is of relevance due to low resistance acquisition rate and an ability to eradicate metabolically dormant bacteria.[12–16] Selectivity towards bacteria is due to differences in lipid compositions between bacteria and mammalian cells.[13] Considering lipid compositions of bacteria, a fraction of lipid head groups contains phosphatidylglycerol (PG), which is not commonly present in mammalian cells.[17] PG head groups can act as a hydrogen bond donors. Thus, introducing groups that have a potential hydrogen bonding ability with PG, in addition to electrostatic interactions from cationic groups, may enhance the selectivity of amphiphilic compounds. Indeed, molecular dynamic simulations of an amphiphilic polymer reveal that amide groups form hydrogen bonds with PG head groups and increase specificity towards bacterial membranes.[18]

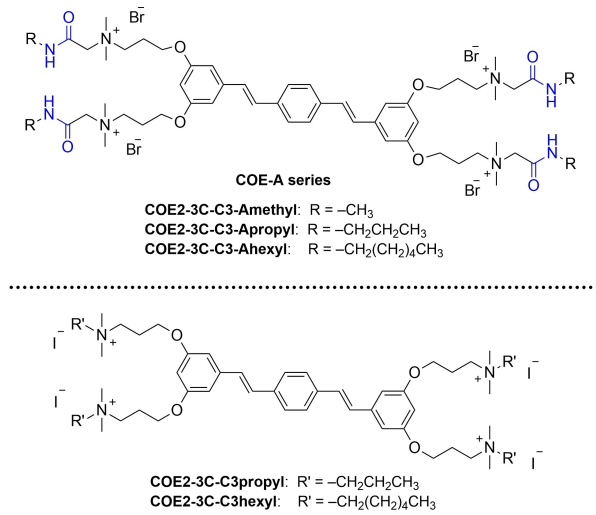
Conjugated oligoelectrolytes (COEs) are being studied in the context of antibiotic development.[19–21] They are a class of amphiphilic compounds bearing a π-conjugated core and cationic pendant groups. In previous work, we showed that cationic COEs with a distyrylbenzene (DSB) framework can be tailored to achieve antimicrobial activities with low cytotoxicity.[22] DSB-COEs reported in the literature only have quaternary ammonium moieties on their side chains. Herein, we report a new series of DSB COEs that include non-peptidic amides on the side chains (Scheme 1, top). Hydrophobicity was modulated by varying the length of R groups on the side chains. Antimicrobial activities and cytotoxicity profiles of these COEs were explored and compared to two representative COEs that only have quaternary ammonium groups (Scheme 1, bottom). We also show that the COEs in this study are membrane-active and can disrupt the cytoplasmic membrane (CM) of *Escherichia coli*, a representative Gram-negative bacterium.

## Results and Discussion

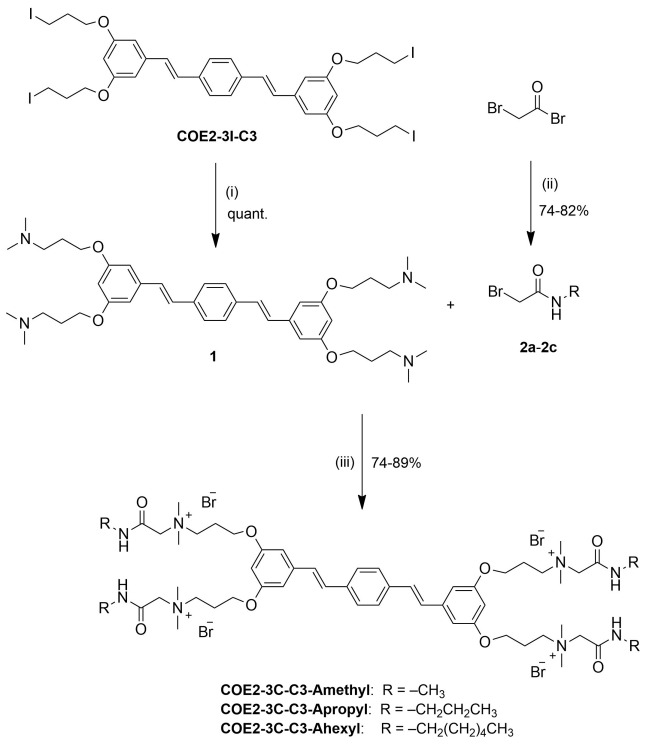
The preparation of amide-containing COEs reported herein relies on **COE2**–**3I**–**C3**[22] as a common starting material, see

[a] J. Limwongyut, Dr. A. S. Moreland, Prof. J. Read de Alaniz, Prof. G. C. Bazan
Center for Polymers and Organic Solids
Department of Chemistry and Biochemistry
University of California, Santa Barbara
Santa Barbara, CA 93106 (USA)
E-mail: chmbgc@nus.edu.sg
[b] Dr. C. Nie, Prof. G. C. Bazan
Department of Chemistry and Chemical Engineering
National University of Singapore
Singapore 117543 (Singapore)

Supporting information for this article is available on the WWW under https://doi.org/10.1002/open.202100260




**Scheme 1.** Amide-containing COEs (top) and quaternary ammonium COEs used for comparisons in this study (bottom).




**Scheme 2.** Synthesis pathway of amide-containing COEs from **COE2**–**3I**–**C3**. Reaction conditions: (i) excess $NH(CH_3)_2$, THF, rt, 48 h; (ii) R–$NH_2$ (0.91 equiv.), $K_2CO_3$ (1.1 equiv.), DCM, −5 °C to rt, 3 h; (iii) DMF, 55 °C, 48 h.

Scheme 2. In brief, **COE2**–**3I**–**C3** was reacted with $HNMe_2$ in THF to yield intermediate **1** in quantitative yield. Bromomethyl-functionalized amides **2b**–**2c** were synthesized from the reaction between bromoacetyl bromide and primary amines in the presence of $K_2CO_3$. Compound **2a** is commercially available. Finally, intermediate **1** was subjected to quaternization reactions with compounds **2a**–**2c** in DMF at 55 °C. Target molecules were obtained in a good yield by precipitating reaction mixtures in diethyl ether, followed by purifications using reverse-phase column chromatography. With one common intermediate **1** and straightforward purification, the synthesis is relatively simple and of low cost. Compounds **COE2**–**3C**–**C3propyl** and **COE2**–**3C**–**C3hexyl** were synthesized according to a previously reported procedures.[22]

Antimicrobial activities were evaluated against *E. coli* K12 (ATCC 47076) by determining their minimum inhibitory concentrations (MICs) in an LB medium (Table 1). The decrease in MIC from **COE2**–**3C**–**C3-Amethyl** (16 μg mL$^{-1}$) to **COE2**–**3C**–**C3-Apropyl** (2 μg mL$^{-1}$) would be reasonably attributed to an increase in hydrophobicity with longer alkyl chains. However, the MIC of **COE2**–**3C**–**C3-Ahexyl** is 8 μg mL$^{-1}$. We note that **COE2**–**3C**–**C3-Ahexyl** solutions turn turbid in LB at concentrations >128 μg mL$^{-1}$, despite its high water solubility (> 10 mg mL$^{-1}$). Since LB broth contains undefined proteins, this could indicate that **COE2**–**3C**–**C3-Ahexyl** binds to proteins in the medium resulting in a lower effective concentration and concomitant decreased antimicrobial efficacy. Such phenomenon has been observed in other antimicrobial agents.[23] **COE2**–**3C**–**C3propyl** and **COE2**–**3C**–**C3hexyl** both have an MIC of 8 μg mL$^{-1}$. To demonstrate that COEs have antimicrobial activities against other Gram-negative bacteria, MICs of COEs against *Klebsiella pneumoniae* and *Salmonella enterica* Typhimurium were also determined (Table S1 in Supporting Information). The relative activities against these two bacteria show a similar trend to the activities against *E. coli* K12.

We measured MIC values in the presence of 40 g L$^{-1}$ human serum albumin (HSA) in LB.[24,25] Table 1 shows that antimicrobial activities of COEs with hexyl chains (**COE2**–**3C**–**C3-Ahexyl** and **COE2**–**3C**–**C3hexyl**) suffer with the presence of HSA with a four-fold increase in MIC (32 μg mL$^{-1}$). **COE2**–**3C**–**C3-Amethyl** has slightly increased in MIC (2-fold increase) while the activities of **COE2**–**3C**–**C3-Apropyl** and **COE2**–**3C**–**C3propyl** were not affected. **COE2**–**3C**–**C3-Apropyl** therefore has the lowest MIC against *E. coli* and its antimicrobial activity was not affected by the presence of HSA.

**Table 1.** Summary of MICs, $IC_{50}$'s, $HC_{50}$'s and selectivity indices of COEs in this study.

| Compound | MIC[a] [μg mL$^{-1}$] | MIC with HSA[b] [μg mL$^{-1}$] | $IC_{50}$[c] [μg mL$^{-1}$] | $HC_{50}$ [μg mL$^{-1}$] | Selectivity index [$HC_{50}$/MIC] |
|---|---|---|---|---|---|
| **COE2**–**3C**–**C3-Amethyl** | 16 | 32 | >1,024 | >1,024 | >64 |
| **COE2**–**3C**–**C3-Apropyl** | 2 | 2 | 740 | >1,024 | >512 |
| **COE2**–**3C**–**C3-Ahexyl** | 8 | 32 | 10 | 40 | 5 |
| **COE2**–**3C**–**C3propyl** | 8 | 8 | >1,024 | >1,024 | >128 |
| **COE2**–**3C**–**C3hexyl** | 8 | 32 | 15 | 197 | 25 |

[a] MIC against *E. coli* K12 in LB. [b] The concentration of HSA was 40 g L$^{-1}$. [c] $IC_{50}$ against the HepG2 cell line.

In vitro cytotoxicities against the human hepatocellular carcinoma cell line (HepG2) were measured and are reported in terms of half maximal inhibitory concentration ($IC_{50}$) values. According to Table 1, $IC_{50}$ values show a correlation to the length of the alkyl groups on the side chains. There is no detectable cytotoxicity from **COE2–3C–C3-Amethyl**, even up to 1,024 μg mL$^{-1}$. **COE2–3C–C3-Apropyl** retains relatively low cytotoxicity with $IC_{50}$ = 740 μg mL$^{-1}$. However, **COE2–3C–C3-Ahexyl** is cytotoxic ($IC_{50}$ = 10 μg mL$^{-1}$). This trend is also observed for **COE2–3C–C3propyl** and **COE2–3C–C3hexyl**, which have $IC_{50}$ values of >1,024 μg mL$^{-1}$ and 15 μg mL$^{-1}$, respectively. These data suggest that considerations of hydrophobicity are particularly useful to minimize undesirable cytotoxicity profiles.[22]

The half maximal hemolytic concentration ($HC_{50}$) value for each compound was determined towards human red blood cells, as described previously.[22] One can observe from Table 1 that among amide-containing COEs, only **COE2–3C–C3-Ahexyl** shows high hemolytic activity ($HC_{50}$ = 40 μg mL$^{-1}$), whereas no hemolytic activity was detected, even up to 1,024 μg mL$^{-1}$, for **COE2–3C–C3-Amethyl** and **COE2–3–C3-Apropyl** (Figure S1). A similar observation was observed with **COE2–3C–C3propyl** ($HC_{50}$ > 1,024 μg mL$^{-1}$) and **COE2–3C–C3hexyl** ($HC_{50}$ = 197 μg mL$^{-1}$). Like cytotoxicity against HepG2 cells, hemolytic activities correlate well to general considerations of hydrophobicity. Taking activity and safety considerations into account, **COE2–3C–C3-Apropyl** was identified to be the optimal compound with a selectivity index ($HC_{50}$/MIC) greater than 512.

Insights into how structural variations impact membrane stability were sought by measuring calcein leakage from model lipid vesicles. Vesicles mimicking bacterial membranes comprised 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG) in a ratio of 3:1.[26] As shown in Figure 1a, **COE2–3C–C3-Ahexyl** induced the highest level of leakage at 57%. **COE2–3C–C3-Apropyl** and **COE2–3C–C3hexyl** induced a similar level of permeabilization, with leakages of 29% and 34%, respectively. **COE2–3C–C3-Amethyl** (11%) and **COE2–3C–C3propyl** (13%) were the least disruptive. In general, amide containing COEs are more effective as illustrated by that **COE2–3C–C3-Apropyl** induced calcein leakage 2.2 times higher than **COE2–3C–C3propyl** and **COE2–3C–C3-Ahexyl** induced 1.7 times more leakage compared to **COE2–3C–C3hexyl**. According to the relative hydrophobicity of COEs, as determined by RP-HPLC retention time measurements, amide containing COEs have similar hydrophobicity compared to non-amide COEs with the same terminal alkyl groups (Table S2). This suggests that significant increases in leakage-inducing activities of amide containing COEs are not due to the increased hydrophobicity of the molecules. That the general trend in permeability in Figure 1a does not correlate to the MIC trend on Table 1 hints to possible non-specific interactions of COEs with components in the LB media (see above) or interactions between COEs and other cell wall components.

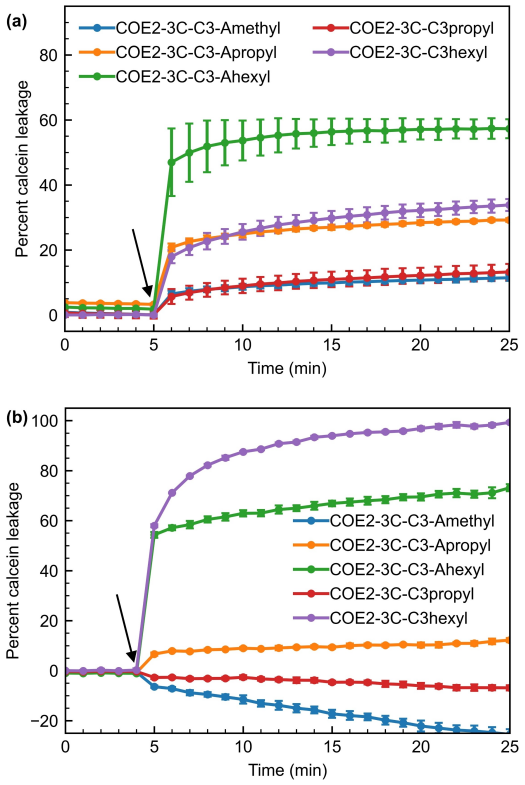



**Figure 1.** Calcein leakage from (a) bacterial lipid model vesicles (3:1 POPE/POPG) and (b) mammalian lipid model vesicles (EYPC). The arrows indicate the instances when COEs were added.

Unlike bacteria, mammalian cell lipids largely consist of the zwitterionic head group phosphatidylcholine (PC).[17] Mammalian cell lipid mimic vesicles were thus prepared from egg yolk L-α-phosphatidylcholine (EYPC). Calcein release measurements (Figure 1b) show a different trend from that observed for the POPE/POPG system. Specifically, **COE2–3C–C3hexyl** treatment resulted in complete release of calcein. **COE2–3C–C3-Ahexyl** also caused a high level of leakage (73%), followed by **COE2–3C–C3-Apropyl** (12%). No increase in calcein signal was observed in vesicles treated with **COE2–3C–C3propyl**. This trend fits well with in vitro cytotoxicity profiles in Table 1. To our surprise, we observed a decrease in calcein emission with **COE2–3C–C3-Amethyl.**

According to dynamic light scattering measurements, there was no observable change in vesicle size compared to control (Figure S7). We found that **COE2–3C–C3-Amethyl** can partially quench calcein emission (Figure S8) and hypothesize that this COE may associate with EYPC vesicles by an unknown mechanism and to interact with calcein.

The outer membrane (OM) is an important barrier before compounds enter or exit Gram-negative bacteria. From Figure 2, the OM of *E. coli* was permeabilized, as indicated by an increase in Nile Red fluorescence compared to controls. The degree of permeabilization is dependent on the length of terminal alkyl groups. We also observed that **COE2–3C–C3-Apropyl** permeabilized the OM slightly more than its non-amide analog, **COE2–3C–C3propyl**. Similarly, **COE2–3C–C3-**

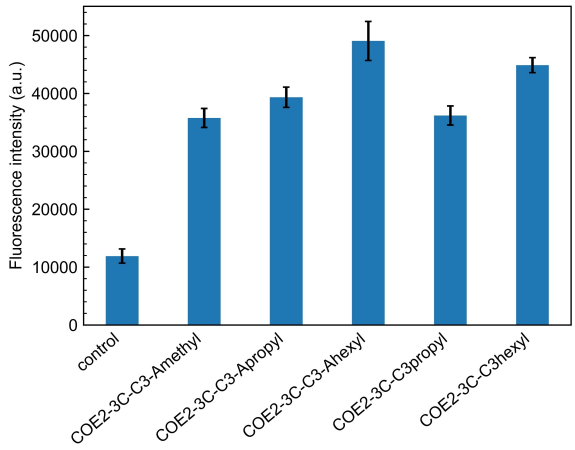



**Figure 2.** Fluorescence signal from Nile Red uptake assay with *E. coli* K12 after COE treatment at 8 μg mL$^{-1}$. An increase in fluorescence intensity indicates OM permeabilization.

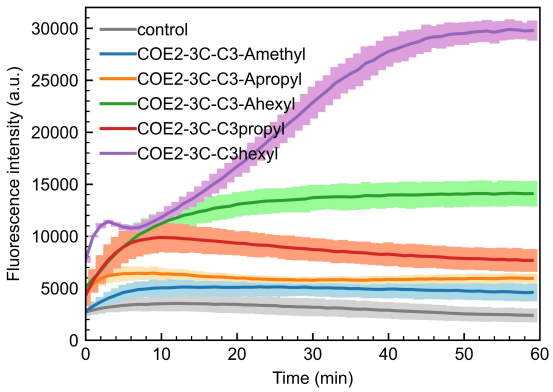



**Figure 3.** Changes in fluorescence signal of $DiSC_3(5)$ in CM depolarization assays with *E. coli* after COE treatment at 8 μg mL$^{-1}$.

**Ahexyl** was more effective than **COE2**—**3C**—**C3hexyl**. The data follow the trend observed in calcein leakage assays. In the buffer for this assay (5 mM HEPES with 20 mM glucose), almost all COEs "associated" to *E. coli* immediately after treatments as shown by time-dependent cell association experiments (Figure S9). Cell association behavior of COEs is in accordance with an immediate increase in Nile Red fluorescence after treatments. This suggests that COEs permeabilize the OM of *E. coli* effectively upon association. The lack of a correlation between the OM permeability and MICs suggests that this is not an important process in bacterial killing mechanism of COEs.

By confining components essential to viability inside the cytoplasm, the cytoplasmic membrane (CM) provides yet another layer of protection in Gram-negative bacteria. CM depolarization assays in the presence of different COEs were thus performed using 3,3'-dipropylthiacarbocyanine iodide ($DiSC_3(5)$). $DiSC_3(5)$ accumulates in CM of bacterial cells and forms self-quenched aggregates. Upon membrane potential disruption, $DiSC_3(5)$ is released to the medium where its fluorescence intensity increases. The results of these studies are provided in Figure 3. One observes that **COE2**—**3C**—**C3-Ahexyl** and **COE2**—**3C**—**C3hexyl** exhibit the strongest effect. **COE2**—**3C**—**C3propyl** and **COE2**—**3C**—**C3hexyl** depolarized the CM to a higher extent than their amide-containing counterparts (**COE2**—**3C**—**C3-Apropyl** and **COE2**—**3C**—**C3-Ahexyl**). Noticeably, the extent of membrane depolarization induced by non-amide COEs is higher than that induced by amide containing COEs with the same alkyl group. It is possible that amide moieties may attenuate depolarizing activities of COEs by an unknown process. However, the absence of correspondence between the rank order of impact in Figure 3 and the MIC values in Table 1 suggests that CM depolarization, as determined by the $DiSC_3(5)$ assay, does not contribute significantly to the COE bactericidal mechanism of action.

Another measure of CM damage is an increase in permeability. Propidium iodide (PI) permeates compromised membranes and binds to DNA in the cytoplasm. An increase in fluorescence from PI in *E. coli* cells thus reflects CM damage. As shown in Figure 4a, **COE2**—**3C**—**C3-Apropyl** and **COE2**—**3C**—**C3hexyl** caused the highest degree of permeabilization. **COE2**—**3C**—**C3-Ahexyl** permeabilized CM less than **COE2**—**3C**—**C3-Apropyl** and **COE2**—**3C**—**C3hexyl**, followed by **COE2**—**3C**—**C3-Amethyl**. According to the calcein leakage and OM permeability assays, one would expect that **COE2**—**3C**—**C3-Ahexyl** should have higher permeabilizing activity than other amide COEs. The unexpectedly lower degree of PI uptake

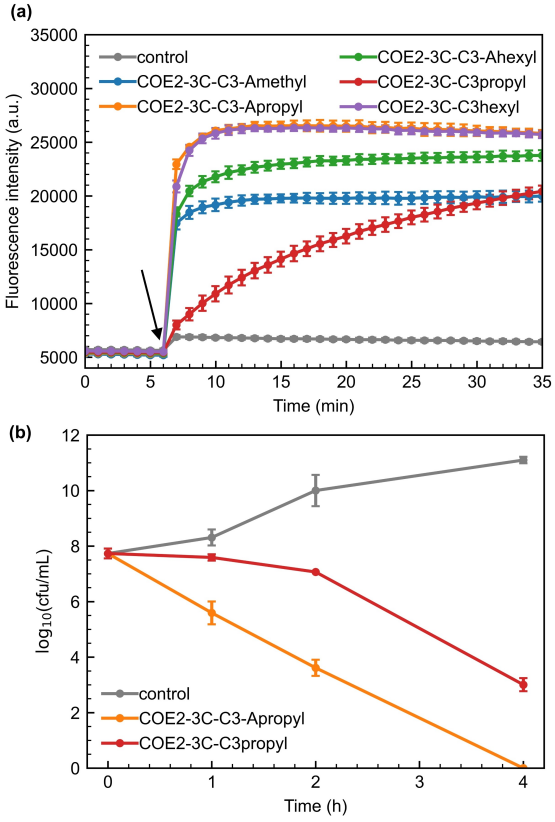



**Figure 4.** (a) Fluorescence signal of propidium iodide (PI) in CM permeabilization assay. The arrow indicates the time when COEs were added; (b) time-kill kinetics studies of **COE2**—**3C**—**C3-Apropyl** and **COE2**—**3C**—**C3propyl** against *E. coli* at 16 μg mL$^{-1}$.

observed could be attributed to high non-specific interactions of the COE to proteins or other cellular components. Interestingly, the effect by **COE2−3C−C3propyl** appears to take a longer time than the other COEs. From Figure 4a, one surmises that the amide moieties help COEs to more effectively permeabilize the CM. According to the trend of CM permeabilization, this is the most diagnostic assay, yet not perfect, for the MICs of COEs in this study.

Time-kill kinetics of **COE2−3C−C3-Apropyl**, the optimal compound in this series, were measured and compared with those of its non-amide analog, **COE2−3C−C3propyl**. *E. coli* in LB was challenged with these two COEs at 16 μg mL$^{-1}$. As shown in Figure 4b, **COE2−3C−C3-Apropyl** eradicated 99.9% of bacteria within 1.5 h. Eradication of bacteria to $<10$ cfu mL$^{-1}$ was also observed 4 h after treatment. **COE2−3C−C3propyl** requires approximately twice of the time in order to achieve the same bactericidal effect. It is also worth noting that **COE2−3C−C3propyl** also permeabilizes the CM of *E. coli* at a slower rate.

## Conclusion

To summarize, we disclose a series of DSB-based COEs with amide moieties on side chains and compared their antimicrobial activities against *E coli* K12 with structural counterparts bearing only quaternary ammonium groups. Among this series, **COE2−3C−C3-Apropyl** was found to be the optimal compound on the basis of the lowest MIC and largest $HC_{50}$/MIC ratio. A series of tests that center on probing membrane perturbations were also carried out to gain insight into the mechanism of action. By and large these experiments, namely calcein release from model vesicles, uptake of Nile Red and PI dyes, and CM depolarization are consistent with the COEs disrupting the integrity and function of the membrane. We found best correspondence between antimicrobial activity (MIC) and the PI uptake, which would imply that permeabilization of the CM is important, although it is too early to make firmer claims. We also found that amide containing COEs rapidly permeabilize the CM of *E. coli* and **COE2−3C−C3-Apropyl** possesses a higher killing rate than its non-amide counterpart. More to the point, the general absence of trends observed for biophysical tests with MIC hints that COEs may have interactions other than lipid bilayer intercalation that warrant future investigations. From a molecular design perspective, this work suggests an important role for hydrogen bonds, a category of intermolecular interactions distinct from electrostatic and hydrophobic interactions, for tuning activity of COEs against bacterial cells and increasing selectivity relative to mammalian cells.

## *Acknowledgements*

*This work was supported by the Institute of Collaborative Biotechnologies (W911NF-09-0001) and by the National University of Singapore start up grant R143-000-A97-133. The authors acknowledge the use of the UCSB Biological Nanostructures Laboratory. J.L. acknowledges support from the Institute for Promotion of Teaching Science and Technology, Thailand.*

## *Conflict of Interest*

The authors declare no conflict of interest.

## Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

**Keywords:** antimicrobial agents · amides · conjugated oligoelectrolytes · membrane disruption · structure-activity relationships

[1] J. O'Neill, Tackling drug-resistant infections globally: final report and recommendations, The review on antimicrobial resistance, London, **2016**.
[2] E. Martens, A. L. Demain, *J. Antibiot.* **2017**, *70*, 520–526.
[3] Federal Task Force on Combating Antibiotic-Resistant Bacteria, *National Action Plan for Combating Antibiotic-Resistant Bacteria 2020–2025*, **2020**.
[4] S. Vijay, N. Bansal, B. K. Rao, B. Veeraraghavan, C. Rodrigues, C. Wattal, J. P. Goyal, K. Tadepalli, P. Mathur, R. Venkateswaran, R. Venkatasubramanian, S. Khadanga, S. Bhattacharya, S. Mukherjee, S. Baveja, S. Sistla, S. Panda, K. Walia, *Infect. Drug Resist.* **2021**, *14*, 1893–1903.
[5] E. Afshinnekoo, C. Bhattacharya, A. Burguete-García, E. Castro-Nallar, Y. Deng, C. Desnues, E. Dias-Neto, E. Elhaik, G. Iraola, S. Jang, P. P. Łabaj, C. E. Mason, N. Nagarajan, M. Poulsen, B. Prithiviraj, R. Siam, T. Shi, H. Suzuki, J. Werner, M. M. Zambrano, M. Bhattacharyya, *Lancet Microb.* **2021**, *2*, e135–e136.
[6] J. Rodríguez-Baño, G. M. Rossolini, C. Schultsz, E. Tacconelli, S. Murthy, N. Ohmagari, A. Holmes, T. Bachmann, H. Goossens, R. Canton, A. P. Roberts, B. Henriques-Normark, C. J. Clancy, B. Huttner, P. Fagerstedt, S. Lahiri, C. Kaushic, S. J. Hoffman, M. Warren, G. Zoubiane, S. Essack, R. Laxminarayan, L. Plant, *J. Glob. Antimicrob. Resist.* **2021**, *25*, 5–7.
[7] D. G. Brown, H. J. Wobst, *J. Med. Chem.* **2021**, *64*, 2312–2338.
[8] T. B. Nielsen, E. P. Brass, D. N. Gilbert, J. G. Bartlett, B. Spellberg, *N. Engl. J. Med.* **2019**, *381*, 503–505.
[9] S. Bai, J. Wang, K. Yang, C. Zhou, Y. Xu, J. Song, Y. Gu, Z. Chen, M. Wang, C. Shoen, B. Andrade, M. Cynamon, K. Zhou, H. Wang, Q. Cai, E. Oldfield, S. C. Zimmerman, Y. Bai, X. Feng, *Sci. Adv.* **2021**, *7*, eabc9917.
[10] N. Zhang, S. Ma, *Eur. J. Med. Chem.* **2019**, *184*, 111743.
[11] B. Findlay, G. G. Zhanel, F. Schweizer, *Antimicrob. Agents Chemother.* **2010**, *54*, 4049–4058.
[12] M. Zhou, M. Zheng, J. Cai, *ACS Appl. Mater. Interfaces* **2020**, *12*, 21292–21299.
[13] M. Zasloff, *Nature* **2002**, *415*, 389–395.
[14] Y.-J. Eun, M. H. Foss, D. Kiekebusch, D. A. Pauw, W. M. Westler, M. Thanbichler, D. B. Weibel, *J. Am. Chem. Soc.* **2012**, *134*, 11322–11325.
[15] T. Yang, W. Moreira, S. A. Nyantakyi, H. Chen, D. binte Aziz, M.-L. Go, T. Dick, *J. Med. Chem.* **2017**, *60*, 2745–2763.
[16] J. G. Hurdle, A. J. O'Neill, I. Chopra, R. E. Lee, *Nat. Rev. Microbiol.* **2011**, *9*, 62–75.
[17] G. J. Nelson, *Biochim. Biophys. Acta Lipids Lipid Metab.* **1967**, *144*, 221–232.
[18] D. S. S. M. Uppu, M. M. Konai, U. Baul, P. Singh, T. K. Siersma, S. Samaddar, S. Vemparala, L. W. Hamoen, C. Narayana, J. Haldar, *Chem. Sci.* **2016**, *7*, 4613–4623.
[19] E. Zamani, S. Chatterjee, T. Changa, C. Immethun, A. Sarella, R. Saha, S. K. Dishari, *Sci. Rep.* **2019**, *9*, 20411.
[20] C. Zhou, G. W. N. Chia, J. C. S. Ho, T. Seviour, T. Sailov, B. Liedberg, S. Kjelleberg, J. Hinks, G. C. Bazan, *Angew. Chem. Int. Ed.* **2018**, *57*, 8069–8072; *Angew. Chem.* **2018**, *130*, 8201–8204.
[21] Y. Wang, Y. Tang, Z. Zhou, E. Ji, G. P. Lopez, E. Y. Chi, K. S. Schanze, D. G. Whitten, *Langmuir* **2010**, *26*, 12509–12514.

[22] J. Limwongyut, C. Nie, A. S. Moreland, G. C. Bazan, *Chem. Sci.* **2020**, *11*, 8138–8144.
[23] A. K. Marr, W. J. Gooderham, R. E. Hancock, *Curr. Opin. Pharmacol.* **2006**, *6*, 468–472.
[24] D. M. Gopal, A. P. Kalogeropoulos, V. V. Georgiopoulou, W. W. H. Tang, A. Methvin, A. L. Smith, D. C. Bauer, A. B. Newman, L. Kim, T. B. Harris, S. B. Kritchevsky, J. Butler, *Am. Heart J.* **2010**, *160*, 279–285.
[25] Health, Aging and Body Composition Study, *Am. J. Clin. Nutr.* **2005**, *82*, 531–537.
[26] W. Dowhan, *Annu. Rev. Biochem.* **1997**, *66*, 199–232.

Manuscript received: December 15, 2021
Revised manuscript received: December 23, 2021