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Design, Synthesis, and Structure-Activity Relationship Studies of the Anaephene Antibiotics

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ABSTRACT:

The natural products, anaephenes A (**1**) and B (**2**), were found to have antimicrobial activity against methicillin resistant *Staphylococcus aureus* (MRSA). In this report, we expanded on our previous synthetic efforts by preparing a library of eighteen analogues in order to understand the structure-activity relationships (SAR) of this interesting class of natural products. These analogues were selected to explore the biological impact of structural variations in the alkyl chain and on the phenol moiety. Lastly, we further assessed the biological activity of anaephene B (**2**) and two additional analogues against other clinically relevant bacterial strains, the hemolytic activity of each and determined that these compounds act via a bactericidal mechanism. These studies led to the identification of compound **7**, which was 4 fold more potent than the natural product (**2**) against MRSA (2 vs. 8 μg/mL) and a 2-hydroxypyridine analogue (**18**) which demonstrated equal potency compared to the natural product (**2**), albeit with a significant reduction in hemolytic activity (< 1% vs. 80% at 100 μM).

KEYWORDS: antibiotics, synthesis, Methicillin-resistant *Staphylococcus aureus*, *Staphylococcus aureus*, natural products

1. Introduction

Multi-drug resistant bacteria present a critical threat to human health and require the development of novel antibiotics for treatment (Marston et al., 2016). *Staphylococcus aureus* was responsible for 119,000 infections and 20,000 deaths in the United States in 2017 (CDC, 2019). Methicillin-resistant *Staphylococcus aureus* (MRSA) first emerged within 2 years of the clinical use of methicillin and more recently has become a community-acquired infectious disease (Kluytmans-Vandenbergh et al., 2006). MRSA has caused epidemics, such as the one involving the USA300 strain, and MRSA infections result in over 23,000 deaths and over 1 million infections per year worldwide (Planet, 2017). There is also an increasing number of MRSA strains resistant to other antibiotics including lipoglycopeptides, lipopeptides, aminoglycosides, oxazolidinones, tetracyclines, quinolones, pleuromutilins and mupirocin (Watkins et al., 2019). Resistance to additional antibiotics makes treatment difficult and presents an urgent need for the development of novel antibiotics.

Natural Products have a vast range of structural diversity and have provided scientists with new molecular scaffolds for the development of new therapeutics for every disease (Patridge et al, 2016). Antibacterial natural products provide excellent starting points for the development of novel therapeutics to treat drug-resistant bacteria, considering their purpose is to interact with biological systems (Shen, 2015). This is highlighted by the fact that several current classes of antibiotics are derived from natural products including, β-lactams, tetracyclines, macrolides, glycopeptides and aminoglycosides. The ability to develop novel drug molecules from natural products is hindered by issues with isolation, supply and characterization. Total Synthesis of naturally occurring molecules provides an opportunity to overcome these issues (Rossiter et al., 2017).

Recently, we reported the first total syntheses of the antibacterial natural products, anaephenes A (**1**) and B (**2**) (Figure 1; Kukla et al., 2020). These compounds are part of the alkylphenol family which have demonstrated a wide variety of biological activities. For example, monochasiol A (**3**), has been shown to selectively inhibit the concanavalin A-induced interleukin-2 production in Jurkat cells, which could have implications in treating autoimmune diseases (Figure 1; Kikuchi et al., 2017). Also, phenolic compounds from essential oils, like eugenol, have demonstrated antibacterial activity against both MSSA and MRSA (Das et al., 2016). While there have been reports of structure-activity

relationship studies against drug-resistant bacteria on similar types of compounds such as, chromones and cannabinoids, there are few reports on alkylphenol natural products (Appendino et al., 2008; Feng et al., 2014; Li et al., 2021).

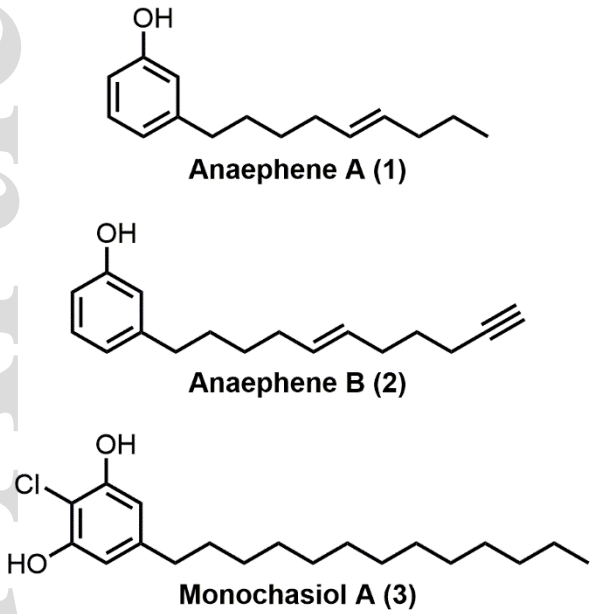


Figure 1. Examples of biologically active alkylphenol natural products.

In this study, our goal was to use our previously reported modular synthetic route towards the anaephene natural products to rapidly generate analogues in order to understand the structure-activity relationships and to improve the overall hydrophilicity of the compounds. Our strategy included examining the chain length, degrees of unsaturation in the chain, the location of the unsaturations in the chain, and three phenolic bioisosteres (Figure 2).

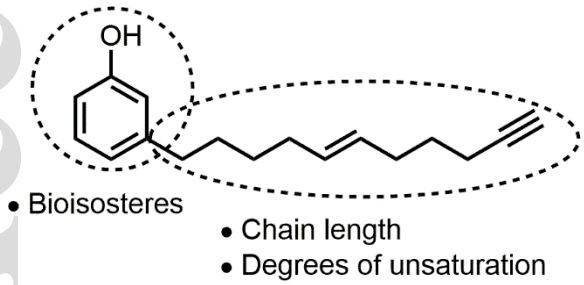


Figure 2. Strategy to understand the structure-activity relationships for the anaephene natural products.

2. Methods

2.1 General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker 400 MHz Avance III spectrometer or Bruker 500 MHz Avance III spectrometer in $CDCl_3$ unless otherwise noted. Chemical shifts are reported with the residual solvent peak used as an internal standard ($CDCl_3$ = 7.26 ppm for ^{1}H and 77.16 ppm for ^{13}C). ^{1}H NMR spectra were tabulated as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, bs = broad singlet, dt = doublet of triplet, dd = doublet of doublets, ddd = doublet of doublets of doublets), number of protons, and coupling constant(s). High-resolution mass spectra were obtained using positive mode electrospray ionization (ESI+) on a Thermo Scientific Q Exactive hybrid quadripole-Orbitrap mass spectrometer. Analytical HPLC was performed with a Thermo Finnigan Surveyor HPLC utilizing a Thermo Scientific Hypersil GOLD column (5 μm, 100 mm × 4.6 mm) run with 80% methanol and 20% 1.0 M acetic acid as the mobile phase at 1.0 mL/min to show the purity of all tested compounds was > 95%. Reactions were monitored by TLC analysis (silica gel 60 F254, 250 mm layer thickness) and visualized with a 254 nm UV light. Flash chromatography on SiO_2 was used to purify the crude reaction mixtures and performed on a flash system utilizing pre-packed cartridges and linear gradients. All starting materials and solvents were purchased from a commercial chemical company and used as received.

2.2 General experimental procedure and characterization of compounds 7, 12a-12i, 13, 15-18.

General Synthetic procedure: To a solution of *tert*-butyl(3-iodophenoxy)dimethylsilane (1 eq) in MeCN (0.2 M) was added terminal alkyne (3 eq) followed by CuI (0.2 eq) and $PdCl_2(PPh_3)_2$ (0.05 eq). Then triethylamine (3 eq) was added and the reaction was heated at 60 °C for 2 hours. Then the reaction was cooled to room temperature and tetrabutylammonium fluoride (TBAF) (3 eq) was added. The reaction was stirred at room temperature overnight and then sat. NH_4Cl was added. The aqueous layer was extracted with EtOAc (3x), the combined organic layers were then washed with brine, dried

($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (10-30% EtOAc:Hex) to afford the desired compound.

3-(undec-1-yn-1-yl)phenol (7). Yield: 39%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 7.14 (t, J = 7.8 Hz, 1H), 6.98 (dt, J = 7.7, 1.2 Hz, 1H), 6.86 (dd, J = 2.5, 1.4 Hz, 1H), 6.75 (dq, J = 2.6, 1.0 Hz, 1H), 4.68 (bs, 1H), 2.39 (t, J = 7.0 Hz, 2H), 1.60 (p, J = 7.3 Hz, 2H), 1.48-1.40 (m, 2H), 1.34-1.26 (m, 10H), 0.89 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 155.3, 129.6, 125.6, 124.5, 118.4, 115.0, 90.9, 80.3, 32.0, 29.7, 29.4, 29.3, 29.1, 28.9, 22.8, 19.5, 14.2; HRESIMS *m/z* 245.1902 $[M+H]^+$ (calcd for $C_{17}H_{25}O$, 245.1905).

3-(pent-1-yn-1-yl)phenol (12a). Yield: 41%; ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 7.15 (t, J = 7.9 Hz, 1H), 6.98 (dt, J = 7.6, 1.1 Hz, 1H), 6.86 (dd, J = 2.5, 1.4 Hz, 1H), 6.75 (ddd, J = 8.2, 2.6, 0.9 Hz, 1H), 4.65 (bs, 1H), 2.37 (t, J = 7.0 Hz, 1H), 1.62-1.58 (m, J = 7.3 Hz, 2H), 1.05 (t, J = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 155.3, 129.6, 125.6, 124.5, 118.4, 115.1, 90.6, 80.5, 22.3, 21.5, 13.7; HRESIMS *m/z* 161.0964 $[M+H]^+$ (calcd for $C_{11}H_{13}O$, 161.0966).

3-(hex-1-yn-1-yl)phenol (12b). Yield: 54%; ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 7.14 (t, J = 7.9 Hz, 1H), 6.98 (dt, J = 7.1, 0.8 Hz, 1H), 6.86 (dd, J = 2.5, 1.4 Hz, 1H), 6.74 (ddd, J = 8.2, 2.6, 0.9 Hz, 1H), 4.73 (bs, 1H), 2.39 (t, J = 7.1 Hz, 1H), 1.59 (p, J = 7.3 Hz, 2H), 1.48-1.44 (m, J = 7.3 Hz, 2H), 0.95 (t, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 155.3, 129.6, 125.6, 124.5, 118.4, 115.0, 90.8, 80.3, 30.9, 22.1, 19.2, 13.8; HRESIMS *m/z* 175.1118 $[M+H]^+$ (calcd for $C_{12}H_{15}O$, 175.1123).

3-(hept-1-yn-1-yl)phenol (12c). Yield: 73%; ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 7.14 (t, J = 7.8 Hz, 1H), 6.98 (dt, J = 7.7, 1.2 Hz, 1H), 6.86 (dd, J = 2.5, 1.4 Hz, 1H), 6.75 (ddd, J = 8.2, 2.6, 0.9 Hz, 1H), 4.70 (bs, 1H), 2.39 (t, J = 7.0 Hz, 2H), 1.61 (p, J = 7.3 Hz, 2H), 1.46-1.42 (m, 2H), 1.40-1.32 (m, 2H), 0.92 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 155.3, 129.6, 125.6, 124.5, 118.4, 115.0, 90.8, 80.3, 31.3, 28.6, 22.4, 19.5, 14.1; HRESIMS *m/z* 189.1275 $[M+H]^+$ (calcd for $C_{13}H_{17}O$, 189.1279).

3-(oct-1-yn-1-yl)phenol (12d). Yield: 70%; NMR data is consistent with the literature (Buckle et al., 1985) ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 7.14 (t, J = 7.8 Hz, 1H), 6.98 (dt, J = 7.7, 1.2 Hz, 1H), 6.86 (dd, J = 2.5, 1.4 Hz, 1H), 6.75 (ddd, J = 8.2, 2.6, 0.9 Hz, 1H), 4.64 (bs, 1H), 2.39 (t, J = 7.0 Hz, 2H),

1.60 (p, J = 7.3 Hz, 2H), 1.48-1.42 (m, 2H), 1.36-1.29 (m, 4H), 0.91 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C ; HRESIMS *m/z* 203.1432 $[M+H]^+$ (calcd for $C_{14}H_{19}O$, 203.1436).

3-(non-1-yn-1-yl)phenol (12e). Yield: 30%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 7.14 (t, J = 7.8 Hz, 1H), 6.98 (dt, J = 7.7, 1.2 Hz, 1H), 6.86 (dd, J = 2.5, 1.4 Hz, 1H), 6.75 (ddd, J = 8.2, 2.6, 0.9 Hz, 1H), 4.66 (bs, 1H), 2.39 (t, J = 7.0 Hz, 2H), 1.62 (p, J = 7.3 Hz, 2H), 1.48-1.41 (m, 2H), 1.37-1.28 (m, 6H), 0.90 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 155.3, 129.6, 125.6, 124.5, 118.4, 115.0, 90.8, 80.3, 31.9, 29.1, 29.0, 28.9, 22.8, 19.5, 14.2; HRESIMS *m/z* 217.1592 $[M+H]^+$ (calcd for $C_{15}H_{21}O$, 217.1592).

3-(dec-1-yn-1-yl)phenol (12f). Yield: 61%; NMR data is consistent with the literature (Pu et al., 2013) ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 7.14 (t, J = 7.8 Hz, 1H), 6.98 (dt, J = 7.7, 1.2 Hz, 1H), 6.86 (dd, J = 2.5, 1.4 Hz, 1H), 6.75 (ddd, J = 8.2, 2.6, 0.9 Hz, 1H), 4.69 (bs, 1H), 2.38 (t, J = 7.1 Hz, 2H), 1.59 (p, J = 7.2 Hz, 2H), 1.44 (p, J = 7.2 Hz, 2H), 1.35-1.26 (m, 8H), 0.89 (t, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 155.3, 129.6, 125.6, 124.5, 118.4, 115.0, 90.8, 80.3, 32.0, 29.4, 29.3, 29.1, 28.9, 22.8, 19.5, 14.2; HRESIMS *m/z* 231.1746 $[M+H]^+$ (calcd for $C_{16}H_{23}O$, 231.1749).

3-(dodec-1-yn-1-yl)phenol (12g). Yield: 38%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 7.14 (t, J = 7.8 Hz, 1H), 6.98 (dt, J = 7.7, 1.2 Hz, 1H), 6.86 (dd, J = 2.5, 1.4 Hz, 1H), 6.75 (ddd, J = 8.2, 2.6, 0.9 Hz, 1H), 4.65 (bs, 1H), 2.39 (t, J = 7.0 Hz, 2H), 1.59 (p, J = 7.3 Hz, 2H), 1.48-1.40 (m, 2H), 1.36-1.25 (m, 12H), 0.88 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 155.3, 129.6, 125.6, 124.5, 118.4, 115.0, 90.8, 80.3, 32.1, 29.7, 29.7, 29.5, 29.3, 29.1, 28.9, 22.8, 19.5, 14.2; HRESIMS *m/z* 259.2058 $[M+H]^+$ (calcd for $C_{18}H_{27}O$, 259.2062).

3-(tridec-1-yn-1-yl)phenol (12h). Yield: 43%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 7.14 (t, J = 7.8 Hz, 1H), 6.98 (dt, J = 7.7, 1.2 Hz, 1H), 6.86 (dd, J = 2.5, 1.4 Hz, 1H), 6.75 (ddd, J = 8.2, 2.6, 0.9 Hz, 1H), 4.79 (bs, 1H), 2.39 (t, J = 7.0 Hz, 2H), 1.60 (p, J = 7.3 Hz, 2H), 1.46-1.40 (m, 2H), 1.36-1.25 (m, 14H), 0.89 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 155.3, 129.6, 125.5, 124.4, 118.4, 115.0, 90.8, 80.3, 32.1, 29.8, 29.7, 29.7, 29.5, 29.3, 29.1, 28.9, 22.8, 19.5, 14.2; HRESIMS *m/z* 273.2214 $[M+H]^+$ (calcd for $C_{19}H_{29}O$, 273.2218).

3-(tetradec-1-yn-1-yl)phenol (12i). Yield: 45%; ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 7.14 (t, *J* = 7.8 Hz, 1H), 6.98 (dt, *J* = 7.7, 1.2 Hz, 1H), 6.86 (dd, *J* = 2.5, 1.4 Hz, 1H), 6.75 (ddd, *J* = 8.2, 2.6, 0.9 Hz, 1H), 4.72 (bs, 1H), 2.39 (t, *J* = 7.0 Hz, 2H), 1.59 (p, *J* = 7.4 Hz, 2H), 1.44 (p, *J* = 7.2 Hz, 2H), 1.34-1.27 (m, 16H), 0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 155.3, 129.6, 125.6, 124.5, 118.4, 115.0, 90.8, 80.3, 32.1, 29.8, 29.8, 29.8, 29.7, 29.5, 29.3, 29.1, 28.9, 22.8, 19.5, 14.2; HRESIMS *m/z* 287.2371 $[M+H]^+$ (calcd for $C_{20}H_{31}O$, 287.2375).

4-(undec-1-yn-1-yl)phenol (13). Yield: 51%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 7.28 (d, *J* = 8.7 Hz, 2H), 6.74 (d, *J* = 8.7 Hz, 2H), 4.76 (bs, 1H), 2.37 (t, *J* = 7.0 Hz, 2H), 1.59 (p, *J* = 7.0 Hz, 2H), 1.44 (p, *J* = 7.4 Hz, 2H), 1.34-1.26 (m, 10H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 155.1, 133.2, 116.7, 115.4, 89.0, 80.2, 32.0, 29.6, 29.4, 29.3, 29.1, 29.0, 22.8, 19.5, 14.2; HRESIMS *m/z* 245.1902 $[M+H]^+$ (calcd for $C_{17}H_{25}O$, 245.1905).

1-methoxy-3-(undec-1-yn-1-yl)benzene (15). 3-iodoanisole was used as the starting material. Yield: 38%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 7.18 (t, *J* = 7.8 Hz, 1H), 6.99 (dt, *J* = 7.7, 1.2 Hz, 1H), 6.93 (dd, *J* = 2.5, 1.4 Hz, 1H), 6.83 (ddd, *J* = 8.2, 2.6, 0.9 Hz, 1H), 3.79 (s, 3H), 2.40 (t, *J* = 7.1 Hz, 2H), 1.61 (p, *J* = 7.0 Hz, 2H), 1.45 (p, *J* = 7.1 Hz, 2H), 1.35-1.27 (m, 10H), 0.89 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 159.4, 129.3, 125.3, 124.3, 116.6, 114.2, 90.5, 80.6, 55.4, 32.0, 29.6, 29.4, 29.3, 29.1, 28.9, 22.8, 19.6, 14.2; HRESIMS *m/z* 259.2062 $[M+H]^+$ (calcd for $C_{18}H_{27}O$, 259.2062).

6-(undec-1-yn-1-yl)-1H-indole (16). 6-iodoindole was used as the starting material. Yield: 48%; ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 8.10 (bs, 1H), 7.54 (d, *J* = 8.2 Hz, 1H), 7.46 (s, 1H), 7.21 (dd, *J* = 3.1, 2.4 Hz, 1H), 7.17 (dd, *J* = 8.2, 1.3 Hz, 1H), 6.53-6.52 (m, 1H), 2.44 (t, *J* = 7.2 Hz, 2H), 1.63, (p, *J* = 7.1 Hz, 2H), 1.48 (p, *J* = 7.2 Hz, 2H), 1.37-1.29 (m, 10H), 0.90 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 135.6, 127.5, 125.2, 123.8, 120.6, 117.5, 114.4, 103.0, 88.8, 81.8, 32.0, 29.7, 29.4, 29.4, 29.1, 29.1, 22.8, 19.7, 14.2; HRESIMS *m/z* 268.2062 $[M+H]^+$ (calcd for $C_{19}H_{26}N$, 268.2065).

N-(3-(undec-1-yn-1-yl)phenyl)methanesulfonamide (17). *N*-(3-iodophenyl)methanesulfonamide was used as the starting material. Yield: 56%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 7.27-7.25 (m, 1H), 7.23-7.21 (m, 2H), 7.14 (dt, *J* = 7.6, 1.6 Hz, 1H), 6.29 (bs, 1H), 3.01 (s, 3H), 2.39 (t, *J* = 7.1 Hz, 2H), 1.60 (p, *J* = 7.0 Hz, 2H), 1.44 (p, *J* = 7.6 Hz, 2H), 1.34-1.26 (m, 10H), 0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR

($CDCl_3$, 100 MHz) δ_C 137.0, 129.8, 128.8, 126.0, 123.6, 119.9, 92.1, 79.7, 39.7, 32.0, 29.6, 29.4, 29.3, 29.1, 28.8, 22.8, 19.5, 14.2; HRESIMS *m/z* 322.1838 $[M+H]^+$ (calcd for $C_{18}H_{28}NO_2S$, 322.1841).

4-(undec-1-yn-1-yl)pyridin-2-ol (18). 4-Iodo-1,2-dihydropyridin-2-one was used as the starting material. Yield: 45%; ^1H NMR ($CDCl_3$, 500 MHz) δ_H 13.04 (bs, 1H), 7.29 (bs, 1H), 6.57 (bs, 1H), 6.22 (bs, 1H), 2.40 (t, *J* = 7.1 Hz, 2H), 1.59 (p, *J* = 7.1 Hz, 2H), 1.42 (p, *J* = 7.3 Hz, 2H), 1.33-1.25 (m, 10H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C ; HRESIMS *m/z* 246.1856 $[M+H]^+$ (calcd for $C_{16}H_{24}NO$, 246.1858).

2.3 Experimental procedures and characterization of compounds 8, 11, 14.

3-undecylphenol (8). Procedure: Compound **7** (54.5 mg, 0.163 mmol) was dissolved in anhydrous EtOH (1.4 mL) and then 10% Pd/C (3.50 mg, 0.0326 mmol) was added. The reaction flask was equipped with a hydrogen ballon and stirred at room temperature overnight. The reaction was then filtered through a pad of Celite and washed with EtOAc (10 mL). The mixture was then concentrated and purified by flash column chromatography (9:1 hexanes:EtOAc) to afford **8** (28.4 mg, 52 % yield); NMR data is consistent with the literature (Takaishi et al., 2004) ^1H NMR ($CDCl_3$, 400 MHz) δ_H 7.14 (t, *J* = 7.9 Hz, 1H), 6.76 (dd, *J* = 7.5, 0.5 Hz, 1H), 6.67-6.63 (m, 2H), 4.61 (bs, 1H), 2.56 (t, *J* = 7.6 Hz, 2H), 1.60 (p, *J* = 7.4 Hz, 2H), 1.34-1.26 (m, 16H), 0.89 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 155.5, 145.1, 129.5, 121.1, 115.4, 112.6, 36.0, 32.1, 31.4, 29.8, 29.8, 29.7, 29.6, 29.5, 29.4, 22.8, 14.2; HRESIMS *m/z* 249.2216 $[M+H]^+$ (calcd for $C_{17}H_{29}O$, 249.2218).

(E)-3-(undeca-5-en-1,10-diyn-1-yl)phenol (11). Procedure: Compound **6** (617 mg, 2.12 mmol) was taken up in MeCN (4.1 mL) and then 4 Å molecular sieves (200 mg), *N*-methyl-morpholine oxide (373 mg, 3.18 mmol) and tetrapropylammonium perruthenate (7.46 mg, 0.0212 mmol) were added. The reaction was stirred for 24 hours at room temperature and then filtered, concentrated and purified by flash column chromatography (3:1 hexanes:EtOAc) to provide compound **9**. To a solution of 5-(hex-5-yn-1-ylsulfonyl)-1-phenyl-1*H*-tetrazole (**10**; Kukla et al. 2020) (161 mg, 0.551 mmol) in THF (3.3 mL) at −78 °C was added dropwise KHMDS (0.66 mL, 1.0 M in THF, 0.66 mmol). The reaction mixture was stirred at −78 °C for 30 min, and then compound **9** (132 mg, 0.459 mmol) was added

dropwise as a 1.0 M solution in THF. The reaction was then stirred at −78 °C for 2 h followed by stirring at 0 °C for 2 h. Saturated NH_4Cl (10 mL) was added, and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (95:5 hexanes/EtOAc) to provide TBS-protected compound **11** (0.551 mmol). This compound (87.6 mg, 0.248 mmol) was then dissolved in THF (1.2 mL), and tetrabutylammonium fluoride was added as a 1 M solution in THF (0.75 mL, 0.75 mmol). The solution was stirred for 45 min at rt. Water (5 mL) was added, and the aqueous layer was extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with brine (5 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (4:1 hexanes/EtOAc) to afford **11** (59.0 mg, 13% yield over three steps); ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 7.14 (t, *J* = 7.8 Hz, 1H), 6.97 (dt, *J* = 7.7, 1.2 Hz, 1H), 6.86 (dd, *J* = 2.5, 1.4 Hz, 1H), 6.75 (ddd, *J* = 8.2, 2.6, 0.9 Hz, 1H), 5.58-5.42 (m, 2H), 4.83 (bs, 1H), 2.45 (t, *J* = 7.2 Hz, 2H), 2.28 (q, *J* = 7.1 Hz, 2H), 2.20 (td, *J* = 7.2, 2.6 Hz, 2H), 2.14 (q, *J* = 7.1 Hz, 2H), 1.96 (t, *J* = 2.7 Hz, 1H), 1.62 (p, *J* = 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 155.3, 130.8, 129.6, 129.5, 125.4, 124.4, 118.4, 115.1, 90.1, 84.8, 80.7, 68.5, 31.9, 31.6, 28.3, 19.9, 17.9; HRESIMS *m/z* 239.1432 $[M+H]^+$ (calcd for $C_{17}H_{19}O$, 239.1436).

2-(undec-1-yn-1-yl)phenol (14). Procedure: To 2-iodophenol (**S1**) (200.0 mg, 0.909 mmol) and K_2CO_3 (503 mg, 3.64 mmol) was added dry DMF (1.4 mL). Then chloromethyl methyl ether (0.10 mL, 1.36 mmol) was added at 0 °C, and the reaction was stirred overnight at room temperature. The reaction mixture was then diluted with water (5 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with brine (5 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash column chromatography (9:1 hexanes:EtOAc) to give 1-iodo-2-(methoxymethoxy)benzene (**S2**) (0.544 mmol). **S2** was then taken up in MeCN (2.6 mL) and 1-undecyne (0.12 mL, 0.598 mmol), CuI (20.7 mg, 0.109 mmol), $PdCl_2(PPh_3)_2$ (19.1 mg, 0.0272 mmol) and triethylamine (0.25 mL, 1.81 mmol) were added. The reaction was heated to 60 °C and stirred for 16 hours. The reaction was cooled to room temperature, filtered and purified by flash column chromatography (100% hexanes) to provide the MOM-protected compound (**S3**) (0.371 mmol). This was then taken up in acetone (1.7 mL) and conc. HCl (0. 20 mL) was added. The reaction was stirred

for 1 hour and then diluted with water. The reaction mixture was extracted with EtOAc (3 x 10 mL) and the combined organic layers were washed with brine (5 mL), dried (MgSO4), filtered and concentrated. The residue was purified by flash column chromatography (9:1 hexanes/EtOAc) to afford **14** (24.7 mg, 12% yield over three steps); ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 7.29 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.19 (td, *J* = 8.3, 1.7 Hz, 1H), 6.92 (dd, *J* = 8.3, 0.9 Hz, 1H), 6.84 (td, *J* = 7.5, 1.0 Hz, 1H), 5.79 (bs, 1H), 2.48 (t, *J* = 7.2 Hz, 2H), 1.63 (p, *J* = 7.6 Hz, 2H), 1.45 (p, *J* = 7.1 Hz, 2H), 1.35-1.25 (m, 10H), 0.89 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 156.7, 131.6, 129.7, 120.3, 114.4, 110.4, 98.2, 74.7, 32.0, 29.6, 29.4, 29.3, 29.1, 28.9, 22.8, 19.7, 14.2; HRESIMS *m/z* 245.1904 $[M+H]^+$ (calcd for $C_{17}H_{25}O$, 245.1905).

2.4 Antibacterial Assays. Minimum inhibitory concentrations (MIC) were determined by broth micro-dilution according to CLSI guidelines (CLSI, 2014). The test medium was lysogeny broth (LB). *S. aureus* (ATCC 25923), MRSA (ATCC 33591), MRSA (ATCC BAA-44), *S. epidermidis* (ATCC 12228), *S. epidermidis* (ATCC 51625), *E. faecalis* (ATCC 29212), *E. faecalis* (ATCC 51299), *P. aeruginosa* (ATCC 27853), and *A. baumannii* (ATCC 19606) were grown in LB for 6-8 h; this culture was used to inoculate fresh LB (5×10^5 CFU/mL). The resulting bacterial suspension was aliquoted (1 mL) and compound was added from a 10 mM DMSO stock to achieve the desired initial starting concentration (128 μg/mL). Linezolid (from a 10 mM DMSO stock) was used as a positive control with final concentrations ranging from 0.063 to 128 μg/mL. Inoculated media not treated with compound served as the negative control. The plate was incubated under stationary conditions at 37 °C. After 16 h, minimum inhibitory concentration (MIC) values were recorded as the lowest concentration of compound at which no visible growth of bacteria was observed, based on duplicate plates performed in three separate experiments. To determine the minimum bactericidal concentration (MBC), 100 μL of culture from wells containing no growth were plated onto tryptic soy agar and incubated at 37 °C overnight. The highest dilution that resulted in 99.9% reduction in the cell count was recorded as the MBC. The MBC values were determined in triplicate.

2.5 Hemolytic Activity. Hemolysis assays were performed on mechanically defibrinated sheep blood (Hemostat Labs: DSB50). Defibrinated blood (1.5 mL) was placed into a microcentrifuge tube and centrifuged for 10 min at 10,000 rpm. The supernatant was then removed and then the cells were

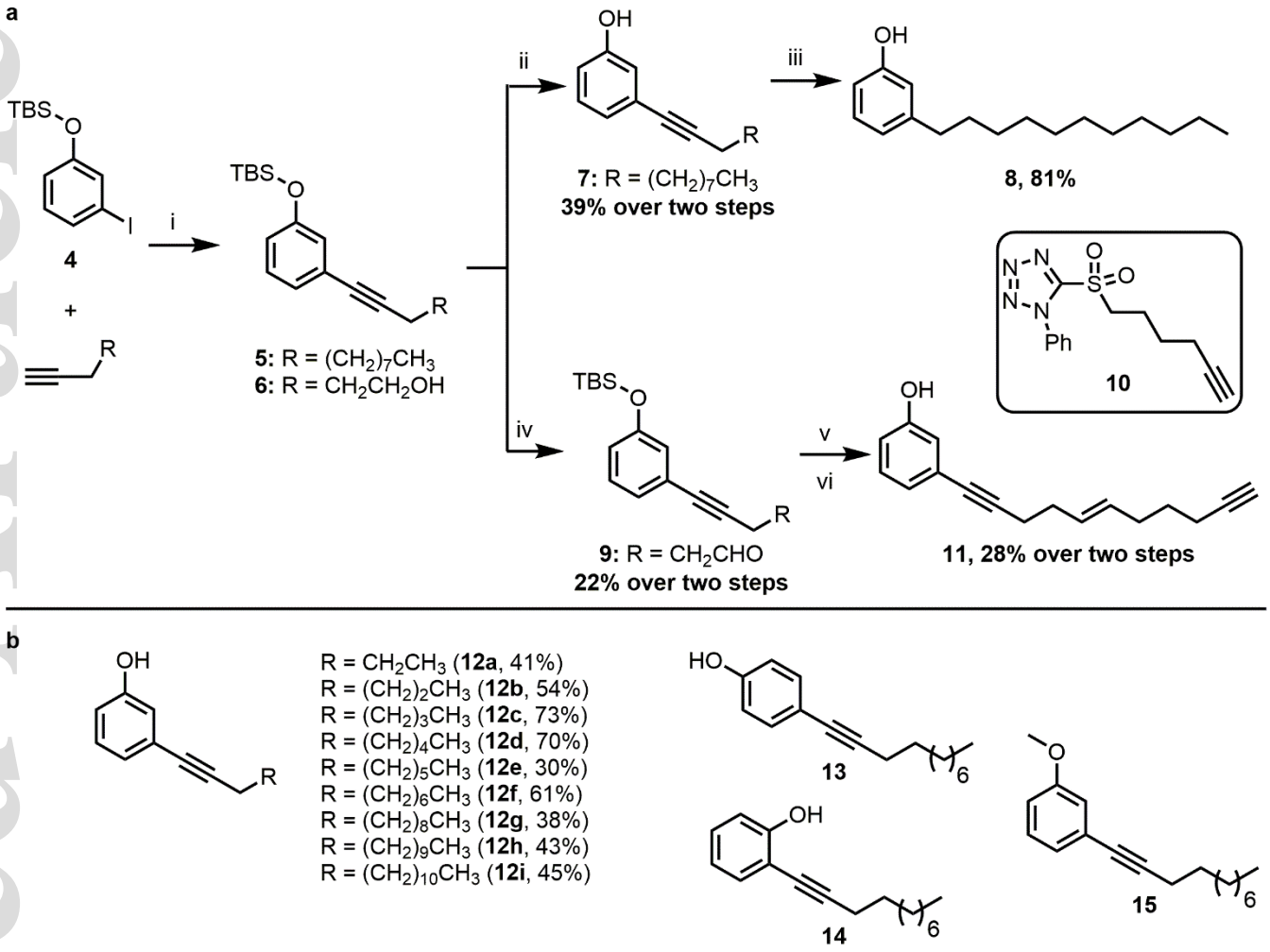
resuspended in 1 mL of phosphate-buffered saline (PBS). The suspension was centrifuged, the supernatant was removed, and cells were re-suspended two additional times. The final cell suspension was then diluted 10-fold. Test compound solutions were made in PBS and then added to aliquots of the 10-fold suspension dilution of blood. PBS was used as a negative control and a zero-hemolysis marker. Triton X (a 1% sample) was used as a positive control serving as the 100% lysis marker. Samples were then placed in an incubator at 37 °C while being shaken at 200 rpm for one hour. After one hour, the samples were transferred to microcentrifuge tubes and centrifuged for 10 min at 10,000 rpm. The resulting supernatant was diluted by a factor of 4 in distilled water. The absorbance of the supernatant was then measured with a UV spectrometer at a 540 nm wavelength.

3. Results

3.1 Synthesis and Antimicrobial Activity of Initial Anaephene Analogues.

Based on the synthetic route previously reported by our group, we first synthesized three analogues that examined the removal and/or addition of unsaturations in the alkyl chain. This was accomplished by starting with commercially available *tert*-butyl(3-iodophenoxy)dimethylsilane (**4**) and subjecting it to a Sonogashira cross-coupling reaction with either 1-undecyne or pent-4-yn-1-ol to provide compounds **5** and **6**, respectively (Scheme 1a). Compound **5** was then treated with tetrabutylammonium fluoride (TBAF) to remove the TBS protecting group, providing compound **7**. Compound **8** was then synthesized by subjecting **7** to hydrogenation conditions to give the fully saturated alkyl chain. In order to synthesize compound **11**, alcohol **6** was oxidized to aldehyde **9**, followed by a Julia-Kocienski olefination with sulfone **10** and followed by TBAF deprotection. The minimum inhibitory concentration (MIC) values for compounds **7**, **8** and **11** were determined against MSSA and MRSA (Table 1). The fully saturated analogue, **8**, had a 2-fold loss in potency compared to anaephene B (**2**). Compound **11**, containing an additional internal alkyne, was equipotent to the natural product **2**. Interestingly, compound **7**, containing only the internal alkyne, was more potent than **2**, with MIC values of 2 μg/mL against both bacterial strains.

Scheme 1. (a) Synthetic route used to construct different anaephene analogues, (b) Structures of analogues that contain variations in length of the alkyl chain and location of the hydroxyl substituent.[a]



[a]Reagents and conditions: (i) CuI, Et_3N, $PdCl_2(PPh_3)_2$, MeCN, 24 h, rt; (ii) **5**, tetrabutylammonium fluoride, THF, 45 min, rt.; (iii) 10% Pd/C, H_2, EtOH, 48 h, rt; (iv) **6**, tetrapropylammonium perruthenate, *N*-methylmorpholine *N*-oxide, MeCN, 6 h, rt; (v) **10**, KHMDS, THF, 4 h, -78 °C to 0 °C; (vi) tetrabutylammonium fluoride, THF, 45 min, rt.

This initial structure-activity relationship data on the varying degrees of unsaturation in the alkyl chain demonstrated that the internal alkyne with no additional unsaturations was the most potent. To further our understanding of the anaephene SAR, we next examined chain length while maintaining the internal alkyne found in compound **7**. To synthesize this set of analogues, a one pot, two-step procedure was developed. *Tert*-butyl(3-iodophenoxy)dimethylsilane (**4**) was subjected to a Sonogashira cross-coupling reaction for 2 hours at 60 °C with the corresponding terminal alkyne.

After cooling to room temperature, TBAF was added and the reaction was stirred overnight to provide compounds **12a**-**12i** (Scheme 1b). The MIC values for compounds **12a**-**12i** were then determined against MSSA and MRSA (Table 1). We found that the potency increased as the alkyl chain became longer from C5 (**12a**) to C11 (**7**) against MSSA and MRSA. Interestingly, as the chain length increased from C12 to C14 (**12g-12i**), the activity quickly decreased against MSSA, but these compounds remained active against MRSA. From this data, it was determined that C10 (**12f**) and C11 (**7**) were the optimal chain lengths for maximum potency. Because anaephene B (**2**) has a C11 alkyl chain, we chose to keep this length for the next round of analogues. We next examined the relationship between the hydroxyl substituent and alkyl chain. To synthesize the *para* analogue **13**, *tert*-butyl(4-iodophenoxy)dimethylsilane was subjected to the same one-pot, two-step procedure (*vide supra*). Unfortunately, the *ortho* analogue **14** was unable to be synthesized via this route due to the steric bulk of the TBS protecting group during the Sonogashira cross-coupling reaction. To circumvent this, we synthesized the MOM protected compound, 1-iodo-2-(methoxymethoxy)benzene. This compound successfully participated in the Sonogashira cross-coupling reaction and after deprotection of the MOM group with HCl, the desired product (**14**) was obtained (Scheme S1). Lastly, we synthesized compound **15** by subjecting 3-iodoanisole to a Sonogashira cross-coupling reaction with 1-undecyne under than same reaction conditions used to synthesize the other analogues. This allowed us to examine the importance of the hydrogen-bond donating ability of the hydroxyl group. The MIC values for these compounds were assessed against MSSA and MRSA (Table 1). The *para* analogue **13** was equipotent to compound **7**, demonstrating no preference between the *meta* and *para* relationships. However, the *ortho* analogue **14** displayed a significant reduction in potency and the methylated analogue **15** was not active at 128 μg/mL.

Table 1. MIC Values of analogues **7**, **8**, **11**, **12a-12i**, and **13-18**.[a]

Compound	***S. aureus* (ATCC 25923)**	**MRSA (ATCC 33591)**
Anaephene B (**2**)	8	8
7	4	2
8	16	16

11	8	8
12a	>128	>128
12b	64	64
12c	16	16
12d	8	8
12e	4	4
12f	4	2
12g	8	2
12h	64	2
12i	128	8
13	4	2
14	128	64
15	>128	>128
16	>128	>128
17	>128	>128
18	8	8
linezolid	0.5	1

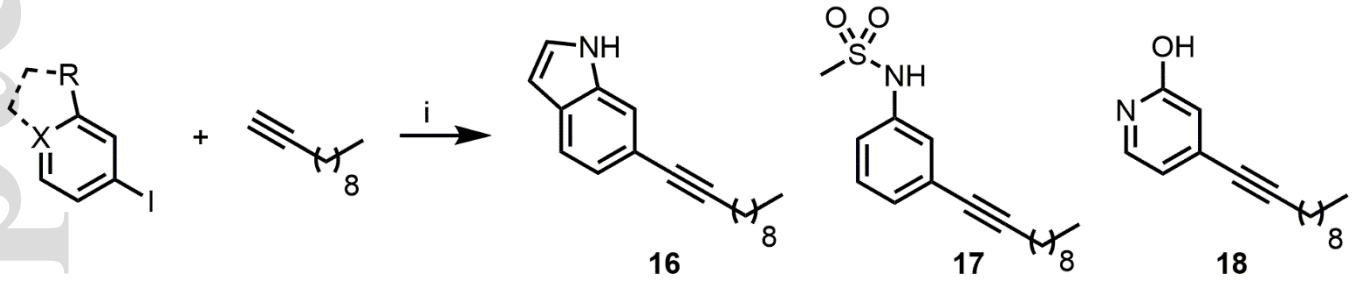
[a]All MIC values in μg/mL.

3.2 Synthesis and Antimicrobial Activity of Phenolic Bioisosteres.

Our initial SAR studies identified the optimal degrees of unsaturation and length of the alkyl chain, as well as, the optimal position of the hydroxyl substituent. It has been well documented in drug discovery that the bioavailability of phenolic compounds is inhibited due to the rapid production of glucuronide and sulfated metabolites (De Souza et al., 2015). One way to overcome this problem is to

utilize phenolic bioisosteres. The term bioisosterism was initially defined by Friedman and more recently defined by Burger as "Compounds or groups that possess near-equal molecular shapes and volumes, approximately the same distribution of electrons, and which exhibit similar physical properties..." (Friedman, 1951; Burger, 1991). Some examples of phenolic bioisosteres include, indoles, alkyl sulfonamides, hydroxypyridines, and benzimidazolones (Kawai et al., 2007). We continued our SAR studies by looking at substituting the phenol moiety for with a bioisostere to improve the overall drug-like properties of this class of molecules. We selected three different isosteres, each with different overall properties, to provide additional SAR information on the hydroxyl group. First, an indole isostere was selected because it maintains the hydrogen-bond donating ability of the hydroxyl group but is not acidic like phenols. Second, a methylsulfonamide isostere was selected because it maintains the hydrogen-bond donating ability and the acidity of phenols. Third, a 2-hydroxypyridine isostere was selected to maintain the oxygen atom and increase the hydrophilicity compared to phenols. Compounds **16**, **17**, and **18** were synthesized in a single step by utilizing a Sonogashira cross-coupling reaction between 1-undecyne and 6-iodoindole, *N*-(3-iodophenyl)methanesulfonamide or 4-iodopyridin-2(1*H*)-one, respectively (Scheme 2).

Scheme 2. Syntheses of phenolic bioisosteres.[a]



[a]Reagents and conditions: (i) CuI, Et_3N, $PdCl_2(PPh_3)_2$, MeCN, 24 h, rt;

With these three isosteres in hand, we determined the MIC values against both MSSA and MRSA (Table 1). Compounds **16** and **17**, which both contain a nitrogen atom in place of the oxygen atom, did not show any activity at 128 μg/mL. However, the 2-hydroxypyridine analogue **18** was equipotent to anaephene B (**2**) and 2-fold weaker than compound **7** against MRSA.

3.3 Additional Biological Testing of Anaephene B (2), analogue 7 and analogue 18.

Following the syntheses and MIC evaluations for each of the 18 analogues, compound **7** was found to be one of the most potent. Compound **7** was 2-fold more potent than anaephene B (**2**) against MSSA and 4-fold more potent against MRSA. Additionally, analogue **18** was equipotent to anaephene B (**2**), which is interesting because the pyridine motif will provide an opportunity to improve the overall drug-like properties of this class of molecules such as, increasing the hydrophilicity. Analogues **7** and **18**, along with anaephene B (**2**), were evaluated against additional clinically relevant strains of bacteria to further define the activity of these compounds.

Compounds **2**, **7**, and **18** all exhibited moderate to good potency against Gram-positive strains, including a multi-drug resistant MRSA strain (BAA-44) and a vancomycin-intermediate *E. faecalis* strain (ATCC 51299). Additionally, we evaluated these compounds against the Gram-negative pathogens, *A. baumannii* (ATCC 19606) and *P. aeruginosa* (ATCC 27853) and they displayed no activity. This may indicate a more specific mechanism of action for this class of compounds rather than non-specific cell lysis.

Table 2. MIC values against additional clinically relevant bacterial strains of **2**, **7**, and **18**.[a]

Strain	Anaephene B (2)	Compound 7	Compound 18	Linezolid	Ciprofloxacin
MRSA (ATCC BAA-44)	4	2	8	1	nt
S. epidermidis (ATCC 51625)	8	2	8	1	nt
E. faecalis (ATCC 29212)	8	4	16	1	nt
E. faecalis (ATCC 51299)	8	4	16	1	nt
P. aeruginosa (ATCC 27853)	128	128	128	nt	0.5
A. baumannii	>128	>128	>128	nt	1

(ATCC 19606)					

[a]nt = not tested. All MIC values in μg/mL.

Given that these molecules are amphipathic, we next evaluated the hemolytic activity of these compounds to examine the effects on eukaryotic membrane integrity. Compounds **2**, **7**, and **18** were subjected to a red blood cell hemolysis assay using mechanically defibrinated sheep blood (Liu et al., 2007). Interestingly, these studies revealed that the hydroxypyridine analogue **18**, possesses significantly less hemolytic activity (< 1% at 100 μM) than anaephene B (**2**) (80% at 100 μM) or analogue **7** (89% at 100 μM). This is a significant result, given the reduction in toxicity and the increase in the therapeutic window. This also demonstrates the usefulness of bioisosteres to improve the overall drug-like properties of a compound. Lastly, we determined the minimum bactericidal concentration (MBC) of compounds **2**, **7**, and **18** against MSSA. The MBC values for **2**, **7**, and **18** were 16 μg/mL, 8 μg/mL, and 16 μg/mL, respectively. These results suggest that these compounds work via a bactericidal mechanism (MBC ≤ 4x MIC; French, 2006).

4. Conclusions

Eighteen analogues (**7**, **8**, **11**, **12a-12i**, **13-18**) of the anaephene natural products were synthesized and characterized. These analogues were found to possess varying antimicrobial activities against MSSA and MRSA. Specifically, in **7**, we found that an internal alkyne with no additional unsaturations in the alkyl chain enhances antimicrobial activity against MRSA compared to anaephene B (**2**) (2 vs. 8 μg/mL). We also found the optimal alkyl chain length for activity against both MSSA and MRSA to be between 10 (**12f**) and 11 (**7**) carbons with a *meta* (**7**) or *para* (**13**) relationship to the hydroxyl substituent. These compounds displayed potent activity with MIC values of 4 μg/mL against MSSA and 2 μg/mL against MRSA, which is only two-fold weaker than the FDA approved antibiotic, linezolid. Lastly, we found that the incorporation of a 2-hydroxypyridine moiety, as in **18**, still possesses equal antimicrobial activity compared to anaephene B (**2**) however, compound **18** displayed minimal hemolytic activity at 100 μM (< 1%), while anaephene B (**2**) and compound **7** displayed significant hemolysis at 100 μM (80% and 89%, respectively). We also determined the MBC of these compounds and these results suggest that these compounds exert their antimicrobial activities via a bactericidal mechanism. This initial SAR study of the anaephene antibiotics provided analogues with

improved potency, less toxicity, and provided a basis for continued study and development of this class of natural products.

DATA AVAILABILTY STATEMENTS

The ^{1}H and ^{13}C NMR spectra and HPLC traces for all final compounds supporting this work are available in the supplementary material of this article.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

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