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Trivalent sulfonium compounds (TSCs): Tetrahydrothiophene-based amphiphiles exhibit similar antimicrobial activity to analogous ammonium-based amphiphiles

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ABSTRACT

Recent advances in the development of quaternary ammonium compounds (QACs) have focused on new structural motifs to increase bioactivity, but significantly less studied has been the change from ammonium- to sulfonium-based disinfectants. Herein, we report the synthesis of structurally analogous series of quaternary ammonium and trivalent sulfonium compounds (TSCs). The bioactivity profiles of these compounds generally mirror each other, and the antibacterial activity of sulfonium-based **THT-18** was found to be comparable to the commercial disinfectant, BAC. The development of these compounds presents a new avenue for further study of disinfectants to combat the growing threat of bacterial resistance.

Quaternary ammonium compounds (QACs) have been commonplace in the past century as a simple means to combat bacterial infections, being found as the active ingredients in disinfecting sprays and mouthwashes.[1,2] To prevent bacterial infection, QACs bind to bacterial cell surfaces via the positively charged nitrogen atom allowing the long aliphatic chains to disrupt the membrane, leading to cell lysis and bacterial death.[3] Due to their robust structure, QACs have found their way into the environment in low concentrations, and bacterial strains have begun to develop resistance to common commercial disinfectants at an alarming rate.[4] To address this concern, there has been a recent resurgence in the study of QAC development. Of particular interest has been the investigation of key structure–activity relationships of these amphiphilic compounds. This has allowed for the generation of QACs that are quite effective against a wide range of both Gram-negative and Gram-positive bacterial strains, including methicillin-resistant *Staphylococcus aureus* (MRSA).[5–11]

From the recent development of novel QACs, trends have emerged regarding structural motifs that increase the bioactivity profiles of these compounds, including the ratio of polar to nonpolar regions as well as the number of positively charged nitrogen atoms.[4,12,13] Less studied, however, are the effects of changing the atom that holds the formal positive charge, typically at the core of the structure. Amphiphilic compounds with phosphonium cations acting as the polar group have been reported; some of these showed superior activity against bacterial strains versus similar nitrogen-based QACs.[14–18] There are even fewer reports of amphiphilic compounds bearing trivalent sulfonium moieties as the polar group. A review of these developments has recently been published.[19]

Sulfonium compounds are prevalent in both plants[20] and animals,[21] one of the most prevalent being S-adenosyl-methionine (SAM). SAM has been utilized as therapeutic to treat depression,[22] osteoarthritis,[23] liver disease,[24] and even as a dietary supplement.[25] The bioactivity of sulfonium compounds as antiseptics, however, has been understudied, therefore offering a unique avenue to expand upon the structural diversity of current amphiphilic antiseptics.[26–29] The incorporation of a sulfonium moiety as a modification has led to promising increases in bioactivity in antimicrobial compounds that are not otherwise amphiphilic. In a recent example, the addition of a sulfonium group to vancomycin increased bioactivity against Gram-positive resistant bacteria (Fig. 1A).[30] Similarly, *N*-chloramines exhibited enhanced inhibitory effects upon the addition of lipophilic sulfonium moieties (Fig. 1B). In both cases, the observed increase in activity was attributed to the amphiphilic nature of the sulfonium moiety leading to membrane disruption and eventual cell lysis.[30,31]

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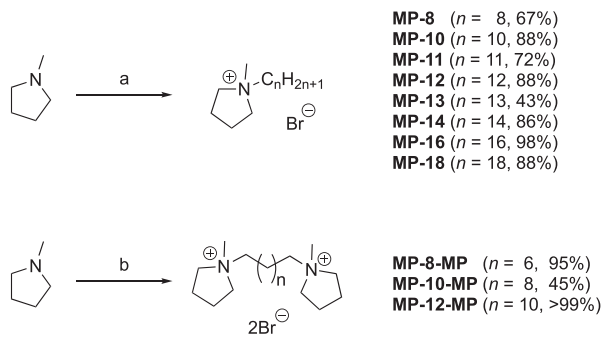
Few sulfonium-based amphiphiles, herein defined as trivalent sulfonium compounds (TSCs), made up of simple aliphatic groups have been previously reported. Hirayama reported symmetrically substituted alkyl and aryl TSCs bearing a central sulfonium moiety that showed marginally increased bioactivity compared to commercially available QACs as well as decreased dermal toxicity (Fig. 1C).[32–34] Reports of simple amphiphilic sulfonium architectures based on commercially available starting materials, however, remain elusive. We therefore sought to design simple sulfonium amphiphiles that could be readily compared to analogous quaternary ammonium amphiphiles analogs to assess potential differences in activity of nitrogen- versus sulfur-based amphiphiles.

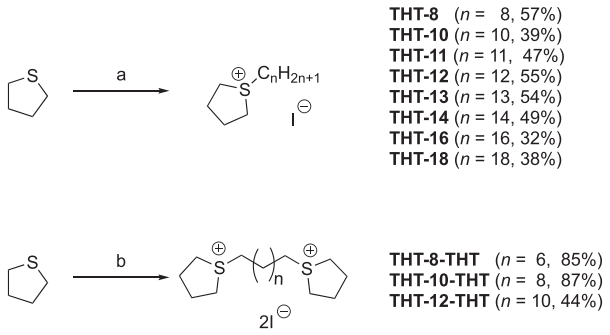
For a direct comparison of QACs and TSCs, we targeted structurally simple *N*-methyl pyrrolidine-based QACs and tetrahydrothiophene-based TSCs. In an initial experiment, *N*-methyl pyrrolidine (MP) was treated with 1.1 equivalents of dodecyl bromide in acetonitrile at reflux for 24 h, affording monoQAC **MP-12** in 88% yield following purification (Scheme 1; see Supporting Information for experimental detail and compound characterization). An analogous procedure was used to synthesize a series of 8 monoQACs, some of which have been previously reported.[35] The resulting **MP-*n*** compounds, where *n* represents the number of carbons in the alkyl chain, were furnished in 43–98% yield. Given the increased bioactivity of bisQACs compared to monoQACs observed by our group and others,[12,14] we also sought to prepare "bola" amphiphiles bearing a central nonpolar region with polar head groups on either end.[36] To this end, heating 1,8-dibromooctane in neat *N*-methyl pyrrolidine for 24 h afforded bolaQAC **MP-8-MP** in 95% yield. Using an analogous procedure, we were able to synthesize 2 other bolaQACs, **MP-10-MP** and **MP-12-MP** (Scheme 1).

With the ammonium-based amphiphiles in hand, we then turned our attention to the synthesis of sulfonium analogues. Our initial attempts of alkylating tetrahydrothiophene (THT) with 1.1 equivalents of dodecyl bromide in acetonitrile at reflux were unsuccessful. However, treatment of dodecyl iodide with a ten-fold excess of tetrahydrothiophene in acetonitrile at reflux led to formation of **THT-12** in 55% yield. An analogous procedure was used to synthesize a series of 8 monoTSCs (**THT-*n***) in modest yields. To synthesize compounds analogous to the **MP-*n*-MP** series, α,ω-substituted diiodoalkanes were heated with a five-fold excess of tetrahydrothiophene in acetonitrile at reflux for 24 h to give bolaTSCs (**THT-*n*-THT**) in 44–87% yield (Scheme 2). An improved, more atom-economical synthesis of THT-based amphiphiles is shown in Scheme 3, with characterization provided in the Supporting Information, taking advantage of an unexpected polar protic solvent, trifluoroacetic acid.[31]

With compounds in hand, we sought to compare the biological activities of the ammonium- and sulfonium-based amphiphiles against a range of bacterial strains. Given previously observed similarities in bioactivity of both iodide and bromide salts,[37,38] the initially prepared TSCs with iodide counterions were assessed for bioactivity. Using standard protocols, we assessed both antimicrobial activity and toxicity, using red blood cell (RBC) lysis as a proxy for the latter. The full set of minimum inhibitory concentration (MIC) values against six bacteria [methicillin-susceptible *Staphylococcus aureus* (MSSA - SH1000), hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA - ATCC 33591), community-acquired methicillin-resistant *S. aureus* (CA-MRSA - USA 300), *Enterococcus faecalis* (OG1RF), *Pseudomonas aeruginosa* (PA01), and *Escherichia coli* (MC4100)] and RBC lysis (presented as $Lysis_{20}$, the highest concentration at which >20% of RBCs are lysed), is



Scheme 1. Synthesis of *N*-methyl pyrrolidine-based QACs. a) 1.1 equiv $C_nH_{2n+1}Br$, CH_3CN, 80 °C, 24 h; b) 0.04 to 0.5 equiv Br–C_nH_{2n}–Br, neat, 80 °C, 24 h.



Scheme 2. Synthesis of tetrahydrothiophene-based TSCs. a) 0.1 equiv $C_nH_{2n+1}I$, CH_3CN, 80 °C, 24 h; b) 0.2 equiv I–C_nH_{2n}–I, CH_3CN, 80 °C, 24 h.

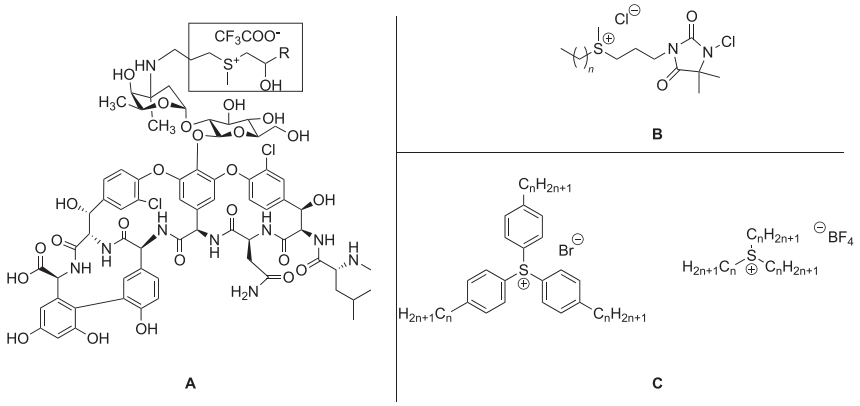
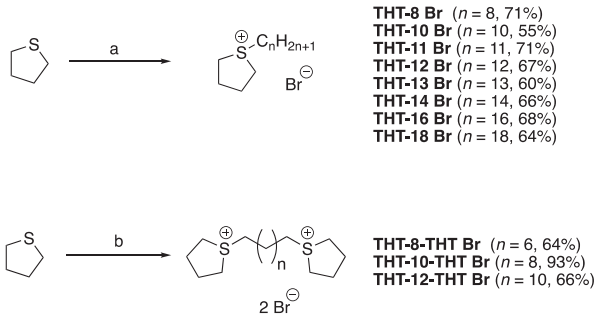


Figure 1. Examples of known amphiphilic sulfonium compounds with antibacterial activity: A) vancomycin derivative with appended sulfonium moiety,[30] (B) *N*-chloramine with appended sulfonium group[31] and (C) bioactive trialkyl and -aryl sulfonium compounds.[32–34]



THT-8 Br (n = 8, 71%)
THT-10 Br (n = 10, 55%)
THT-11 Br (n = 11, 71%)
THT-12 Br (n = 12, 67%)
THT-13 Br (n = 13, 60%)
THT-14 Br (n = 14, 66%)
THT-16 Br (n = 16, 68%)
THT-18 Br (n = 18, 64%)

THT-8-THT Br (n = 6, 64%)
THT-10-THT Br (n = 8, 93%)
THT-12-THT Br (n = 10, 66%)

Scheme 3. Improved synthesis of tetrahydrothiophene-based TSCs. a) 1.1 equiv $C_nH_{2n+1}Br$, trifluoroacetic acid, 73 °C, 24 h; b) 0.5 equiv Br-C_nH_{2n}-Br, trifluoroacetic acid, 73 °C, 24 h.

shown in Table 1.

The antimicrobial activity data promised to address a direct question; is there differing activity for amphiphiles built around a sulfonium or an ammonium core? The answer was a resounding no; structurally analogous QACs and TSCs showed remarkably similar activity against all bacterial strains tested. Longer chained monocationic amphiphiles (**MP-*n*** and **THT-*n***) were effective against Gram-positive strains, but were less effective against Gram-negative bacterial strains, compared to commercial monoQACs BAC and CPC. These findings are consistent with previous reports showing that TSCs have greater activity against Gram-positive strains compared to Gram-negative strains.[32,33]

Increase in alkyl chain length beyond 14 carbons was necessary to observe good antibacterial activity. The most potent compounds of the series were **MP-18** and **THT-18**, both of which exhibited low micromolar MIC values against MSSA and CA-MRSA strains. This is consistent with other structure–activity studies on monoQACs where activity is best at elongated chain lengths (16–18 carbons).[39] Somewhat unsurprisingly, the biscationic bola compounds (**MP-*n*-MP** and **THT-*n*-THT**) showed poor activity against all strains tested. This likely results from the structural composition of the bola amphiphiles that contain a short nonpolar region and lack a significant hydrophobic moiety, the latter of which likely contributes to its activity via membrane disruption. The development of longer chained sulfonium-based bola-amphiphiles will be of future interest.

As antibacterial activity increased, hemolytic activity also increased as evidenced by lower $Lysis_{20}$ values. However, none of the reported compounds were as hemolytic as commercial disinfectant CPC ($Lysis_{20}$ = 8 μM). The therapeutic indices of the tested commercial ammonium-based disinfectants are similar to that of **THT-18**. These data point to potential utility of TSCs as alternatives to these traditional ammonium-based antiseptics.

Most compounds tested, except CPC, exhibited decreased activity against HA-MRSA compared to both MSSA and CA-MRSA. The decreased activity of all QACs and TSCs tested against HA-MRSA points to the importance of addressing bacterial resistance in the development of novel disinfectants. Nonetheless, the unique properties of sulfur, including its greater polarizability compared to nitrogen, may be an asset moving forward in addressing the growing concern of bacterial resistance towards commercial QACs.[4,40]

Overall, this work sought to expand the architecture of amphiphilic disinfectants by comparing the activity of sulfonium-based TSCs and structurally analogous QACs. To this end, we synthesized a series of QACs and TSCs based on *N*-methyl pyrrolidine and tetrahydrothiophene nucleophiles, respectively. A comparison of the bioactivity profiles of these compounds revealed striking similarities in activity against Gram-

Table 1
Antimicrobial activity and toxicity data for ammonium- and sulfonium-based amphiphiles.[a]

Compound	Minimum Inhibitory Concentration (μM)						$Lysis_{20}$ (μM)
	MSSA	HA-MRSA	CA-MRSA	*E. faecalis*	*P. aeruginosa*	*E. coli*	
BAC	2	8	2	250	125	32	32
CPC	1	1	1	125	125	32	8
MP-8	>250	>250	>250	>250	>250	>250	125
MP-10	>250	>250	>250	>250	>250	>250	250
MP-11	125	>250	125	>250	>250	>250	125
MP-12	32	125	32	>250	>250	>250	125
MP-13	8	63	16	>250	>250	>250	63
MP-14	4	16	8	>250	>250	250	32
MP-16	4	8	2	>250	>250	125	16
MP-18	2	8	4	>250	250	63	16
MP-8-MP	>250	>250	>250	>250	>250	>250	125
MP-10-MP	>250	>250	>250	>250	>250	>250	125
MP-12-MP	>250	>250	>250	>250	>250	>250	125
THT-8	>250	>250	>250	>250	>250	>250	125
THT-10	250	>250	250	>250	>250	>250	125
THT-11	125	250	250	>250	>250	>250	125
THT-12	16	125	16	>250	>250	>250	125
THT-13	8	125	32	>250	>250	>250	63
THT-14	4	32	4	>250	>250	250	16
THT-16	2	8	4	>250	>250	125	16
THT-18	1	8	4	250	250	63	16
THT-8-THT	>250	>250	>250	>250	>250	>250	125
THT-10-THT	250	>250	250	>250	>250	>250	125
THT-12-THT	250	>250	250	>250	>250	>250	250

[a] Gram-negative bacterial strains *P. aeruginosa* and *E. coli* are shaded. All MIC and $Lysis_{20}$ data were acquired through compilations of the highest value of three independent trials; all trials were within one dilution.

positive strains, with **THT-18** as the best compound overall with low micromolar MICs against both MSSA and CA-MRSA, as well as therapeutic indices similar to commercial disinfectants tested. The bioactivity profile of **MP-18** and **THT-18** against Gram-positive strains is comparable to commercial disinfectant BAC. Given the overall similarities in bioactivity between the synthesized QACs and TSCs, further study is warranted into TSCs to expand our current library of disinfectants to combat bacterial infections and the rapid development of bacterial resistance to commercial disinfectants. In light of the superior activity of bisQACs versus traditional ammonium amphiphiles, bisTSCs represent an area ripe for exploration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127809.

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