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Research paper

Synthesis and bioactivity investigation of quinone-based dimeric cationic triazolium amphiphiles selective against resistant fungal and bacterial pathogens

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ABSTRACT

A series of synthetic dimeric cationic anthraquinone analogs (CAAs) with potent antimicrobial activities against a broad range of fungi and bacteria were developed. These compounds were prepared in 2–3 steps with high overall yield and possess alkyl chain, azole, quinone, and quaternary ammonium complexes (QACs). *In vitro* biological evaluations reveal prominent inhibitory activities of lead compounds against several drug-susceptible and drug-resistant fungal and bacterial strains, including MRSA, VRE, *Candida albicans* and *Aspergillus flavus*. Mode of action investigation reveals that the synthesized dimeric CAA's can disrupt the membrane integrity of fungi. Computational studies reveal possible designs that can revive the activity of QACs against drug-resistant bacteria. Cytotoxicity assays in SKOV-3, a cancer cell line, show that the lead compounds are selectively toxic to fungi and bacteria over human cells.

1. Introduction

Amphiphilic molecules have attracted great interest as antifungal or antibacterial agents for many years. Recently, many studies on aminoglycoside-based amphiphilic molecules have been published (Fig. 1) [1–3]. The protonated amines on an aminoglycoside form a cationic head and together with a long alkyl chain form an amphiphilic molecule. Similarly, amphiphilic molecules based on quaternary ammonium complexes (QAC) and hydrophobic alkyl chain have been known for many years, and it is one of the most common antimicrobial agents used as disinfectants (Fig. 1) [4,5]. They are widely used in domestic, industrial, agricultural and clinical applications [6,7]. Several different types of QACs either possessing mono- or multi-cationic functional groups can be obtained in the market. Due to the strong positively charged cationic head and long lipophilic tail, these compounds act as amphiphilic molecules and are similar to phospholipids found in the cell membrane. Disruption of the phospholipid bilayer to compromise cell membrane has been attributed as the primary mode of action [8].

Our laboratory is dedicated to synthesizing cationic amphiphiles based on aminoglycoside and 1,4-naphthoquionone moieties that resemble QACs [9,10]. These compounds, called cationic anthraquinone analogs (CAAs), are antimicrobial when substituted with an alkyl chain [10], but anticancer when substituted with the aromatic ring [11]. The antimicrobial CAAs, which are much more active against Gram positive (G+) bacteria than Gram negative(G-) bacteria, act as redox inhibitors at minimum inhibitory concentrations (MICs) and act as membrane disrupters at higher concentrations [12]. The anticancer CAAs act as ROS-generating molecules [13]. Our studies have shown that the biological activities of CAAs are tunable depending on the attached substituents. Dequalinium chloride which contains two cationic motifs has been noted recently for its new antimicrobial applications different from mono-cationic QACs [14]. Inspired by the design of dequalinium chloride, we decided to explore the activities of the dimeric form of CAAs (Fig. 2). In this article, we are describing novel small multi-cationic QACs which possess two redox active cationic anthraquinone heads and two alkyl lipophilic tails.

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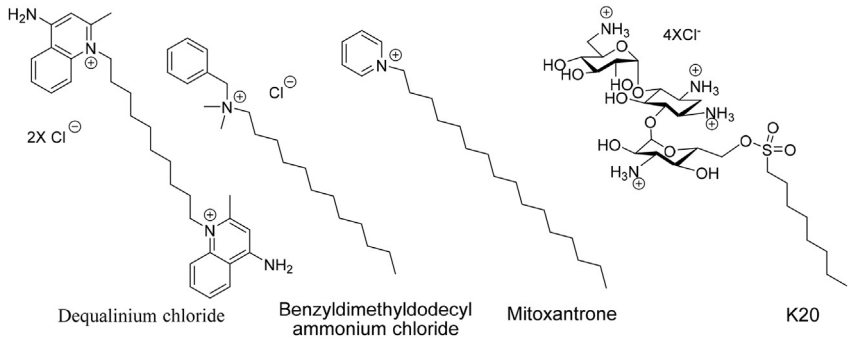

Fig. 1. Cationic amphiphiles.

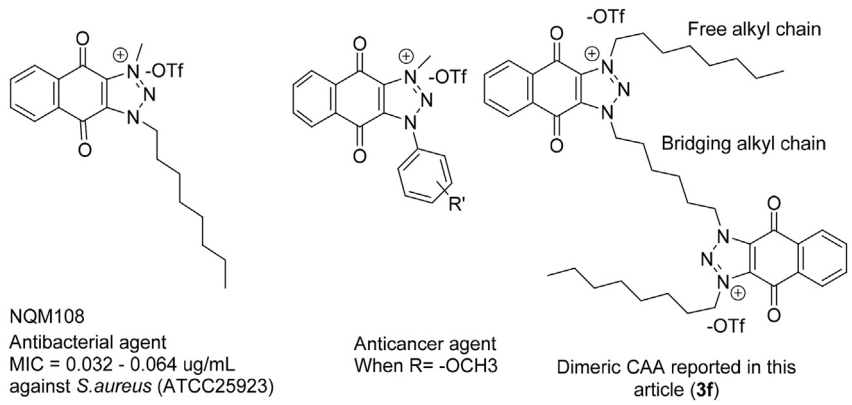

Fig. 2. Structural comparison of CAAs.

2. Results and discussion

2.1. Design and chemical synthesis

The redox-active quinone heads are connected with an alkyl linker via triazole moiety. Alkylation at the triazole ring generates the cationic property, and the positive charge generated by alkylation is delocalized around the triazole ring. The molecule is hybrid in nature as it is the combination of several popular antimicrobial scaffolds, such as quinone [15–18], triazole [19–21], and QAC [22].

The synthesis began with cyclization/oxidation using 1,4-naphthoquinone and 1,6-diazidohexane, **2** following the reported procedure to form **3** (Scheme 1). The linker, **2** can be prepared by a substitution reaction using 1,6-dibromohexane or 1,6-ditosylhexane and sodium azide. Dialkylation of **3** using alkyl triflates with various chain lengths, including methyl, ethyl, butyl, hexyl, octyl and decyl, provided the desired dimeric forms of CAAs. The alkyl triflates are commercially available or can be synthesized from the triflation of the corresponding alcohols. Because we observed solubility issues for the dimeric CAAs with chain length longer than decyl group, no derivatives with carbon chain longer than C_{10} were synthesized. All the compounds (**3a-f**) were characterized by ^{1}H NMR, ^{13}C NMR, and high-resolution mass spectrometry. HPLC analysis determined the purity level of all the final compounds that were found to be >95% pure. It is worth mentioning that all of the compounds can be synthesized from a low-cost starting material, 1,4-naphthoquinone, and do not require cumbersome column chromatography purification. Therefore, large quantity of lead compounds can be synthesized easily for future applications.

2.2. Antibacterial activity

The newly synthesized compounds were tested for antibacterial and antifungal activities. The antibacterial activities of the dimeric CAAs against *Staphylococcus aureus* (ATCC25923 G+), Methicillin-resistant *S. aureus* (MRSA) (ATCC33591, G+) and *Escherichia coli* (ATCC25922, G-) using vancomycin and kanamycin A as controls are summarized in Table 1. No antibacterial activity was observed for intermediate compound **3** at 5 mg/mL, which emphasizes the importance of the cationic property and alkylation. The final compounds (**3a-3f**) exhibited strong activities against drug susceptible *S. aureus* (ATCC25923) with compounds **3a-3c** being the most active (MIC 0.125 μg/mL). The MIC value is higher than the MIC value (0.032–0.064 μg/mL) for mono-cationic compound (NQM108, Fig. 2); the drop-off in MIC value is most likely due to increase in molecular weight of di-cationic compounds. The MICs decreased by 32–64 fold as the alkyl chain length increased to hexyl (C6) or longer. Interestingly, the trend was observed in reverse order for *S. aureus* (MRSA) (ATCC33591) and *E. coli* (ATCC25922). In both of the latter cases, the MICs for **3a-3c** (C1, C2 and C4) were >250 μg/mL while **3d-3f** (C6, C8 and C10) were more active with MICs ranging from 4 to 16 μg/mL.

We have reported that antibacterial CAAs may exert two types of antibacterial mode of actions: 1) interference with bacterial redox processes at MIC levels, and 2) membrane disruption at higher concentration [12]. Since increasing alkyl length will enhance the lipophilicity of the dimeric CAAs, the tendency to cause membrane disruption by these compounds is predicted to increase. Therefore, we conclude that **3a-3c** manifest antibacterial activities by inhibiting redox processes in *S. aureus*. However, this mode of action does not appear to apply to resistant *S. aureus* (MRSA) and G- *E. coli*