Cationic Anthraquinone Analogs as Selective Antimicrobials

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ABSTRACT: Development of new antibiotics is always needed in the fight against growing threat from multiple drug–resistant bacteria, such as resistant Gram-negative (G–) *Escherichia coli* and *Klebsiella pneumoniae*. While the development of broad-spectrum antibiotics has attracted great attention, careful administration of these antibiotics is important to avoid adverse effects, like *Clostridium difficile* infection (CDI). The use of broad-spectrum antibiotics, for example, quinolones, can increase the risk of CDI by eradicating the protective bacteria in intestine and encouraging *C difficile* spore germination. Many common intestine bacteria are G– or anaerobic, including *Enterococcus faecalis, Bacteroides fragilis*, and *E coli*. Hence, it may be advantageous in certain therapeutic practices to employ selective antimicrobials. For instance, Gram-positive (G+) methicillin-resistant *Staphylococcus aureus* (MRSA) that can cause life-threatening sepsis can be controlled with the use of selective antibiotic, vancomycin. Nevertheless, its effectiveness has been limited with the emerging of vancomycin-resistant *Staphylococcus aureus* (VRSA). A recent report on antimicrobial cationic anthraquinone analogs (CAAs) that show tunable activity and selectivity may provide new hope in the search for selective antimicrobials. In particular, the lead CAA displays prominent activity against MRSA while manifesting low activity against *E coli* and low cytotoxicity toward normal mammalian cells.

KEYWORDS: cationic anthraquinone analogs, antibiotic, methicillin-resistant ***Staphylococcus aureus***, vancomycin-resistant ***Staphylococcus aureus***, ***Clostridium difficile*** infection, cytotoxicity

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Continuous incidents reported worldwide regarding the bacterial pathogens that are resistant to single or multiple drugs have prompted the call for re-devoting effort into the discovery of new antibiotic.[1] As highlighted in the news, the emergence of multiple drug–resistant (MDR) Gram-negative (G–) *Escherichia coli* and *Klebsiella pneumonia* equipped with New Delhi metallo-β-lactamase 1 (NDM-1) that makes bacteria resistant to various antibiotics has attracted great attention for the development of new broad-spectrum antibiotics.[2] Broad-spectrum antibiotics have the advantage of exerting antibacterial activity against both G– and Gram-positive (G+) bacteria, while selective antibiotics may have activity against only G+ or specific strains of bacteria. Vancomycin, for example, is a well-known selective, or narrow-spectrum, antibiotic with effectiveness only toward G+ bacteria, such as *Staphylococcus aureus* but not G– bacteria.

While the development of broad-spectrum antibiotics has attracted great attention, careful administration of these antibiotics is important to avoid adverse effects, like *Clostridium difficile* infection (CDI). The use of broad-spectrum antibiotics, for example, quinolones, clindamycin, and cephalosporins, can increase the risk of CDI by eradicating the protective bacteria in intestine and encouraging *C difficile* spore germination.[3,4] Many common intestine bacteria are either G– or anaerobic, including *Enterococcus faecalis, Bacteroides fragilis*, and *E coli*. Hence, it may be advantageous in certain therapeutic practices to employ selective antimicrobials. For instance, Gram-positive (G+) methicillin-resistant *Staphylococcus aureus* (MRSA) that can cause life-threatening sepsis can be controlled with the use of selective antibiotic, vancomycin. Nevertheless, its effectiveness has been limited with the emerging of vancomycin-resistant *Staphylococcus aureus* (VRSA).[5] Even the newer selective antibiotics, linezolid and daptomycin, have encountered the problem of bacterial resistance.[6,7] In short, there is a significant need in the development of new selective antibiotics against formidable bacterial pathogens, like MRSA, while minimizing the risk of disrupting human gut flora.

We have recently reported that latest version of antimicrobial cationic anthraquinone analogs (CAAs) that show tunable activity and selectivity may provide new hope in the search for selective antimicrobials against serious human pathogens, like MRSA or VRSA.[8] In particular, the lead CAA displays prominent activity against MRSA while manifesting low activity against *E coli* and low cytotoxicity toward normal mammalian cells. The development of CAAs resides on 2 essential guidelines in drug development: (1) low cost of production and (2) accessibility to diverse structural variations with biologically relevant moiety. The cost of production is seldom the focus of early-stage drug development, especially for the laboratories in academia. Nevertheless, a recently Food and Drug Administration (FDA)-approved antibiotic, fidaxomicin, a new class of narrow-spectrum macrocyclic antibiotic, has not been able to compete with vancomycin or other generic drugs in the market due to its higher price.[9]

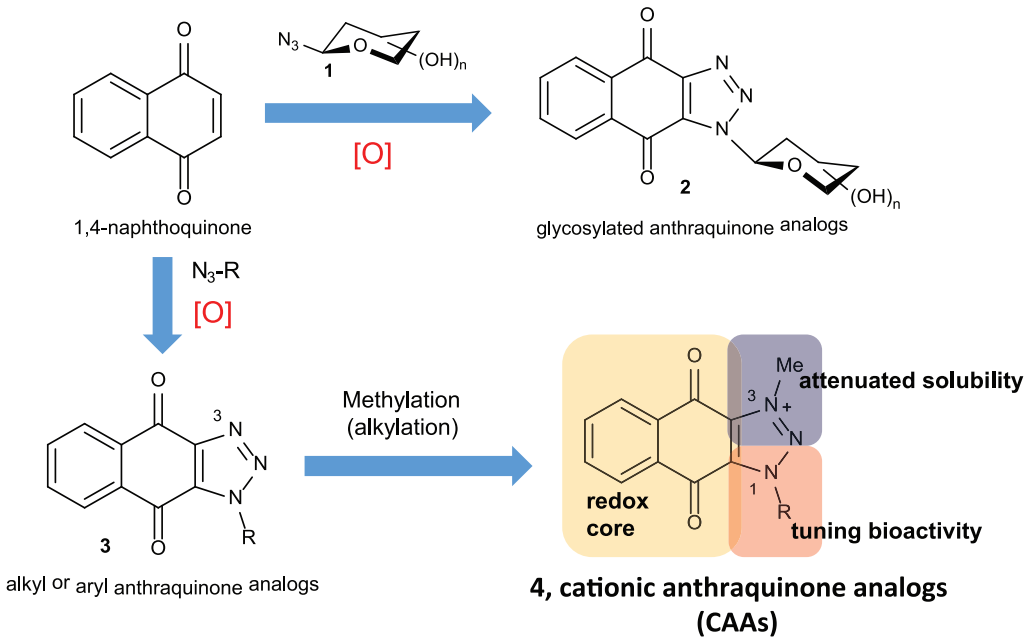


Figure 1. Development of CAAs. CAAs indicate cationic anthraquinone analogs.

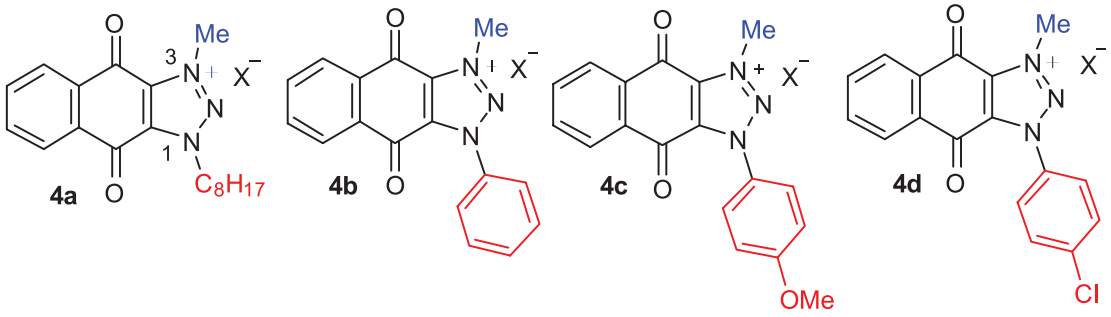
Accordingly, we decide to employ 1,4-naphthoquinone and "Click" chemistry to meet these 2 guidelines; 1,4-naphthoquinone, a redox active scaffold, is a common core in many bioactive molecules.[10] It can react with azides via a [2 + 3] cycloaddition, a Click reaction to enable the introduction of diverse structural moieties readily and lower the cost of production. The inception of CAAs began with [2 + 3] cycloaddition of 1,4-naphthoquinone and glycosyl azides (**1**; Figure 1).[11] The cycloaddition was found to undergo immediate oxidation in situ to yield glycosylated anthraquinone analogs (**2**) with anticancer activity. Attempts to employ alkyl or aryl azides for the same cycloaddition/oxidation also offer similar adducts (**3**). However, these alkyl or aryl anthraquinone analogs were insoluble in aqueous media and thereby had poor bioavailability. To offer remedy, alkylation (eg, methylation) at the *N*-3 position was conducted successfully leading to the production of CAAs (**4**).[12] The cationic nature makes CAAs to have better bioavailability. The quinone scaffold of CAAs exerts biological activity through redox process like 1,4-naphthoquinone. More importantly, we have discovered that the attached alkyl or aryl groups at *N*-1 play a key role in tuning the bioactivity or biological profile of these molecules.[13,14] Specifically, the CAAs with linear alkyl groups show good antibacterial activity, while those with aryl group have superior anticancer activity.

Following the structure-activity relationship (SAR) study of CAAs, the analog with *n*-octyl group at *N*-1 (**4a**) was found to be highly active against MRSA and VRSA while being less active against G- *E coli* as indicated with the minimum inhibitory concentrations (MICs; Table 1). However, **4a** also displayed significant cytotoxicity against both normal and cancerous human cells. In contrary, the CAA with phenyl group at *N*-1 (**4b**) manifested potent anticancer activity. More interestingly, **4b** showed selective antibacterial activity against G+ bacteria and higher potency against cancerous cells. On further examination, we noticed that the attachment of electron-withdrawing substituent at the para position of the *N*-1 phenyl group (**4d**) can decrease the cytotoxicity of CAA while maintaining similar selective antibacterial activity. However, compound **4c** with electron-donating substituent at the para position of the *N*-1 phenyl group showed higher potency against both human cells. The results suggest that it is possible to attenuate the cytotoxicity and still hold similar selective antibacterial activity.

The revealed SAR led to the synthesis of **4e** and **4f** that equipped with stronger electron-withdrawing substituents (CN and NO_2, respectively) than Cl. The outcomes support our design and meet the expectation (Table 2). Both **4e** and **4f** display selective antibacterial activity: active against MRSA, but inactive against *E coli* and *E faecalis*. Furthermore, these 2 compounds are much less toxic toward normal human cells than the cancer cells as indicated with the selectivity. All of these features make compounds of **4e** and **4f** as the prominent leads for the development of selective (narrow-spectrum) antibiotics.

In conclusion, the development of narrow-spectrum antibiotics is important for certain therapeutic applications albeit not receiving as much attention as the development of broad-spectrum antibiotics. Through years of investigation, we have identified 2 lead compounds, which can be prepared with simple chemistry, as the potential selective or narrow-spectrum antibiotics. Such narrow-spectrum antibiotics have the potential of controlling the infections caused by MRSA while not increasing the risk factor of CDI.

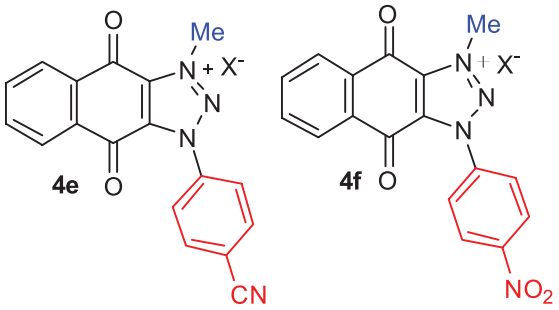
Table 1. Structure and activity of CAAs[a].



	VANCOMYCIN	4A	4B	4C	4D
MIC against *Staphylococcus aureus* (ATCC 25923)	1	0.032	1	1-2	1
MIC against MRSA (ATCC 33591)	2	1–2	0.5	0.5	0.5
MIC against VRSA (VRS1)[14]	>32	1–2	ND	ND	ND
MIC against *Escherichia coli* (ATCC 25922)	>256	8	128	>128	>128
IC_{50} against BEAS-2B (normal cell)	ND	0.60	5.76	2.72	6.43
IC_{50} against A549 (cancer cell)	ND	0.43	1.0	0.37	2.95

Abbreviations: CAAs, cationic anthraquinone analogs; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; ND, not determined; VRSA, vancomycin-resistant *Staphylococcus aureus*.
[a]Unit: μg/mL.

Table 2. Structure and activity of CAAs[a].



	4E	4F
MIC against *Staphylococcus aureus* (ATCC 25923)	2	2
MIC against MRSA (ATCC 33591)	1	1
MIC against MRSA (ATCC 43300)	2	2
MIC against *Escherichia coli* (ATCC 25922)	>128	>128
MIC against *Enterococcus faecalis*	>256	>256
IC_{50} against BEAS-2B (lungs normal cell)	30.15	52.86
IC_{50} against A549 (lungs cancer cell)	5.20	3.50
Selectivity[b]	2.37	15.09
CCD-841-CoN (CRL-1790; colon normal cells)	32.65	55.75
Colo205 (CCL-222; colon cancer cells)	5.91	4.64
Selectivity[c]	5.52	11.99

Abbreviations: CAAs, cationic anthraquinone analogs; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*.
[a]Unit: μg/mL.
[b]Selectivity is calculated as IC_{50} (lungs normal cell)/IC_{50} (lungs cancer cell).
[c]Selectivity is calculated as IC_{50} (colon normal cell)/IC_{50} (colon cancer cell).

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Author Contributions

YPS performed the experiments and assisted in manuscript preparation. CWTC designed the experiments and instructed the data analysis.

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