Bioorganic & Medicinal Chemistry Letters 27 (2017) 2107–2112

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Ester- and amide-containing multiQACs: Exploring multicationic soft antimicrobial agents

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ARTICLE INFO

Article history:
Received 12 February 2017
Revised 23 March 2017
Accepted 24 March 2017
Available online 29 March 2017

Keywords:
Antiseptics
Methicillin-resistant *Staphylococcus aureus* (MRSA)
Quaternary ammonium compounds
Soft antimicrobials

ABSTRACT

Quaternary ammonium compounds (QACs) are ubiquitous antiseptics whose chemical stability is both an aid to prolonged antibacterial activity and a liability to the environment. Soft antimicrobials, such as QACs designed to decompose in relatively short times, show the promise to kill bacteria effectively but not leave a lasting footprint. We have designed and prepared 40 soft QAC compounds based on both ester and amide linkages, in a systematic study of mono-, bis-, and tris-cationic QAC species. Antimicrobial activity, red blood cell lysis, and chemical stability were assessed. Antiseptic activity was strong against a panel of six bacteria including two MRSA strains, with low micromolar activity seen in many compounds; amide analogs showed superior activity over ester analogs, with one bisQAC displaying average MIC activity of ~1 μM. For a small subset of highly bioactive compounds, hydrolysis rates in pure water as well as buffers of pH = 4, 7, and 10 were tracked by LCMS, and indicated good stability for amides while rapid hydrolysis was observed for all compounds in acidic conditions.

Quaternary ammonium cations (QACs) are one of the more historic classes of antimicrobial agents, having been reported as early as 1916, when Jacobs noted the antibacterial activity of alkylated derivatives of hexamethylene tetraamine.[1] A major advance in the field was reported in 1935, when Domagk disclosed the activity of benzyldodecyldimethyl ammonium chloride, which has become the backbone of benzalkonium chloride (BAC) based antiseptics.[2] Many iterations of QAC structures have been developed over the last 100 years, and countless applications of QACs pepper the industrial and consumer product landscape.[3]

To both their benefit and detriment, QACs are relatively robust compounds. BAC is reported to have a ~9-month half-life in the environment,[4,5] with somewhat limited microbial degradation.[6,7] Such stability can be ideal in select applications (water cooling tower protection, wood preservation) but not in other scenarios (environmental contamination, select consumer products).[3] In fact, it is estimated that 700,000 tons of QACs are used and released into the environment annually, where it is commonly found at levels of ~0.05 mg/L in surface waters, and at 5 mg/L in hospital wastewater![8] This may result in an increase in microbes harboring QAC resistance genes,[9] whose prevalence is steadily increasing in drug-resistant bacteria.[10]

Steps have been taken to purposefully limit the stability of QACs, leading to a field of "soft" antimicrobials designed to decompose after a select survival time.[11,12] Esterquats are the leading group in this field, using esters on the non-polar chains of QACs to lead to controlled hydrolysis, which has been well studied. Examples include the pioneering work of Bodor, who coined the term "soft antimicrobials"[13] and developed hydrolyzable QACs with imidazole- or DABCO-based core structures. Select ester-based QAC species have been shown to be effective and rapid antiseptics, with biocidal activity in two minutes and a half-life of five hours.[9] Other examples of related QACs include the alkanoylcholines.[14,15] Haldar and co-workers have long been interested in evaluating the antibacterial properties of cleavable QACs utilizing ester and then amide moieties. Initial esterquats were capable of killing bacterial cells within 20–40 min of application while remaining readily hydrolyzable.[16] Continuing their campaign of cleavable QACs, they found that cleavable amidequats displayed antibacterial activity in the low micromolar range with decent therapeutic indexes against eukaryotic cell lines.[17] Quite recently, they reported the activity of a series of amide-substituted QACs bearing two cationic residues, noting strong activity in compounds presumably more resistant to decomposition than esterquats

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http://dx.doi.org/10.1016/j.bmcl.2017.03.077
0960-894X/

previously reported.[18] Other gemini esterquats have also been published.[19,20]

We recognized that there are multiple approaches to the installation of instability into multiQAC structures. We viewed two competing tactics as the preparation of "edge-destruct" or "center-destruct" functionality. In the case where a labile moiety is incorporated between the two (or more) cationic residues, then fragmentation would lead to two (or more) daughter QACs (Fig. 1, bottom). Alternatively, when an "edge-destruct" strategy would be taken, then a multicationic residue from the center of the molecule is produced; it would no longer be an amphiphile and may have no residual biological activity. We thus pursued an "edge-destruct" strategy, and chose to apply it to a number of our previously prepared QAC architectures.

Having prepared over 400 novel QACs in our laboratories,[21] with a particular focus on muticationic variations (multiQACs), but not having investigated rationally-designed instability, we elected to apply the principles of soft antimicrobials to our compounds via the installation of multiple alkyl chains bearing cleavable moieties. In light of the previous contributions to the field, we sought to methodically assess the antimicrobial activity of a number of different multiQAC architectures bearing either ester- or amide-based sidechains. The survey of architectures would teach us which mono- or multi-QACs showed the most antimicrobial promise; the anticipated enhanced stability of amide analogs would serve to contrast with esters.

We thus decided to prepare a series of soft QACs from four different structural classes developed in our group, as well as BAC-type soft QACs (Fig. 2). Our esterquat naming system is based on the existing names we had for our previously prepared analogs; for esters, we added an E at the start of the name, and indicated the total number of atoms in the side chain (for example, giving the hexyl ester a total number of 9). We used the following 5 categories for esters: E-BAC (based on benzalkonium chloride),[22] E-TMEDA (based on a simple tetramethyl ethylenediamine core),[23] E-P (based on a piperazine backbone), E-linear (based on a linear triamine backbone), and E-sT (based on a branched T-shaped core). Amide analogs were given the designation of A. In total, we prepared 35 esterquats and 5 amidequats[18] and evaluated them for bioactivity and toxicity, as indicated by red blood cell lysis. Lead compounds were examined for their stability in water and buffers of different pH.

The assembly of 40 soft multiQACs is shown in Fig. 3. For esterquat formation, all reactions were performed by simple alkylations of bromoesters of varying chain length; these electrophiles were in turn prepared via simple Fischer esterifications according to standard methods.[24] Alkylation reaction conditions generally involved stirring in acetonitrile under ambient atmosphere and high-concentration conditions (~1 M); when heating was employed, reaction times were brief (≤3 h). This stands in contrast to the alkylation conditions we typically observe with simple alkyl halides (Δ, 24–48 h), so we infer enhanced reactivity at the position α to the carbonyl. Recrystallization or trituration afforded the compounds in good to high yields. Analogous reactions were accomplished for amidequats, again in high yields. Complete experimental detail, including compound characterization, for all 40 compounds is presented in the Supporting Information.

With 40 ester- and amide-based QACs in hand, varying in both alkyl chain length and number of cationic residues, we began to inspect both antimicrobial activity and toxicity, using red blood cell (RBC) lysis as a model. These assessments followed standard protocols.[21,25] The complete set of MIC values against six bacteria [*Staphylococcus aureus* (SA), *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, community-acquired methicillin-resistant

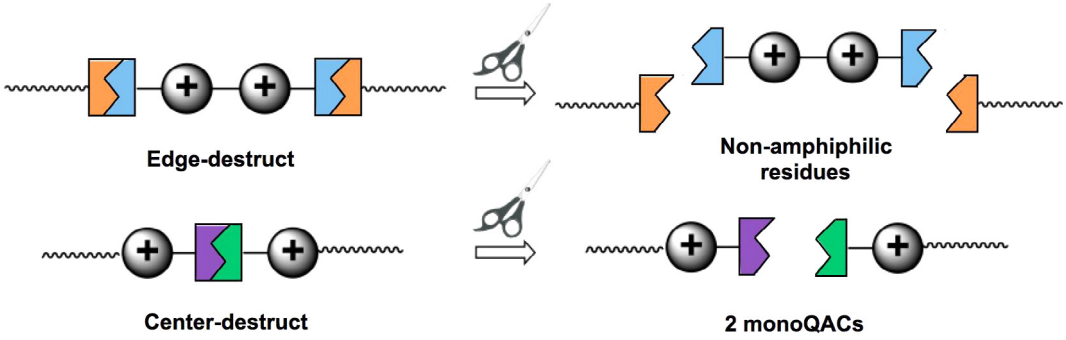

Fig. 1. Conceptualization of self-destruct strategies for multiQACs.

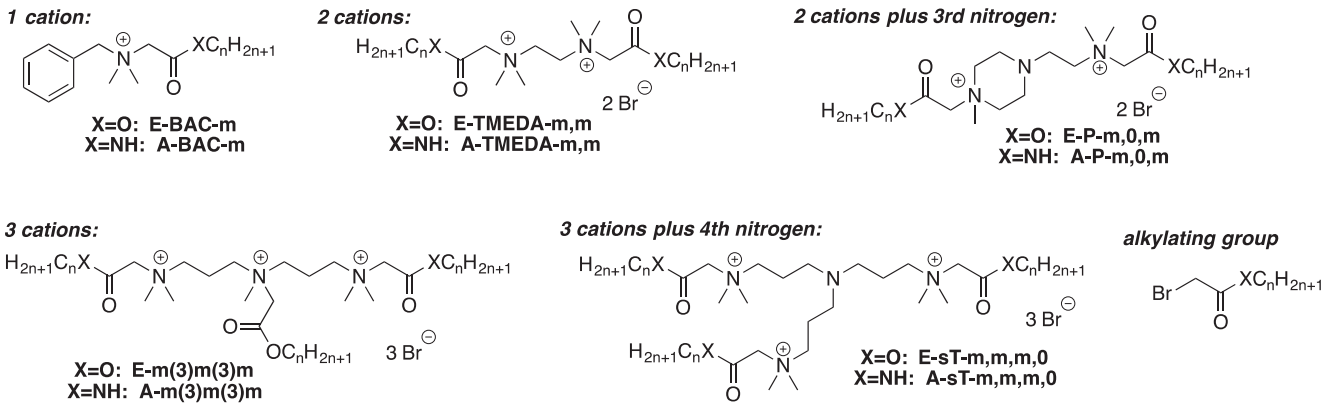

Fig. 2. Designed multicationic soft antimicrobial QACs for this investigation.