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Article

Highly Effective and Broad-Spectrum Antimicrobial Quaternary Ammonium Salts Containing Camphene Structure: Preparation, Surface-Active Properties, and Bioassay

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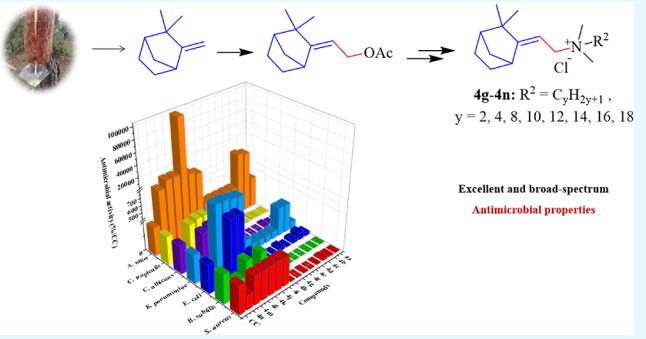
ABSTRACT: A series of novel quaternary ammonium salts (QASs) (**4a−4n**) comprising a camphene moiety were synthesized for the first time. Fourteen examples were prepared from camphene through Prins reaction, halogenation, and quaternarization, successively. The structures of the synthesized QASs were analyzed by Fourier transform infrared spectroscopy, ^{1}H NMR, ^{13}C NMR and high-resolution mass spectrometry. Surface-active properties, emulsifying abilities, and foaming properties of the investigated compounds were then studied. The antimicrobial activities of these QASs against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*), and fungi species (*Candida albicans*, *Candida tropicalis*, and *Aspergillus niger*) were determined by the microdilution method. The results showed that the chemical structural factors, including types of substitutes and alkyl chain length, might be correlated with the lipid−water partition coefficient (cLog *P*), which played a critical role in the antimicrobial process. Compounds with alkyl chain lengths (*N*) in the range of 10−14 were relatively more active, while compounds bearing pyridinium, benzyl, methylimidazolium groups, or varied alkyl chain lengths ($N < 5$ and $N > 16$) were almost inactive. Compound **4k** possessing a dodecyl group exhibited the most effective and broad-spectrum antimicrobial activity against almost all tested bacteria and fungi with the minimal inhibitory concentration values ranging from 0.24 to 0.98 μg/mL.



INTRODUCTION

Infectious diseases triggered by bacteria, viruses, and fungi emerge suddenly and spread quickly, posing an ongoing threat that could strike us at any time. In particular, an outbreak of pneumonia caused by a novel, highly contagious coronavirus has led to over one-sixth of global deaths in the past 3 years.[1] Under those circumstances, disinfectants are commonly used in hospitals, industries, and households, which play a pivotal role on epidemic prevention and control.[2] Quaternary ammonium salts (QASs) are one of the most favorable and effective classes of disinfectants due to their excellent antibacterial activity, relatively low toxicity, easy preparation, and low price.[3,4]

With the lucubrating of research, the development of modern QASs has been in rapid progress over the past 2 decades. The types of cations have evolved from the simplest alkyl chain to diverse organic skeleton, and the change of the anions has also varied from halogen ions to other inorganic anions, such as hydrogen sulfate, tetrafluoroborate, hexafluorophosphate, etc.[5−7] Moreover, the number of carbon chain present has upgraded from single-chain to double-chain QASs, and the number of positive charges present can be divided into mono-, bis (gemini)-, and multi-QAS.[8,9] These led to the enhancement of broad-spectrum antimicrobial properties of the following developed QASs.

Unfortunately, the fight against infections still faces difficulties since the widespread use of these QASs may induce environmental issues and the emergence of new strains of antimicrobial-resistant microorganisms.[10,11] For example, the results obtained by Amangeldykyzy and coworkers have shown that the most frequently used QASs, cetalkonium chloride (**CC**) and benzalkonium chloride, were ineffective to a number of strains, such as*Pseudomonas aeruginosa* and *Staphylococcus* spp.[12] Besides, it has been reported that benzalkonium chloride has significantly low antimicrobial action against *Escherichia coli* and *P. aeruginosa*, with the minimal inhibitory concentration (MIC) over 1 μg/mL.[13−15] To handle these problems, enhanced QASs capable of killing bacteria effectively and moderately are encouraged.

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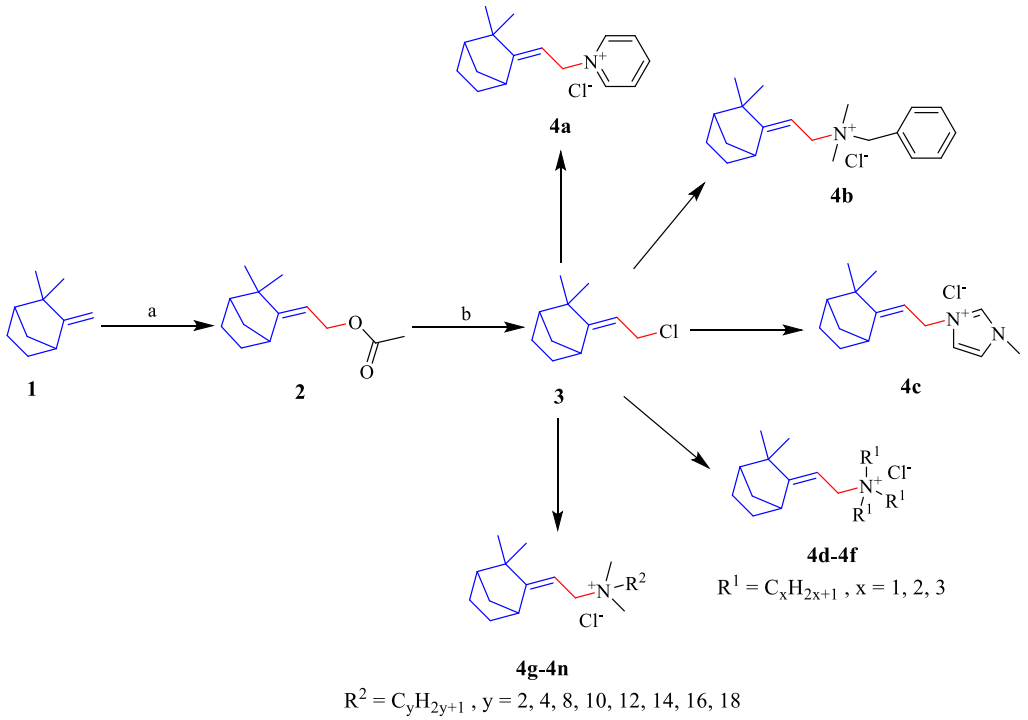
Scheme 1. Synthetic Route of Target Compounds 4a−4n[a]



[a]Reagents and conditions: (a) $(HCHO)_n$, HAc, 110 °C, 24 h; (b) AcCl, EtOH, 30 °C, reflux, 5 min.

In terms of the principle of green chemistry, green and sustainable products made from biomass resources have become a popular trend. Recently, β-pinene, one of the components of turpentine, was successfully introduced to novel QASs. The majority of those salts displayed high and broad-spectrum antifungal, antibacterial, and anticancer activities.[16] This finding indicates that β-pinene and its derivatives have significant research and application values in microbial action. Similarly, camphene, a minor constituent of many essential oils derived from plants, such as turpentine oil, cypress oil, citronella oil, etc., can be produced by the isomerization reaction of α-pinene.[17−20] It has been well-demonstrated that camphene possesses a wide range of pharmacological activities, including antimicrobial, antitumor, antihyperlipidemia, and insecticidal activities, so that it has been widely applied in the field of agriculture, forestry, and pharmaceutical.[21−24] Therefore, camphene is an ideal resource for developing novel QASs.

However, camphene cannot be applied directly to show its high efficacy because of the nature of volatility and water insolubility.[25] To overcome these shortcomings, research efforts have been directed toward molecular structure modification or derivation. Taking advantages of its chemical properties through variety of reactions, such as addition, oxidation, isomerization, acetyl methylation, and formylation, several functional groups with synergistic or enhanced hydrophilicity and biological activities could be introduced into the molecular structure.[21,26−30] It is worth mentioning that ω-acetoxymethyl camphene (**2**) synthesized via Prins condensation reaction retains the molecular structure of camphene with high bioactive functions. In the previous work of our group, ω-chloromethyl camphene (**3**), belonging to allyl chloride compounds, was synthesized from compound **2** and acetyl chloride with anhydrous ethanol by a facile halogenation procedure. The studies revealed that the N-substituted derivative of 1,2-benzo[*d*]isothiazol-3(2*H*)-one prepared from compound **3** is more soluble in nonpolar organic solvents than 1,2-benzo[*d*]isothiazol-3(2*H*)-one and presented higher antimicrobial activity than camphene and compound **2** against bacteria and fungi.[31]

Motivated by the continuous interest in developing novel QASs and further exploring the use of camphene derivatives for antimicrobial agents as well, the introduction of the camphene moiety into QASs was carried out for the first time in this work. A library of novel camphene-based QASs were synthesized from camphene through multiple steps and were all verified by Fourier transform infrared spectroscopy (FTIR), ^{1}H NMR, ^{13}C NMR, and high-resolution mass spectrometry (HRMS). Their microbehavior in the solution through surface tension measurements, emulsification measurements, foamability, and foam stability tests was also determined. In the end, the antibacterial and antifungal properties of the series of camphene-based QASs have been tested, which not only makes us have a deeper understanding of the synthesized products but also facilitates the development of biomedicines.

RESULTS AND DISCUSSION

Synthesis. Compound **3** is one of the derivatives of compound **2**, comprising camphene, allyl, and a chloride atom in its molecular structure. Compound **3** belongs to allyl chloride compounds and thereby shows good chemical reaction activity. Previous studies found that this compound is likely to react with organic tertiary amine easily through quaternarization under normal heating reflux conditions with high yields of QASs. The products are soluble in water and easy for separation and purification but insoluble in ethyl acetate, petroleum ether, ether, and other weak polar or nonpolar organic solvents.[31] In this study, compounds **2** and **3**

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were synthesized from camphene (**1**) according to our previous report.[31] Different organic tertiary amines were quaternized with compound **3** by using ethyl acetate as a solvent to give a series of novel quaternary ammonium salts (**4a**−**4n**). The synthetic route for the preparation of the target compounds (**4a**−**4n**) is depicted in Scheme 1. These 14 QASs were obtained in good yields with up to 70%. The best results (yields of salts **4g** and **4j**−**4l** 80−86%) were produced using *N*,*N*-dimethylethylamine, *N*,*N*-dimethyldecylamine, *N*,*N*-dimethyldodecylamine, and *N*,*N*-dimethyltetradecylamine, respectively. The yields of QASs **4a**−**4c** from the corresponding pyridine, *N*,*N*-dimethylbenzylamine, and 1-methylimidazole did not exceed 75%. Different kinds of amines formed QASs **4d**−**4h** and **4m**−**4n** with a close yield (around 75%).

Structural Analysis. The chemical structures of the synthesized QASs were characterized by FTIR, ^{1}H NMR, ^{13}C NMR, and HRMS, respectively. The FTIR spectrum data showed that the absorption band of the =C−H group from the camphene molecular structure was at 3048 cm^{-1} from Figures S1−S14 (Supporting Information). The characteristic features for compounds **2** and **3** were absorption of C−H stretching vibrations at 2959 and 2870 cm^{-1}, the skeletal vibration band of the C=C group at 1672 cm^{-1}, and the bending vibration band of −CH_2− group at 1460 cm^{-1} as well as the stretching vibration band of C−Cl at 660 cm^{-1}. Moreover, the disappearance of the stretching vibration bands of C=O and C−O groups at 1740 and 1231 cm^{-1}, respectively, confirmed the formation of compound **3**. Furthermore, the absorption bands at 2976−2700 cm^{-1} were attributed to the C−H stretching vibration bands of the methyl and methylene groups for the synthesized compounds **4a**−**4n**. The characteristic absorption band of the C=C group from camphene was at 1670 cm^{-1}, and the absorption band of multiple −CH_2−CH_2−groups was observed at 1487−1456 cm^{-1}. Thus, the FTIR spectra confirmed that 14 camphenyl QASs were successfully synthesized.

According to ^{1}H NMR spectra, the proton chemical shift of the carbon−carbon double bond was around δ 5.20 and that of the two methyl groups on camphene was around δ 0.9 (Figures S15−S28, Supporting Information). Moreover, as can be seen in the ^{13}C NMR spectra (Figures S29−S42), the chemical shift of the carbon−carbon double bond appeared at δ 169 and δ 101, respectively. Furthermore, from electrospray ionization-mass spectrometry (ESI-MS), the ion peaks of investigated compounds were consistent with their theoretical values (Figures S43−S56).

Surface-Active Properties. As the surfactant concentration in water increases, the surfactant molecules gathered on the surface form an oriented compact monolayer. The formation of micelles is a reflection of the repulsion occurring between the different hydrophobic chains and the aqueous medium, and the minimum concentration at which micelles begin to form is called critical micelle concentration (CMC). Plots of the surface tension against the molar concentration are shown in Figures S57−S71, and the CMC values of the synthesized compounds and the corresponding γ_{CMC} values are listed in Table 1. The data revealed that both the CMC and γ_{CMC} values of compound **4b** were higher than those of compounds **4a** and **4c**. The reason is that nitrogen heterocycle may play a significant role in the micellization process. Moreover, the CMC and γ_{CMC} values of compounds **4d**−**4f** showed an upward trend as the chain length of substituted groups at the nitrogen atom increased. It suggested that the change of the alkyl substituent at the central nitrogen atom was likely to have a strong effect on molecular electrostatic interactions. Furthermore, it was noteworthy that the CMC and γ_{CMC} values of compounds **4g**−**4n** decreased with the elongation of the hydrophobic chain length. This is caused by the gradual increase in the hydrophobicity of the compounds as the length of the hydrocarbon chain increased from 2 to 18. It can also be clearly seen that the values of γ_{CMC} of compounds **4i**−**4l** were lower than that of the commercial QAS, **CC**, and thereby these four compounds presented outstanding surface-active properties.

Table 1. Surface Tension and CMC of Compounds 4a−4n

compounds	CMC (mmol/L)	γ_{CMC} (mN/m)
water	0	71.84
4a	4.00	27.65
4b	8.00	39.02
4c	2.50	25.82
4d	9.00	29.84
4e	10.00	33.98
4f	12.00	45.55
4g	15.00	35.05
4h	15.00	28.36
4i	1.75	18.72
4j	0.40	18.34
4k	0.15	18.60
4l	0.02	17.61
4m	0.03	22.44
4n	0.005	22.66
CC	0.004	19.49

Emulsifying Activities. The emulsifying activity is one of the most considerable parameters in the study of surfactants.[32−34] Figure 1 illustrates the time required to separate 10 mL of water from the emulsion formed between surfactant aqueous solution and benzene/α-pinene. It is known that the longer the time is, the better is the emulsifying power of the measured surfactant.[35] The data showed that the emulsification activity of compounds **4g**−**4n** enhanced significantly first and then decreased with increasing alkyl chain length at the quaternary nitrogen. This probably results from an increase in the hydrophobicity of the molecules (−CH_2−CH_2-groups

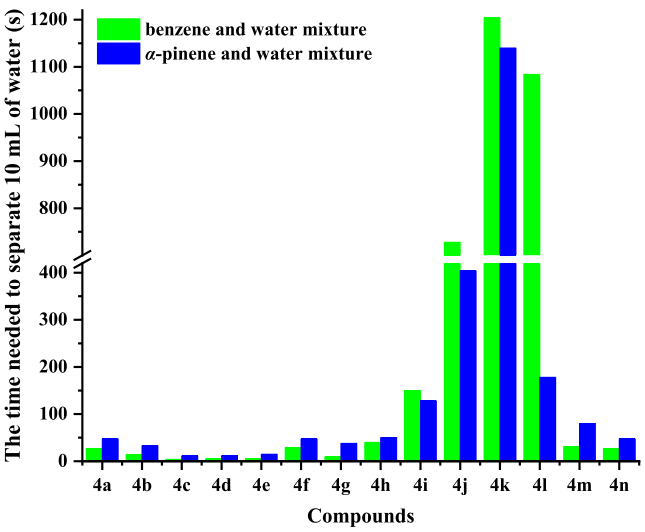

Figure 1. Emulsifying activities of tested compounds **4a**−**4n**.

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within the alkyl chain), which contributes to the emulsion stability. However, compared to that of compounds with long alkyl chain lengths, compounds **4m**−**4n** were less effective for emulsifying benzene and α-pinene, possibly because the impaired electrostatic repulsion at the surface of emulsion droplets led to unstable emulsion. It is worth noting that compound **4k** exhibited maximum emulsification power for benzene and α-pinene, which could be due to its adequate hydrophile−lipophile balance. The obtained results might be attributed to the CMC and γ_{CMC} values of these surfactants.

Foaming Properties. The foaming properties of the prepared camphenyl QASs have been measured in different aqueous media as described above. The experimental results of the foaming capacity (FC) and foaming stability (FS) of 14 synthesized compounds are given in Figure 2. It can be found

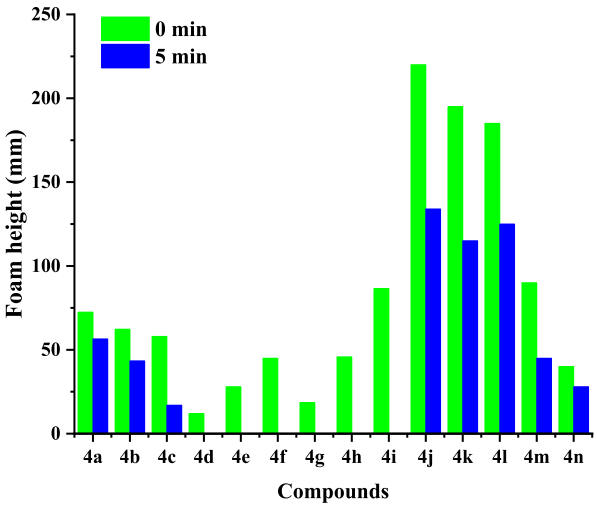

Figure 2. Foaming height of compounds **4a**−**4n** at 0 and 5 min.

that the FC and FS of compound **4a** were more superior than those of compounds **4b**−**4c**; this might be due to the structural differences of compounds **4b**−**4c** containing a benzene ring and an imidazole ring, respectively. On the other hand, the FC of compounds **4d**−**4f** gradually enhanced, which indicated that the growth of alkyl chain length could enhance the cohesive force between the molecules and then influence the elasticity and strength of the liquid film. Conversely, the FS of compounds **4d**−**4i** was poor as there was zero foam height after 5 min. For compounds **4g**−**4n**, the FC of compounds **4g**−**4j** improved rapidly where the number of alkyl-chain-lengths at the quaternary nitrogen increased from 2 to 10 but that of compounds **4k**−**4n** declined steadily as the number of alkyl-chain-length was beyond 11. The weak FC of those compounds with longer hydrophobic carbon chains originated from the high surface tension, lack of elasticity, and strong steric hindrance. In addition, compounds **4j**−**4l** showed moderate foam heights ranging between 115 and 134 mm at 5 min; thus, these compounds have better FS than others. There is a possibility that the stronger surface activity (lower γ_{CMC}) is beneficial to the stability of the foam. It can be concluded that the foam properties of the synthesized compounds were not only ascribed to their surface tension and structure but also related to other factors, such as gas diffusion and gravity drainage.

Antimicrobial Activities. The data of MIC for 14 QASs **4a**−**4n** against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*), and fungi (*Candida albicans*, *Candida tropicalis*, and *Aspergillus niger*) were obtained, as shown in Table 2. Compound **1** and commercial **CC** were chosen as the key reference compounds in the MIC test. The MIC values of compounds **4a**−**4n** were further used to assess their antimicrobial activities by comparison to that of **CC**. The lipophilicity of the synthesized compounds was expressed in terms of their cLog *P* values calculated using ChemBioDraw Ultra 12.0.[36−38]

As can be seen from Table 2, a majority of investigated compounds displayed inhibitory and bactericidal effects against the four bacterial strains and fungal species. In particular,

Table 2. Summary Data of MIC and *N* and cLog *P* Values of Compounds 4a−4n against Bacteria and Fungi[a]

compounds	MIC (μg/mL)							*N*	cLog *P*
	Gram-positive bacteria		Gram-negative bacteria		fungi				
	S. aureus	*B. subtilis*	*E. coli*	*K. pneumoniae*	*C. albicans*	*C. tropicalis*	*A. niger*		
1	15.6	250	125	62.5	31.3	31.3	31.3		4.702
4a	15.6	15.6	62.5	62.5	62.5	31.3	125		0.009
4b	7.81	31.3	31.23	15.6	125	31.3	62.5		1.57
4c	3.91	15.6	62.5	15.6	15.6	15.6	32.3		0.351
4d	31.3	250	250	62.5	>500	62.5	>500	1	−1.048
4e	62.5	250	62.5	62.5	>500	>500	>500	2	0.539
4f	15.6	15.6	250	62.5	>500	62.5	>500	3	2.126
4g	125	31.3	250	62.5	>500	250	>500	2	−0.519
4h	31.3	1.95	125	31.3	>500	125	>500	4	0.539
4i	0.242	0.483	15.6	0.977	15.6	1.95	31.3	8	2.655
4j	0.242	0.242	3.91	0.483	1.95	0.242	7.81	10	3.713
4k	0.242	0.483	0.977	0.242	0.242	0.242	0.483	12	4.771
4l	0.242	0.242	0.977	0.242	0.977	0.977	3.91	14	5.829
4m	0.242	0.483	>500	3.91	3.91	3.91	3.91	16	6.887
4n	0.483	3.91	>500	>500	31.3	15.6	31.3	18	7.945
CC	0.242	0.242	7.81	15.6	0.977	0.977	500	16	5.297

[a]Abbreviations: MIC, minimum inhibitory concentration; *N*, alkyl chain length at quaternary nitrogen atom; and cLog *P*, lipid−water partition coefficient.

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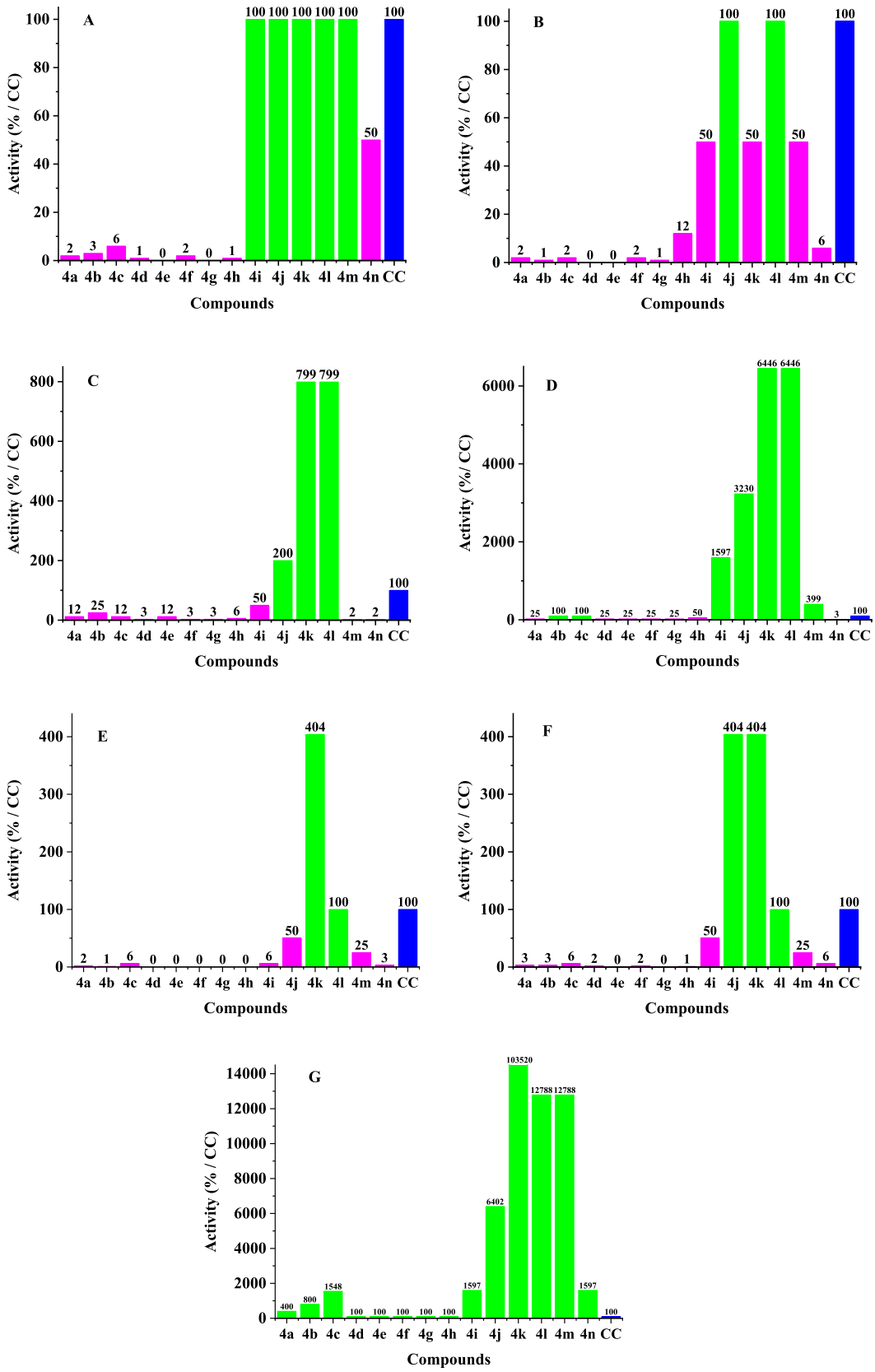

Figure 3. Antimicrobial activities of synthesized compounds **4a**−**4n** against (A) *S. aureus*, (B) *B. subtilis*, (C) *E. coli*, (D) *K. pneumoniae*, (E) *C. albicans*, (F) *C. tropicalis*, and (G) *A. niger* compared with that of **CC** (the MIC value of **CC**/ the MIC value of the tested compound).

compound **4k** showed the highest activity with the values of MIC ranging between 0.24 and 0.98 μg/mL, and the best results were found against *K. pneumoniae*, *S. aureus*, and *C. tropicalis*(0.242 μg/mL). Similar results were obtained for compound **4l** with the values of MIC in the range of 0.24−3.91 μg/mL with particularly higher efficiency against *K. pneumo-*

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niae, *S. aureus*, and *B. subtilis*. However, compounds **4i**−**4j** exhibited the same efficiency against *S. aureus* with the value of MIC at 0.24 μg/mL, but higher concentrations were mandatory to inhibit *E. coli*, *C. albicans*, and *A. niger*. Compounds **4a**−**4h** displayed less activity against all bacterial strains and fungal species, and relatively low antimicrobial activity (MIC > 62.5 μg/mL) could be observed from compounds **4d**−**4h** against three fungal species.

These findings were consistent with the observation achieved in Figure 3. It is clear that the synthesized compounds **4a**−**4h** containing pyridinium, benzyl, methylimidazolium, methyl, ethyl, propyl, and butyl groups at the quaternary nitrogen were almost inactive. For compounds with 8−18 carbon atoms in the alkyl substituent at the quaternary nitrogen, comparable antibacterial and fungi inhibition activity was reached by compounds **4j**−**4l**, except in a few limited circumstances. Moreover, compounds with *N* values in the range of 16−18 (**4m**−**4n**) were less inactive than compounds with *N* values in the range of 10−14 (**4j**−**4l**) on *E. coli*, *K. pneumoniae*, and partially fungal species. Furthermore, when compared to the antimicrobial potency of **CC** and other compounds, the compound with the dodecyl group at the quaternary nitrogen (**4k**) seems to be the most active against fungi, Gram-positive and Gram-negative strains except for *B. subtilis*. This is in line with previous studies which showed that the lipophilicity (cLog *P*) is strongly associated with antimicrobial activity of the corresponding QASs.[39]

More interestingly, for fatty alkyl QASs with short chains, an increase in a fat alkyl carbon chain led to a rise in their lipophilicity, which made them more active toward bacteria and fungi. When the carbon chain length is up to 16, the antimicrobial activity of this compound weakens. This phenomenon is possible because a decrease in hydrophilicity of a target compound leads to an increase in its lipophilicity, as the growth of the carbon chain. It can also be seen that compounds with cLog $P < 2$ and compounds with cLog $P > 6$ both presented almost inactive antimicrobial performance, while compounds having cLog *P* values ranging from 3 to 5 were more active in inhibiting bacteria. Similar results have been obtained for the other studied fungal species. Overall, the antimicrobial potency of the synthesized compounds highly depended on substituents at the quaternary nitrogen atom, and the antimicrobial activity of compound **4k** against three fungal species was likely to be the most effective among all prepared compounds and **CC**, which suggests the potential of using this compound for antimicrobial treatment further.

EXPERIMENTAL SECTION

Materials. Camphene [gas chromatography (GC) purity of 97%] was purchased by Wuzhou Huangpu Chemical Pharmaceutical Co., Ltd.; acetyl chloride, trichloromethane, anhydrous ethanol, anhydrous sodium sulfate, paraformaldehyde, glacial acetic acid, ethyl acetate, petroleum ether, sodium bicarbonate, sodium chloride, benzene, α-pinene, pyridine (a), *N*,*N*-dimethylbenzylamine (b), 1-methylimidazole (c), trimethylamine (d), triethylamine (e), tri-*n*-propylamine (f), *N*,*N*-dimethylethylamine (g), *N*,*N*-dimethylbutylamine (h), *N*,*N*-dimethyloctylamine (i), *N*,*N*-dimethyldecylamine (j), *N*,*N*-dimethyldodecylamine (k), *N*,*N*-dimethyltetradecylamine (l), *N*,*N*-dimethylhexadecylamine (m), *N*,*N*-dimethyloctadecylamine (n), and **CC** (also known as *N*-hexadecyl-*N*,*N*-dimethylbenzenemethanaminium chloride) were used. These reagents used were commercially available.

Synthesis of ω-Acetyloxymethyl Camphene (2). According to the method reported by Nayak et al.,[40] in a 1000 mL round-bottomed flask, fitted with a reflux condenser and a mechanical stirrer, compound **2** was synthesized from compound **1** (136.23 g, 1.00 mol, purity of 84.6%), paraformaldehyde (36.05 g, 1.00 mol), and glacial acetic acid (292.86 g, 4.88 mol) in an oil bath at 110 °C for 24 h. The reaction mixture was heated to 145 °C to distill unreacted glacial acetic acid and cooled to room temperature; an appropriate amount of distilled water was then added into the solution. The oily organic layer was separated, and the aqueous portion was extracted with petroleum ether three times. The combined organic portions were washed with saturated sodium bicarbonate until they became neutral, then washed with saturated sodium chloride, and dried with anhydrous sodium sulfate. After removal of the solvent, the product with a yield of 98.0% and a GC purity of 91.0% was obtained via reduced pressure distillation (bp 108 °C/5 mmHg). IR (KBr) ν_{max}: 3048, 2959, 1740, 1231 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): δ 5.17−5.19 (dd, 1H, *J* = 10.1, 5.9 Hz, H-10), 4.07−4.14 (dd, 2H, *J* = 11.0, 8.0 Hz, H-11), 3.01−3.02 (d, 1H, *J* = 4.5 Hz, H-4), 1.91−1.92 (m, 1H, H-1), 1.63−1.70 (m, 4H, H-5, H-6), 1.16−1.22 (m, 2H, H-7), 1.03 (s, 3H, H-8), 1.01 (s, 3H, H-9); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 163.07 (C_3), 111.93 (C_{10}), 47.94 (C_1), 42.85 (C_4), 42.38 (C_{11}), 41.43 (C_7), 37.37 (C_2), 29.01 (C_8), 28.16 (C_9), 25.76 (C_5), 23.78 (C_6).

Synthesis of ω-Chloromethylcamphene (3). Compound **3** was synthesized from compound **2**. Compound **2** (2.08 g, 0.01 mol) was transferred to a 100 mL three-necked, round-bottomed flask with anhydrous ethanol (0.02 mol). After adding acetyl chloride (1.57 g, 0.02 mol) dropwise, the round-bottomed flask in the water bath was heated to reflux while stirring magnetically at 30 °C for 5 min. Once the reaction stopped, the unreacted acetyl chloride and ethanol were removed by rotary evaporator. Then, an appropriate amount of distilled water and 20 mL of trichloromethane were poured slowly and stirred fully into the reaction mixture. In the next step, the solution was kept for a while before organic phase was separated and aqueous phase was extracted with 10 mL of trichloromethane three times. Solutions in the organic phase were all collected and washed to neutral with distilled water. In the last step, tan sticky liquid was obtained after the solution mixture was dried with anhydrous sodium sulfate and removed trichloromethane by reduced pressure distillation. After vacuum drying, the final product with a yield of 95.2% and a GC purity of 95.0% was obtained. IR (KBr) ν_{max}: 3048, 2959, 2870, 1672, 1460, 660 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): δ 5.17−5.19 (dd, 1H, *J* = 10.1, 5.9 Hz, H-10), 4.07−4.14 (dd, 2H, *J* = 11.0, 8.0 Hz, H-11), 3.01−3.02 (d, 1H, *J* = 4.5 Hz, H-4), 1.91−1.92 (1H, m, H-1), 1.63−1.70 (4H, m, H-5, H-6), 1.16−1.22 (2H, m, H-7), 1.03 (3H, s, H-8), 1.01 (3H, s, H-9); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 163.07 (C_3), 111.93 (C_{10}), 47.94 (C_1), 42.85 (C_4), 42.38 (C_{11}), 41.43 (C_7), 37.37 (C_2), 29.01 (C_8), 28.16 (C_9), 25.76 (C_5), 23.78 (C_6); high-resolution electron ionization mass spectrometry (HREIMS) *m*/*z*: 149.1332 (calcd for $[C_{11}H_{17}]^+$, 149.1330).

Synthesis of Camphenyl Quaternary Ammonium Salt Compounds (4a−4n). The preparation of camphenyl quaternary ammonium salt compounds **4b**−**n** followed the typical procedure described below for the preparation of compound **4a**.

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*(E)-1-(2-(3,3-Dimethylbicyclo[2.2.1]heptan-2-ylidene)-ethyl)pyridine-1-ium Chloride (**4a**).* Compound **3** (3.68 g, 20 mmol) was dissolved in 30 mL of ethyl acetate, and then pyridine (1.90 g, 24 mmol) was added into the solution. The reaction solution was refluxed at 85 °C for 4 h. Once the reaction was completed, the solution was cooled to room temperature; the solvent and the residue were removed. The brown solids were dried under vacuum at 50 °C for 3.5 h after being washed with ethyl acetate for several times to give corresponding salts, yield = 74.47%. mp 126.21−126.56 °C; IR (KBr) ν_{max}: 3070, 3052, 2961, 2869, 1677, 1633, 1615, 1500, 1477, 1148, 887, 774, 676 cm^{-1}; ^1H NMR (D_2O, 600 MHz): δ 8.72 (2H, d, J = 5.7, H-1′, H-5′), 8.47 (1H, t, J = 7.8, H-3′), 7.98 (2H, t, J = 7.0, H-2′, H-4′), 5.32 (1H, t, J = 7.6, H-10), 5.17 (2H, t, J = 7.3, H-11), 3.12 (1H, d, J = 4.1, H-4), 1.95 (1H, d, J = 2.2, H-1), 1.76−1.59 (4H, m, H-5, H-6), 1.45−1.27 (2H, m, H-7), 1.01 (6H, d, J = 8.2, H-8, H-9); ^{13}C NMR (D_2O, 150 MHz): δ 169.23 (C_3), 145.28 ($C_{1'}$, $C_{5'}$), 143.48 ($C_{3'}$), 127.92 ($C_{2'}$, $C_{4'}$), 106.14 (C_{10}), 60.01 (C_{11}), 47.23 (C_1), 42.13 (C_2), 41.54 (C_4), 36.79 (C_7), 27.95 (C_8), 27.30 (C_9), 24.77 (C_6), 23.00 (C_5); HREIMS m/z: 228.1751 (calcd for $[C_{16}H_{22}N]^+$, 228.1752).

*(E)-N-Benzyl-2-(3,3-dimethylbicyclo[2.2.1]heptan-2-ylidene)-N,N-dimethylethan-1-aminium Chloride (**4b**).* White solids, yield = 72.62%. mp 188.87−189.27 °C; IR ν_{max}: 3003, 2963, 2942, 2867, 1674, 1486, 1457, 1365, 1342, 1108, 861, 736, 713 cm^{-1}; ^1H NMR (D_2O, 600 MHz): δ 7.36 (5H, t, J = 12.3 Hz, H-3′, H-4′, H-5′, H-6′, H-7′), 5.10 (1H, t, J = 7.5 Hz, H-10), 4.23 (2H, s, H-1′), 3.92−3.62 (2H, m, H-11), 2.85 (1H, s, H-4), 2.82−2.49 (6H, m, H-1″, H-2″), 1.80 (1H, s, H-1), 1.52 (4H, d, J = 8.0 Hz, H-5, H-6), 1.24 (1H, d, J = 12.2 Hz, H-7), 1.15 (1H, d, J = 9.8 Hz, H-7), 0.92 (6H, t, J = 15.2 Hz, H-8, H-9); ^{13}C NMR (D_2O, 150 MHz): δ 172.60 (C_3), 132.71 ($C_{3'}$, $C_{7'}$), 130.51 ($C_{2'}$), 128.97 ($C_{4'}$, $C_{6'}$), 127.25 ($C_{5'}$), 100.98 (C_{10}), 67.03 ($C_{1'}$), 63.48 (C_{11}), 48.34 ($C_{1''}$), 47.15 (C_1), 42.39 (C_2), 41.66 (C_4), 36.69 (C_7), 28.10 (C_6), 26.80 (C_8), 24.75 (C_9), 22.99 (C_5); HREIMS m/z: 284.2380 (calcd for $[C_{20}H_{30}N]^+$, 284.2378).

*(E)-3-(2-(3,3-Dimethylbicyclo[2.2.1]heptan-2-ylidene)-ethyl)-1-methyl-1H-imidazol-3-ium Chloride (**4c**).* Dark brown oily liquid, yield = 63.42%. IR ν_{max}: 3072, 2959, 2868, 1680, 1568, 1458, 1384, 1160, 851 cm^{-1}; ^1H NMR (D_2O, 600 MHz): δ 7.60 (1H, s, H-1′), 7.36 (1H, s, H-2′), 7.27 (1H, s, H-3′), 5.12 (1H, t, J = 7.6 Hz, H-10), 4.69 (2H, dd, J = 31.2, 5.9 Hz, H-11), 3.76 (3H, s, H-4′), 3.73 (1H, s, H-4), 3.01 (1H, d, J = 3.4 Hz, H-1), 1.66−1.23 (4H, m, H-5, H-6), 0.98 (2H, dd, J = 29.1, 15.8 Hz, H-7), 0.87 (6H, d, J = 10.0 Hz, H-8, H-9); ^{13}C NMR (D_2O, 150 MHz): δ 165.88 (C_3), 135.31 ($C_{1'}$), 123.68 ($C_{2'}$), 121.70 ($C_{3'}$), 107.12 (C_{10}), 47.99 (C_1), 47.55 (C_{11}), 42.10 (C_2), 41.36 (C_4), 37.03 (C_7), 35.81 ($C_{4'}$), 28.50 (C_6), 27.62 (C_8), 25.27 (C_9), 23.42 (C_5); HREIMS m/z: 231.1864 (calcd for $[C_{15}H_{23}N_2]^+$, 231.1861).

*(E)-2-(3,3-Dimethylbicyclo[2.2.1]heptan-2-ylidene)-N,N,N-trimethylethan-1-aminium Chloride (**4d**).* Light gray solids, yield = 73.63%. mp 251.4−252.07 °C; IR ν_{max}: 2956, 2700, 1676, 1479, 1258, 897 cm^{-1}; ^1H NMR (600 MHz, D_2O): δ 5.11−5.02 (1H, m, H-10), 3.80−3.65 (2H, m, H-11), 2.93−2.90 (1H, m, H-4), 2.87−2.84 (9H, m, H-1′, H-2′, H-3′), 1.82 (1H, s, H-1), 1.63−1.46 (4H, m, H-5, H-6), 1.08−0.92 (2H, m, H-7), 0.91 (6H, d, J = 11.2, H-8, H-9); ^{13}C NMR (150 MHz, D_2O): δ δ172.70 (C_3), 101.25 (C_{10}), 5.05 (C_{11}), 51.70 ($C_{1'}$, $C_{2'}$, $C_{3'}$), 47.16 (C_1), 44.58 (C_2), 41.57 (C_4), 36.72 (C_7), 28.12 (C_6), 26.87 (C_8), 24.75 (C_9), 23.01 (C_5); HREIMS m/z: 208.2068 (calcd for $[C_{14}H_{26}N]^+$, 208.2060).

*(E)-2-(3,3-Dimethylbicyclo[2.2.1]heptan-2-ylidene)-N,N,N-triethylethan-1-aminium Chloride (**4e**).* White solids, yield = 71.81%. mp 167.13−167.6 °C; IR ν_{max}: 2976, 2868, 1676, 1487, 1013, 793 cm^{-1}; ^1H NMR (D_2O, 600 MHz): δ 4.91 (1H, t, J = 7.8 Hz, H-10), 3.62 (2H, q, J = 14.1, 7.8 Hz, H-11), 3.04 (6H, q, J = 7.3 Hz, H-1′), 2.90 (1H, d, J = 4.0 Hz, H-4), 1.81 (1H, s, H-1), 1.59−1.48 (3H, m, H-5, H-6), 1.30−1.23 (1H, m, H-7), 1.17 (1H, d, J = 9.9 Hz, H-7), 1.14−1.08 (9H, m, H-2′), 0.89 (6H, d, J = 16.5 Hz, H-8, H-9); ^{13}C NMR (D_2O, 150 MHz): δ 171.02 (C_3), 100.50 (C_{10}), 56.07 (C_{11}), 51.82 ($C_{1'}$), 47.18 (C_1), 42.27 (C_2), 41.76 (C_4), 36.75 (C_7), 28.09 (C_6), 27.03 (C_5), 24.74 (C_8), 23.05 (C_9), 6.67 ($C_{2'}$); HREIMS m/z: 250.2534 (calcd for $[C_{17}H_{32}N]^+$, 250.2535).

*(E)-N-(2-(3,3-Dimethylbicyclo[2.2.1]heptan-2-ylidene)-ethyl)-N,N-dipropylpropan-1-aminium Chloride (**4f**).* White solids, yield = 70.90%. mp 163.03−163.4 °C; IR ν_{max}: 2968, 2868, 1676, 1487, 1107, 854 cm^{-1}; ^1H NMR (D_2O, 600 MHz): δ 4.89 (1H, t, J = 7.7 Hz, H-10), 3.67 (2H, d, J = 7.7 Hz, H-11), 2.92 (6H, dd, J = 11.0, 6.1 Hz, H-1′), 2.88 (1H, d, J = 3.3 Hz, H-4), 1.81 (1H, s, H-4, H-5, H-6), 1.73−1.41 (8H, m, H-1, $H_{2'}$), 1.17 (1H, d, J = 9.8 Hz, H-7), 0.95 (1H, dd, J = 15.8, 10.7 Hz, H-7), 0.89 (6H, d, J = 13.0 Hz, H-8, H-9), 0.76 (9H, t, J = 7.2 Hz, H-3′); ^{13}C NMR (D_2O, 150 MHz): δ 170.81 (C_3), 100.64 (C_{10}), 59.22 ($C_{1'}$), 57.86 (C_{11}), 47.19 (C_1), 42.26 (C_2), 41.80 (C_4), 36.77 (C_7), 28.14 (C_6), 27.09 (C_5), 24.75 (C_8), 23.07 (C_9), 14.89 ($C_{2'}$), 9.77 ($C_{3'}$); HREIMS m/z: 292.3008 (calcd for $[C_{20}H_{38}N]^+$, 292.3004).

*(E)-2-(3,3-Dimethylbicyclo[2.2.1]heptan-2-ylidene)-N-ethyl-N,N-dimethylethan-1-aminium Chloride (**4g**).* White solids, yield = 80.25%. mp 207.9−208.5 °C; IR ν_{max}: 3001, 2985, 2867, 1674, 1470, 1032, 891 cm^{-1}; ^1H NMR (D_2O, 600 MHz): δ 5.01 (1H, t, J = 8.0 Hz, H-10), 3.69 (2H, dd, J = 8.0, 3.4 Hz, H-11), 3.12 (2H, q, J = 7.3 Hz, H-1′), 2.90 (1H, t, J = 6.7 Hz, H-4), 2.77 (6H, s, H-1″, H-2″), 1.81 (1H, d, J = 1.8 Hz, H-1), 1.61−1.46 (4H, m, H-5, H-6), 1.16 (2H, t, J = 7.8 Hz, H-7), 1.03−0.89 (6H, m, H-8, H-9), 0.88 (3H, s, H-2′); ^{13}C NMR (D_2O, 150 MHz): δ 172.22 (C_3), 100.97 (C_{10}), 62.65 (C_{11}), 58.58 ($C_{1'}$), 48.64 (C_1), 47.16 ($C_{1''}$, $C_{2''}$), 42.3 (C_4), 47.16 (C_2), 36.71 (C_7), 28.09 (C_6), 26.87 (C_8), 24.74 (C_9), 23.01 (C_5), 7.37 ($C_{2'}$); HREIMS m/z: 222.2222 (calcd for $[C_{15}H_{28}N]^+$, 222.2222).

*(E)-N-(2-(3,3-Dimethylbicyco[2.2.1]heptan-2-ylidene)-ethyl)-N,N-dimethylbutan-1-aminium Chloride (**4h**).* White solids, yield = 76.32%. mp 196.83−197.5 °C; IR ν_{max}: 2961, 2867, 1670, 1465, 1106, 881 cm^{-1}; ^1H NMR (D_2O, 600 MHz): δ 4.99 (1H, t, J = 8.0 Hz, H-10), 3.76−3.62 (2H, m, H-11), 3.04−2.96 (2H, m, H-1′), 2.89 (1H, d, J = 3.9 Hz, H-4), 2.79 (6H, dd, J = 22.2, 6.7 Hz, H-1″, H-2″), 1.80 (1H, s, H-1), 1.65−1.44 (4H, m, H-5, H-6), 1.37−1.11 (4H, m, H-7, H-2′), 1.05−0.85 (8H, m, H-8, H-9, H-3′), 0.75 (3H, t, J = 7.4 Hz, H-4′); ^{13}C NMR (D_2O, 150 MHz): δ 172.08 (C_3), 101.01 (C_{10}), 63.04 ($C_{1'}$), 62.92 (C_{11}), 49.32 ($C_{1''}$, $C_{2''}$), 47.14 (C_1), 42.27 (C_2), 41.64 (C_4), 36.68 (C_7), 28.07 (C_6), 26.86 (C_5), 24.71 (C_8), 23.81 (C_9), 22.98 ($C_{2'}$), 19.05 ($C_{3'}$), 12.68 ($C_{4'}$); HREIMS m/z: 250.2535, (calcd for $[C_{17}H_{32}N]^+$, 250.2535).

*(E)-N-(2-(3,3-Dimethylbicyclo[2.2.1]heptan-2-ylidene)-ethyl)-N,N-dimethyloctan-1-aminium Chloride (**4i**).* White solids, yield = 73.34%. mp 140.13−141.07 °C; IR ν_{max}: 2927, 2919, 2849, 1672, 1469, 1107, 883, 725 cm^{-1}; ^1H NMR (D_2O, 600 MHz): δ 4.99 (1H, t, J = 8.0 Hz, H-10), 3.70 (2H, qd, J = 13.3, 8.1 Hz, H-11), 3.03−2.94 (2H, m, H-1′), 2.89 (1H, d, J =

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3.8 Hz, H-4), 2.79 (6H, d, J = 3.9 Hz, H-1″, H-2″), 1.81 (1H, s, H-1), 1.69−1.45 (6H, m, H-5, H-6, H-7), 1.10 (12H, ddd, J = 62.0, 39.5, 10.3 Hz, H-2′, H-3′, H-4′, H-5′, H- 6′, H-7′), 0.89 (6H, d, J = 11.8 Hz, H-8, H-9), 0.67 (3H, t, J = 6.9 Hz, H-8′); ^{13}C NMR (D_2O, 150 MHz): δ 171.96 (C_3), 101.04 (C_{10}), 62.97 ($C_{1'}$), 62.89 (C_{11}), 49.51 ($C_{1''}$, $C_{2''}$), 47.14 (C_1), 42.28 (C_2), 41.66 (C_4), 36.72 (C_7), 30.85 ($C_{6'}$), 28.10 (C_6), 28.03−28.01 ($C_{4'}$, $C_{5'}$), 26.90 ($C_{3'}$), 25.42 (C_8), 24.73 (C_9), 23.00 ($C_{2'}$), 21.85 (C_5), 21.72 ($C_{7'}$), 13.25 ($C_{8'}$); HREIMS m/z: 306.3165, (calcd for $[C_{21}H_{40}N]^+$, 306.3161).

*(E)-N-(2-(3,3-Dimethylbicyclo[2.2.1]heptan-2-ylidene)-ethyl)-N,N-dimethyldecan-1-aminium Chloride (**4j**).* White solids, yield = 82.31%. mp 161.13−161.9 °C; IR ν_{max}: 2925, 2855, 1672, 1466, 1107, 894, 721 cm^{-1}; ^{1}H NMR (D_2O, 600 MHz): δ 5.00 (1H, t, J = 7.9 Hz, H-10), 3.74 (2H, dd, J = 32.3, 6.6 Hz, H-11), 2.94 (3H, s, H-1′, H-4), 2.86 (6H, s, H-1″, H-2″), 1.77 (1H, s, H-1), 1.60−1.49 (4H, m, H-5, H-6), 1.32−0.92 (18H, m, H-7, H-2′, H-3′, H-4′, H-5′, H-6′, H-7′, H-8′, H-9′), 0.90 (d, 6H, J = 14.2 Hz, H-8, H-9), 0.68 (t, 3H, J = 6.2 Hz, H-10′); ^{13}C NMR (D_2O, 150 MHz): δ 169.85 (C_3), 102.06 (C_{10}), 62.10 ($C_{1'}$), 61.25 (C_{11}), 50.89 ($C_{1''}$), 50.63 ($C_{2''}$), 47.51 (C_1), 42.44 (C_2), 41.78 (C_4), 37.03 (C_7), 31.86 ($C_{8'}$), 29.45 (C_6), 29.31 ($C_{5'}$), 29.26 ($C_{6'}$), 28.64 ($C_{4'}$), 28.60 ($C_{7'}$), 27.17 ($C_{3'}$), 25.84 (C_8), 25.13 (C_9), 23.42 (C_5), 22.53 ($C_{2'}$), 21.88 ($C_{9'}$), 13.75 ($C_{10'}$); HREIMS m/z: 334.3474, (calcd for $[C_{23}H_{44}N]^+$, 334.3474).

*(E)-N-(2-(3,3-Dimethylbicyclo[2.2.1]heptan-2-ylidene)-ethyl)-N,N-dimethyldodecan-1-aminium Chloride (**4k**).* White solids, yield = 81.34%. mp 156.0−156.67 °C; IR ν_{max}: 2957, 2919, 2849, 1672, 1469, 1107, 864, 724 cm^{-1}; ^{1}H NMR (600 MHz, D_2O): δ 5.02 (1H, t, J = 7.7 Hz, H-10), 3.86−3.69 (2H, m, H-11), 2.99 (1H, s, H-4), 2.93 (8H, dd, J = 16.7, 7.2 Hz, H-1′, H-1″, H-2″), 1.74 (1H, s, H-1), 1.61−1.49 (5H, m, H-5, H-6), 1.29−0.99 (23H, m, H-7, H-2′− H-11′), 0.92 (6H, d, J = 15.5 Hz, H-8, H-9), 0.70 (3H, t, J = 6.7 Hz, H-12′); ^{13}C NMR (D_2O, 150 MHz): δ 169.58 (C_6), 102.22 (C_{10}), 61.99 ($C_{1'}$), 61.13 (C_{11}), 50.99 ($C_{2''}$), 50.73 ($C_{1''}$), 47.58 (C_1), 42.44 (C_2), 41.79 (C_4), 37.07 (C_7), 31.91 ($C_{10'}$), 29.72−29.44 ($C_{4'}$, $C_{5'}$, $C_{6'}$, $C_{7'}$, $C_{8'}$), 28.70 (C_6), 27.19 ($C_{3'}$, $C_{9'}$), 25.88 (C_8), 25.19 (C_9), 23.48 ($C_{2'}$), 22.56 (C_5), 21.91 ($C_{11'}$), 13.75 ($C_{12'}$); HREIMS m/z: 362.3786, (calcd for $[C_{25}H_{48}N]^+$, 362.3787).

*(E)-N-(2-(3,3-Dimethylbicyclo[2.2.1]heptan-2-ylidene)-ethyl)-N,N-dimethyltetradecan-1-aminium Chloride (**4l**).* White solids, yield = 85.34%. mp 151.0−151.8 °C; IR ν_{max}: 3015, 2918, 2854, 1667, 1470, 1111, 904, 721 cm^{-1}; ^{1}H NMR (D_2O, 600 MHz): δ 5.04 (1H, t, J = 7.6 Hz, H-10), 3.91−3.70 (2H, m, H-11), 3.02 (1H, s, H-4), 2.94 (8H, d, J = 8.9 Hz, H-1′, H-1″, H-2″), 1.73 (1H, s, H-1), 1.56 (5H, dd, J = 23.7, 12.1 Hz, H-5, H-6), 1.30−1.01 (27H, m, H-7, H-2′− H-13′), 0.93 (6H, d, J = 15.5 Hz, H-8, H-9), 0.71 (3H, t, J = 6.7 Hz, H-14′); ^{13}C NMR (D_2O, 150 MHz): δ 169.24 (C_3), 102.45 (C_{10}), 61.93 ($C_{1'}$), 61.12 (C_{11}), 51.07 ($C_{1''}$), 50.81 ($C_{2''}$), 47.65 (C_1), 42.45 (C_2), 41.80 (C_4), 37.11 (C_7), 31.92 (C_6), 29.88 ($C_{12'}$), 29.76−29.34 ($C_{5'}$, $C_{6'}$, $C_{7'}$, $C_{8'}$, $C_{9'}$, $C_{10'}$), 28.77 ($C_{4'}$, $C_{11'}$), 27.23 (C_5), 25.96 (C_8), 25.25 (C_9), 23.54 ($C_{3'}$), 22.58 ($C_{2'}$), 21.98 ($C_{13'}$), 13.78 ($C_{14'}$); HREIMS m/z: 390.4109, (calcd for $[C_{27}H_{52}N]^+$, 390.4100).

*(E)-N-(2-(3,3-Dimethylbicyclo[2.2.1]heptan-2-ylidene)-ethyl)-N,N-dimethylhexadecan-1-aminium Chloride (**4m**).* White solids, yield = 70.56%. mp 146.93−147.1 °C; IR ν_{max}: 2918, 2849, 1673, 1470, 1134, 895, 721 cm^{-1}; ^{1}H NMR (D_2O, 600 MHz): δ 5.03 (1H, t, J = 7.5 Hz, H-10), 3.89−3.71 (2H, m, H-11), 3.02 (1H, s, H-4), 2.93 (8H, d, J = 8.9 Hz, H-1′, H-1″, H-2″), 1.73 (1H, s, H-1), 1.56 (5H, dd, J = 28.7, 6.9 Hz, H-5, H-6), 1.28−1.00 (32H, m, H-7, H-2′ − H-15′), 0.93 (6H, d, J = 15.7 Hz, H-8, H-9), 0.71 (3H, t, J = 6.7 Hz, H-16′); ^{13}C NMR (150 MHz, D_2O): δ 169.26 (C_3), 102.43 (C_{10}), 61.91 ($C_{1'}$), 61.11 (C_{11}), 51.06 ($C_{1''}$), 50.80 ($C_{2''}$), 47.64 (C_1), 42.45 (C_2), 41.79 (C_4), 37.11 (C_7), 31.93($C_{14'}$), 29.92 ($C_{5'}$, $C_{6'}$, $C_{7'}$, $C_{8'}$, $C_{9'}$, $C_{10'}$, $C_{11'}$, $C_{12'}$), 29.50 (C_6), 28.76 ($C_{4'}$, $C_{13'}$), 27.22 (C_5), 25.94 (C_8), 25.25 (C_9), 23.55 ($C_{3'}$), 22.59 ($C_{2'}$), 21.96 ($C_{15'}$), 13.78 ($C_{16'}$); HREIMS m/z: 416.4417, (calcd for $[C_{29}H_{56}N]^+$, 416.4413).

*(E)-N-(2-(3,3-Dimethylbicyclo[2.2.1]heptan-2-ylidene)-ethyl)-N,N-dimethyloctadecan-1-aminium Chloride (**4n**).* White solids, yield = 75.59%. mp 82.3−82.67 °C; IR ν_{max}: 2918, 2849, 1673, 1470, 1110, 907, 721 cm^{-1}; ^{1}H NMR (D_2O, 600 MHz): δ 5.01 (1H, s, H-10), 3.78 (2H, d, J = 36.4, H-11), 3.00 (1H, s, H-4), 2.91 (8H, d, J = 8.5, H-1′, H-1″, H-2″), 1.70 (1H, s, H-1), 1.60−1.47 (4H, m, H-5, H-6), 1.26−0.98 (36H, m, H-7, H-2′− H-17′), 0.90 (6H, d, J = 15.6, H-8, H-9), 0.69 (3H, t, J = 6.5, H-18′); ^{13}C NMR (D_2O, 150 MHz): δ 169.16 (C_3), 102.46 (C_{10}), 61.84 ($C_{1'}$), 61.08 (C_{11}), 51.05 ($C_{1''}$), 50.78 ($C_{2''}$), 47.64 (C_1), 42.42 (C_2), 41.77 (C_4), 37.10 (C_7), 31.94 ($C_{16'}$), 30.01 ($C_{5'}$, $C_{6'}$, $C_{7'}$, $C_{8'}$, $C_{9'}$, $C_{10'}$, $C_{11'}$, $C_{12'}$, $C_{13'}$, $C_{14'}$), 29.82 (C_6), 28.75 ($C_{4'}$, $C_{15'}$), 27.20 ($C_{3'}$), 25.93 (C_8), 25.24 (C_9), 23.54 ($C_{2'}$), 22.59 (C_5), 21.95 ($C_{17'}$), 13.79 ($C_{18'}$); HREIMS m/z: 446.4718, (calcd for $[C_{31}H_{60}N]^+$, 446.4726).

Analytical Methods. The structures of the prepared compounds were confirmed by their spectral data. ^{1}H NMR and ^{13}C NMR spectra were recorded on an Avance II 600 MHz spectrometer (Bruker Co., Ltd., Switzerland) using D_2O or $CDCl_3$ solutions and tetramethylsilane as the internal reference. IR spectra were obtained on a Magna-IR 550 FTIR spectrometer (Nicolet Co., Ltd., USA) in the 400−4000 cm^{-1} region using KBr pellets. The melting point was determined on an X-6 apparatus (Beijing Taike Corp., Beijing, China) without correction for the thermometer. High-resolution mass spectra (ESI^+) were determined by Xevo G2-S QTOF ultra-performance liquid chromatography-quadrupole-time-of-flight-mass spectrometry (UPLC-Q-TOF-MS) (Waters Ltd., USA). The value of the lipid−water partition coefficient (cLog P) was calculated by using ChemBioDraw Ultra 12.0 software.

Surface Tension Measurements. Surface tension of all surfactant sample solutions was measured using a BZY-2 automatic surface tensiometer by the platinum ring detachment method. All 14 synthesized QASs **4a**−**4n**, commercial surfactant, and ultrapure water were used to prepare fresh aqueous solutions at 26 °C. The surface tension (γ) values were measured five times, and the recorded values were taken as the average of these values. The CMC values were taken at the intersection of the linear portion of the curve of γ versus the logarithm concentration (lnc).[41]

Emulsification Measurements. At room temperature (26 °C), a 40 mL amount of aqueous sample solutions with a mass fraction of 0.1% was placed in a 100 mL mixing cylinder. 40 mL of α-pinene and benzene was then added, respectively. The cylinder was shaken vigorously around 45 times and then allowed to settle. The time required to separate 10 mL of water from emulsion was recorded, and the experiment was repeated five times for each sample. The average separation time of the three experiments was taken as an indication of the emulsification power of each surfactant.[42]

Foamability and Foam Stability Tests. The foaming abilities and foam stability of the synthesized compounds were measured according to the procedure described in the

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literature.[43] At room temperature (26 °C), a 20 mL amount of aqueous sample solutions with a mass fraction of 0.5% was placed in a 100 mL mixing cylinder with a stopper. Warm water was then added to the volume of 30 mL. The cylinder was shaken vigorously 30 times and allowed to settle for 5 min. Foam heights at 0 and 5 min were recorded, respectively. The FC was determined by the foam height at 0 min. The FS was evaluated by the foam height after 5 min.

Antimicrobial Activity Evaluation. Antibacterial and antifungal activities of the prepared 14 compounds **4a−4n** were all tested via the microdilution method against two Gram-positive bacterial species (*S. aureus* and *B. subtilis*), Gram-negative bacterial species (*E. coli* and *K. pneumoniae*), and three fungal species, namely, *C. albicans*, *C. tropicalis*, and *A. niger*. These were purchased from the Beijing Beina Chuanglian Biotechnology Research Institute. 100 μL of axenic liquid culture medium containing 2% dimethyl sulfoxide (DMSO) and 100 μL of bacterial suspension was used as the negative control. Rifampicin was used as the positive control for bacteria, and ketoconazole was used as the positive control for fungal species. Hydrolyzed casein medium (MH) and potato glucose water compound medium were obtained from Qingdao Haibo Biotechnology company.

In a sterile 96-well plate, 100 μL of prepared sample solutions was injected by a sterile micropipette into holes no.1 and no.2, and 100 μL of axenic liquid culture medium containing 2% DMSO was successively injected into holes no.2 to no.12. A 100 μL amount of sample mixture in hole no.2 was then transferred into hole no.3, and 100 μL of sample mixture in hole no.3 was transferred into hole no.4; all the sample mixtures in the holes by analogy were conducted via the twofold dilution method, except for hole no.12.[44] The sample mixture in hole no.12 was then removed. The sample concentrations of holes no.1 to no.12 were 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.9063, 1.9531, 0.9766, and 0.4833 μg/mL, respectively. 100 μL of bacterial suspension was injected into holes no.1 to no.12 consecutively to give a final sample concentration of holes no.1 to no.12 ranging from 500, 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.9063, 1.9531, 0.9766, and 0.4833 to 0.2417 μg/mL.

The incubation was carried out at 37 °C for Gram-positive and Gram-negative bacteria and at 30 °C for fungi in the microbial incubator for 24 h. Turbidity can be observed in the negative control group after the incubation. The antimicrobial activity of the prepared QASs tested was evaluated on the basis of their MICs. MIC was defined as the lowest concentration of compounds at which the microorganisms tested do not show visible growth.[45] Three parallel experiments were carried out for each sample. On the basis of the determination of MIC, 100 μL of sample solutions with higher MIC (including MIC) was taken into 96-well plates and observed after culturing at 37 °C for 24 h.

CONCLUSIONS

In this study, a series of novel camphene-based QASs were successfully synthesized using camphene as the starting material based on our previous work and characterized by FTIR, ^{1}H NMR, ^{13}C NMR, and HRMS. The surface tension and CMC values of prepared camphenyl QASs were assessed by surface tension measurements. It has been found that the introduction of a long alkyl chain as the hydrophobic moiety at the quaternary nitrogen atom resulted in an enhanced surface-active property of investigated compounds. Moreover, it was shown that the alkyl chain length also affects the emulsifying activity of the measured compounds, and the emulsification of benzene and α-pinene by compound**4k** was outstanding. Although the trends for foaming ability and foam stability were not consistent with the compound structure, compound **4j** showed excellent foaming ability and foam stability as the height of foam production reached 220 mm at 0 min and 134 mm at 5 min, respectively. Furthermore, the majority of synthesized camphenyl compounds presented substantial antimicrobial activity against Gram-positive and Gram-negative bacteria and different fungal species. The results indicated that there was a strong correlation between the lipophilicity and the antimicrobial activity of investigated compounds. The synthesized QASs showed exceptional antimicrobial activity when their alkyl chain lengths (N) were in the range of 10−14, while compounds bearing pyridinium, benzyl, methylimidazolium groups, or varied alkyl chain lengths ($N < 5$ and $N > 16$) were almost inactive. Especially, compound **4k** displayed a pronounced and broad-spectrum antimicrobial property against almost all the chosen bacteria and fungi, which has great potential to be used as an antimicrobial agent.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c03599.

IR spectrum, ^{1}H and ^{13}C NMR spectra, HRMS of compounds **4a−4n**, surface tension, and logarithm of concentration curves (PDF)

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Notes

The authors declare no competing financial interest.

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