Highlights

- Olympicin A from *Hypericum olympicum* has potent activity against MRSA clinical isolates.
- Olympicin A and a series of *ortho* alkyloxy and acyl derivatives were synthesized.
- The most potent compounds had MICs of 0.25-0.5 mg/L against all strains tested.
- A 10-carbon alkyloxy group *ortho* to a 5-carbon acyl group is key to potent activity.
A series of new structurally-related acylphloroglucinols were synthesized and screened for their antibacterial activities against a panel of multi-drug resistant (MDR) and methicillin-resistant *Staphylococcus aureus* (MRSA) strains. 12 showed strongest anti-staphylococcal activity (MIC 0.25-1 mg/L).
Total synthesis of acylphloroglucinols and their antibacterial activities against clinical isolates of multi-drug resistant (MDR) and methicillin-resistant strains of *Staphylococcus aureus*

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ABSTRACT

Bioassay-directed drug discovery efforts focusing on various species of the genus *Hypericum* led to the discovery of a number of new acylphloroglucinols including \((S,E)-1-(2-((3,7\text{-dimethyl}o)ct-2,6\text{-dien}-1\text{-yl})\text{oxy})-4,6\text{-dihydroxyphenyl})-2\text{-methylbutan}-1\text{-one}\) \((6,\text{ olympicin A})\) from *H. olympicum*, with MICs ranging from 0.5 to 1 mg/L against a series of clinical isolates of multi-drug-resistant (MDR) and methicillin-resistant *Staphylococcus aureus* (MRSA) strains. The promising activity and interesting chemistry of olympicin A prompted us to carry out the total synthesis of \(6\) and a series of analogues in order to assess their structure-activity profile as a new group of antibacterial agents. Following the synthesis of \(6\) and structurally-related acylphloroglucinols \(7-15\) and \(18-24\), their antibacterial activities against a panel of *S. aureus* strains were evaluated. The presence of an alkyloxy group consisting of 8-10 carbon atoms ortho to a five-carbon acyl substituent on the phloroglucinol core are important structural features for promising anti-staphylococcal activity.

*Key words:* Acylphloroglucinols; Total synthesis; Anti-staphylococcal; MRSA
1. Introduction

In the era of antibiotic resistance, natural products notably from plants, microbes and marine organisms continue to be an important source of lead compounds for drug discovery. Since the discovery of penicillin from *Penicillium notatum*, researchers have focused on natural resources, mostly microorganisms, for effective and safe antibiotics. Although plants have been well documented for their production of biologically-active metabolites including anticancer agents such as paclitaxel (Taxol®) [1], antimalarial drugs (artemisinin) [2], narcotic analgesics (morphine) [3] and cardioactive agents such as digoxin [4], this area has been under-exploited for antimicrobial drug discovery. However, there are numerous reports of plants being used as systemic and topical antimicrobial agents in Ayurvedic [5] and Traditional Chinese Medicine [6] as well as in western herbal medicine [7] because of their self-protection strategy to counter bacteria and fungi in their own environment [8].

Due to the burgeoning global problem of anti-microbial resistance (AMR), there is an increasing need for new chemistries to supplement the dwindling antibiotic arsenal [9]. St John’s Wort (*Hypericum perforatum*), a medicinal plant used widely as an anti-depressant in herbal medicine, has been reported to produce hyperforin, an antibacterial metabolite with a minimum inhibitory concentration (MIC) value of 0.1 mg/L against methicillin-resistant *Staphylococcus aureus* (MRSA) and penicillin-resistant variants [10]. Our bioassay-directed drug discovery efforts to identify potent anti-staphylococcal agents from various species of the genus *Hypericum* led to the discovery of a number of acylphloroglucinols including (S,E)-1-((3,7-dimethyl-2,6-dien-1-yloxy)-4,6-dihydroxyphenyl)-2-methylbutan-1-one (6) (trivial name olympicin A) from *H. olympicum* [11] with MIC values of 0.5 to 1 mg/L against a series of MRSA strains. The potential activity and the complex but interesting chemistry of such acylphloroglucinol compounds prompted us to carry out the total synthesis of olympicin A and its related analogues in order to assess their structure-activity profile as a new group of
antibacterial agents. Here we report the total synthesis of olympicin A (6) and a series of acylphloroglucinols (7-22) that are structurally related to 6, as well as their antibacterial activities against a panel of multi-drug-resistant (MDR) and methicillin-resistant Staphylococcus aureus strains.

2. Results and discussion

2.1. Synthesis of (S)-(2-methylbutanoyl)phloroglucinols with ortho alkyloxy variants

(S,E)-1-((3,7-Dimethylocta-2,6-dien-1-yl)oxy)-4,6-dihydroxyphenyl)-2-methylbutan-1-one (6) (trivial name olympicin A), a promising antibacterial acylphloroglucinol isolated from H. olympicum, and its analogues with variable ortho alkyloxy substituents were synthesised in four steps starting from (S)-2-methylbutanoic acid (1) (Scheme 1) with typical overall yields of 10-12%. This general approach involved Friedel-Crafts acylation of phloroglucinol (3) with (S)-2-methylbutanoyl chloride (2) to give the intermediate ketone (S)-2-methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one (4), which was regioselectively protected as the bis-silyl derivative 5 prior to alkylation of the remaining free phenolic hydroxyl group (and simultaneous deprotection). The structures of all intermediate compounds were confirmed unambiguously by NMR spectral data.

In the first step, commercially available (S)-2-methylbutanoic acid (1) was treated with thionyl chloride to form the corresponding acid chloride (S)-2-methylbutanoyl chloride (2; 93% yield), which was isolated by distillation. The boiling point of 2 was 119-120 °C, which was in good agreement with the literature [12]. The structural identity of 2 was confirmed by mass spectrometry, and $^1$H and $^{13}$C NMR spectroscopy (section 4.2.1).

Friedel-Crafts acylation of phloroglucinol (3) with (S)-2-methylbutanoyl chloride (2) in the presence of AlCl$_3$, CS$_2$ and nitrobenzene led to the formation of (S)-2-methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one (4; 54% yield), which was purified by VLC. Unreacted phloroglucinol and nitrobenzene were easily separated from 4 during purification and
successful acylation was verified by mass spectrometry and NMR spectral data (section 4.2.2). In addition to the presence of the signals that were typical for a 2-methylbutanoyl substituent, the $^1$H NMR spectrum showed a singlet at $\delta_H 5.81$, integrating for two equivalent meta aromatic hydrogen atoms, consistent with the plane of symmetry in 4.

Initial efforts to protect two (para and one ortho) of the three phenolic hydroxyl groups in 4 utilised methoxymethyl (MOM) ether protecting groups. Isolated yields were generally poor (below 20%) and this approach was ultimately abandoned as the acidic conditions required for MOM ether deprotection also resulted in cleavage of the required geranyl ether in the target compound 6. The use of silyl ethers for the desired bis-protection proved more successful. Compound 4 was treated with TBDMS-Cl (2.1 molar equivalents) under basic reaction conditions, forming (S)-1-(2,4-bis((tert-butyldimethylsilyl)oxy)-6-hydroxyphenyl)-2-methylbutan-1-one (5) in an improved 81% yield, after purification by VLC over silica gel. Small quantities of mono-protected derivatives were also identified in the reaction mixture. The presence of a hydrogen-bonded hydroxyl ($\delta_H 13.43$) and two meta-coupled ($J = 2.0$ Hz) hydrogen atoms with two different chemical shifts ($\delta_H 5.85$ and 6.04) confirmed the protection of the hydroxyl groups with TBDMS at one ortho and the para position. Intramolecular hydrogen bonding between the hydrogen of the one remaining free phenolic hydroxyl and the carbonyl oxygen of the acyl substituent, as well as its lower steric accessibility, are likely to account for the excellent regioselectivity of this bis-silyl ether protection.

The final step in the synthesis of the (S)-2-methylbutanoylphloroglucinol series involved the alkylation of the free ortho phenolic hydroxyl of 5 using the appropriate alkyl bromide. Treatment of a solution of (S)-1-(2,4-bis((tert-butyldimethylsilyl)oxy)-6-hydroxyphenyl)-2-methylbutan-1-one (5) with geranyl bromide in the presence of K$_2$CO$_3$ yielded (S,E)-1-(2-((3,7-dimethylocta-2,6-dien-1-yl)oxy)-4,6-dihydroxyphenyl)-2-
methylbutan-1-one (6; typically 9-16% yield), which was purified by VLC over silica gel. The original intention had been to alkylate the free ortho phenolic hydroxyl followed by silyl ether deprotection in a subsequent step, but the conditions used in the alkylation unexpectedly led to simultaneous TBDMS group removal. While this suggested the possibility of direct alkylation (geranylation) of unprotected 4, attempts to achieve this generally resulted in very poor conversion (less than 5% yields) of starting acylphloroglucinols to typically ca. 1:1 mixtures of ortho and para mono-alkylated products, which were difficult to separate from unreacted starting materials.

The NMR data of 6 were identical to those of the new acylphloroglucinol we reported from *H. olympicum* [11]. The alkyl (geranyl) substituent included an oxymethylene group (δ_H 4.56 d, J = 6.5 Hz, geranyl C-1), the hydrogen atoms of which showed long-range (^3J_H-C) ^1H-^13C correlations with a deshielded aromatic carbon atom (δ 162.6, aromatic C-2) of the acylphloroglucinol nucleus and two carbons associated with an olefinic group (δ 118.2, geranyl C-2; δ 142.4, geranyl C-3). Together with the non-equivalence (asymmetry) of the aromatic C-H signals, this confirmed successful ortho O-alkylation. In a similar manner, a series of other ortho alkoxy acylphloroglucinols (7-15, Figure 1) was synthesised, purified and characterised. The final ether substituents were varied to include both saturated and unsaturated linear and branched alkyl/alkenyl groups such as prenyl (7), farnesyl (8), 3,7-dimethyloctyl (reduced geranyl; 9), 3-methylbutyl (reduced prenyl; 10), benzyl (11), octyl (12), decyl (13), dodecyl (14) and octadecyl (15) groups. In each analogue, the C-1 oxymethylene hydrogen atoms of the alkyl substituent demonstrated key HMBC interactions with the aromatic C-2 (ortho) carbon atom of the acylphloroglucinol core, allowing confirmation of the successful desired ortho alkylation in compounds 7-15. The yield of the final alkylation/deprotection step was typically quite low at around 30% (though much lower
for 8 and 10). Isolated by-products often included recovered 4, along with small quantities of bis-alkylated (ortho plus para) and occasionally para alkylated derivatives.

2.2. Synthesis of ortho geranylated acylphloroglucinols with acyl variants

The acyl chloride used in the initial Friedel-Crafts acylation of phloroglucinol was varied to generate, after elaboration as described above, a series of geranylated acyolphloroglucinol analogues (Figure 2), incorporating simple linear acyl substituents such as propanoyl (19), butanoyl (20), pentanoyl (21), decanoyl (22) and octadecanoyl (23) as well as aromatic acyl substituents such as benzoyl (24). Commercially available acetylphloroglucinol was also used to synthesise ortho geranylated acetylphloroglucinol (18). Regioselective di-protection of two (para and one ortho) of the three hydroxyl groups of the acylphloroglucinol (16) moiety again using TBDMS-Cl, followed by alkylation with geranyl bromide and simultaneous deprotection of the silyl ethers (17) yielded the corresponding ortho geranylated acyolphloroglucinols 18-24 (Scheme 2). Final products were purified by SPE over silica gel and confirmed by NMR spectroscopy. Again, a key aspect in the confirmation of the structures of each of the compounds was the use of 2D-HMBC, in which the C-1 oxymethylene hydrogen atoms of the geranyl substituent demonstrated $^3J$ correlations with the C-2 (ortho) carbon of the acylphloroglucinol.

2.3. Antibacterial activity of acylphloroglucinols

The antibacterial activities (Table 1) of the acylphloroglucinol series were assessed against a panel of clinical isolates of multidrug-resistant (MDR) and methicillin-resistant Staphylococcus aureus (MRSA), notably SA1199B, XU212, RN4220, EMRSA15 and EMSA16. These organisms represent a group of effluxing strains that are resistant to common antibiotics including certain fluoroquinolones (SA1199B), tetracycline (XU212) and macrolides (erythromycin; RN4220). These strains were chosen as antibacterials that are not substrates for these MDR pumps are clinically desirable. It was evident from Table 1 that the
(S)-2-methylbutanoylphloroglucinol derivatives with geranyl (6; the natural product olympicin A) and 3,7-dimethyloctyl (reduced geranyl; 9) ether substituents at the ortho position were found to be highly active with MIC values of 0.5-1 mg/L. The effect on activity of replacing the geranyl substituent with shorter and longer alkyl/alkenyl groups (prenyl, farnesyl, 3-methylbutyl, 3,7-dimethyloctyl) can also be observed. We found that increasing the chain length from geranyl (6) to farnesyl (8) resulted in a dramatic loss of activity, evidenced by an increase in MIC values from 0.5-1 mg/L to in excess of 512 mg/L (no inhibition of the growth of any of the strains of bacteria was seen at the highest concentration of 8 tested), while decreasing the chain length from geranyl to prenyl (7) showed at least a two-fold reduction in activity. These results suggested an optimal length for the alkylxoxy group in terms of antibacterial activity, which may imply a specific molecular target or a requirement for a particular lipophilicity for the mechanism of action. In addition, the 3-methylbutyl (10) and 3,7-dimethyloctyl (9) derivatives were as active as the prenyl or geranyl derivatives respectively, suggesting that the presence of the double bonds in the alkyl side chain (and the consequent reduction in conformational freedom) might not have any significant role in antibacterial activity. The most potent compound in terms of its activity against the six *S. aureus* strains was 12, an (S)-(2-methylbutanoyl)phloroglucinol with a straight chain octyl ether substituent – the same continuous linear chain length as the geranyl and 3,7-dimethyloctyl derivatives – at the ortho position phenolic hydroxyl.

Among the ortho geranylated acylphloroglucinol series, the pentanoyl derivative (21) demonstrated the highest activity with MICs of 0.5-1 mg/L, making it comparable in activity to the parent natural product 6. The octadecanoyl analogue (23) did not show any inhibition of bacterial growth at any of the concentrations tested, indicating that there was clearly an optimal chain length (number of carbon atoms) for the acyl substituent.
Geranylated analogues containing three to seven carbon atoms in their acyl substituents (aliphatic or aromatic) seem to be most active.

3. Conclusion

The acylphloroglucinol natural product olympicin A was synthesised from commercial phloroglucinol over three straightforward steps (Friedel-Crafts acylation, regioselective silyl protection, and simultaneous deprotection/O-alkylation). The same approach provided access to a series of ortho alkyloxy and acyl analogues. Evaluation of the activities of the analogues against several strains of MRSA seemed to indicate that the presence of an alkyloxy group consisting of 8-10 carbon atoms ortho to a five-carbon-atom acyl substituent is an important structural feature for promising anti-staphylococcal activity within this class of acylphloroglucinol antibiotics. The most potent derivative 12 – the octyl ether analogue of olympicin A – demonstrated improved activity over the natural product against all MRSA strains tested. The most promising acylphloroglucinols identified in this study are being evaluated for their clinical potential as systemic and topical antibacterial agents. A more diverse range of structural analogues, including those incorporating side-chain functionality, is also being synthesised in an effort to better understand the structure-activity relationships of this class of agents. At present we do not fully understand how these compounds function. However, given that the most active compounds all have a hydrophobic portion, comprised of an ether and an acyl functionality, capable of membrane interaction, and a hydrophilic diphenolic moiety, it is probable that their target is cell-wall located.

4. Experimental section

4.1. General methods

All chemicals and reagents used for the syntheses were purchased from Sigma-Aldrich, Gillingham, UK. UV spectra were recorded on a Thermo Electron Corporation Helios spectrophotometer and IR spectra were recorded on a Nicolet 360 FT-IR
spectrophotometer. NMR spectra (both 1D and 2D) were recorded on a Bruker AVANCE 500 MHz spectrometer (500 MHz for $^1$H and 125 MHz for $^{13}$C). Chemical shift values ($\delta$) are reported in parts per million (ppm) and are calibrated relative to residual solvent peaks as internal standards, and coupling constants ($J$ values) are expressed in Hz. Detailed assignments of NMR spectra can be found in the Supporting Information. Mass spectra were recorded on a Finnigan MAT 95 high resolution, double focusing, magnetic sector mass spectrometer. Accurate mass measurement was achieved using voltage scanning of the accelerating voltage. This was nominally 5 kV and an internal reference of heptacosa was used. Resolution was set between 5000 and 10000. Both TLC and preparative TLC were performed using silica gel 60 PF$_{254}$ plates (Merck). Vacuum liquid chromatography (VLC) columns were packed with silica gel 60 PF$_{254}$ (Merck), while solid phase extraction (SPE) was performed using 10 g pre-packed SPE columns (SiGel60) using mobile phases of hexane and ethyl acetate of increasing polarity.

4.2. Synthesis of intermediate and final compounds

4.2.1. Synthesis of (S)-2-methylbutanoyl chloride (2) [12]

(S)-2-Methylbutanoic acid (1; 10 g, 97.91 mmol) and thionyl chloride (10.71 mL, 146.9 mmol, 1.5 equiv) were heated together at 80-90 °C under reflux for 2 h. Distillation of the reaction mixture afforded (S)-2-methylbutanoyl chloride as a colourless liquid (10.89 g, 90.72 mmol, 93%). $[\alpha]_D^{22} +10.1$ (C 0.54, CHCl$_3$); b.p. 119-120 °C; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$H 0.95 (3H, t, $J = 7.5$ Hz), 1.26 (3H, d, $J = 7.5$ Hz), 1.59 (1H, m), 1.80 (1H, m), 2.80 (1H, q, $J = 7.0$ Hz); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$C 11.3, 16.7, 26.7, 53.1, 177.8; HRMS (ESI) m/z: [M-H]$^-$ Calcd for C$_5$H$_8$OCl 119.0264; Found 119.0263.

4.2.2. Synthesis of (S)-2-methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one (4) [13]

Phloroglucinol (3; 10.81 g, 85.8 mmol) in carbon disulphide (50 mL) was transferred into a two-necked round-bottomed flask and allowed to stir while aluminium trichloride
(46.43 g, 351.8 mmol, 4.1 equiv) was added. Nitrobenzene (40 mL) was then added to the solution over 30 min. The solution was then heated under reflux at 55 °C for 30 min. A solution of 2-methylbutanoyl chloride (2) (10.89 g, 85.8 mmol) dissolved in 5 mL nitrobenzene was added to the reaction mixture over 30 min, followed by heating for another 30 min. The reaction mixture was allowed to cool with stirring and then poured into an ice-water bath (400 mL). 100 mL of 3 M hydrochloric acid was then added and the mixture extracted with diethyl ether (3 × 500 mL). The organic solvents were removed under reduced pressure. The oily residue containing the acylphloroglucinol was subjected to VLC over SiGel PF254 using hexane and EtOAc of increasing polarity. The VLC fraction eluted with 30-45% EtOAc in hexane gave (S)-2-methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one as a pale yellow oil (9.71 g, 46.19 mmol, 54%). $[\alpha]_D^{22} +8.5$ (c 0.35, CHCl$_3$); UV (CHCl$_3$) $\lambda_{\text{max}}$ (log $\varepsilon$): 240 (4.17), 290 (3.97) nm; IR $\nu_{\text{max}}$ (thin film) cm$^{-1}$: 3297, 1628, 1602, 1222; $^1$H NMR (500 MHz, CD$_3$OD): $\delta$H 0.88 (3H, t, $J = 7.5$ Hz), 1.11 (3H, d, $J = 6.5$ Hz), 1.33 (1H, m), 1.78 (1H, m), 3.84 (1H, m), 5.81 (2H, s); $^{13}$C NMR (125 MHz, CD$_3$OD): $\delta$C 12.4, 17.2, 28.2, 46.7, 96.0, 96.0, 105.3, 165.8, 165.8, 211.4; HRMS (ESI) m/z: [M-H] Calcd. for C$_{11}$H$_{13}$O$_4$ 209.0814; Found 209.0813.

4.2.3. Synthesis of (S)-1-(2,4-bis(tert-butyldimethylsilyl)oxy)-6-hydroxyphenyl)-2-methylbutan-1-one (5) [14]

Acylphloroglucinol 4 (9.71 g, 46.19 mmol) was dissolved in part in dry acetone (150 mL) and transferred into a 250 mL round bottom flask. Imidazole (3.43 g, 138.6 mmol, 3 equiv) was added to the solution and the reaction mixture stirred for 5 min followed by the addition of TBDMS-Cl (14.61 g, 97.0 mmol, 2.1 equiv). The reaction mixture was stirred for 2 h at room temperature. Acetone was removed from the reaction mixture under reduced pressure and the residue taken up in chloroform and washed with 1 M HCl (150 mL). The organic layer was dried using anhydrous magnesium sulphate, filtered and the solvent was
removed under reduced pressure. The crude product purified by VLC over silica gel using hexane and EtOAc of increasing polarity. VLC fractions eluted with 2.5-7.5% EtOAc in hexane afforded (S)-1-(2,4-bis((tert-butyldimethylsilyl)oxy)-6-hydroxyphenyl)-2-methylbutan-1-one as a pale yellow oil (16.4 g, 37.26 mmol, 81%). \[\alpha\]D22 +4.9 (c 0.39, CHCl3); UV (CHCl3) \(\lambda_{\text{max}}\) (log e): 239 (4.26), 290 (4.23) nm; IR \(\nu_{\text{max}}\) (thin film) cm\(^{-1}\): 3276, 2973, 1688, 1572, 1531, 1256, 1131, 1072, 850; \(^1\)H NMR (500 MHz, CDCl3): \(\delta\)H 0.23 (2 × 3H, s), 0.32 (2 × 3H, s), 0.88 (3H, t, \(J = 7.5\) Hz), 0.97 (3 × 3H, s), 0.99 (3 × 3H, s), 1.12 (3H, d, \(J = 6.5\)Hz), 1.43 (1H, m), 1.78 (1H, m), 3.82 (1H, m), 5.85 (1H, d, \(J = 2.0\) Hz), 6.04 (1H, d, \(J = 2.0\) Hz), 13.43 (1H, s); \(^13\)C NMR (125 MHz, CDCl3): \(\delta\)C -4.2, -3.7, 11.0, 16.9, 18.1, 18.9, 25.5, 26.5, 26.1, 45.0, 102.0, 103.1, 108.4, 158.8, 161.7, 166.5, 210.5; HRM (ESI) m/z: [M-H]\(^{-}\) Calcd. for C\(_{23}\)H\(_{41}\)O\(_4\)Si\(_2\) 437.2549; Found 437.2554.

4.2.4. Synthesis of (S,E)-1-(2-((3,7-dimethylocta-2,6-dien-1-yl)oxy)-4,6-dihydroxy-phenyl)-2-methylbutan-1-one (olympinic A) (6)

TBDMS-protected acylphloroglucinol 5 (6.6 g, 15.0 mmol) was dissolved in dry DMF (100 mL) and anhydrous potassium carbonate (3.1 g, 22.5 mmol, 1.5 equiv) was added. The mixture was stirred for approximately 5 min followed by the addition of geranyl bromide (3.43 mL, 18 mmol, 1.2 equiv). The mixture was heated at 80 °C for 3 h with stirring. The reaction mixture was poured over water and extracted with chloroform. The solvent in the organic layer was removed under reduced pressure. The crude product was purified by chromatography over silica gel by VLC. Compound 6 was eluted with 9:1 hexane-ethyl acetate and removal of the solvents under reduced pressure yielded the title compound as a pale yellow oil (450 mg, 1.3 mmol, 8.7%). All spectral data were identical to those of the natural product [11]. \[\alpha\]D22 +6.0 (c 0.30, CHCl3); UV (CHCl3) \(\lambda_{\text{max}}\) (log e): 239 (4.06), 240 (4.23) nm; IR \(\nu_{\text{max}}\) (thin film) cm\(^{-1}\): 3357, 2965, 2931, 1623, 1589, 1458, 1212, 1165, 1087, 825; \(^1\)H NMR (500 MHz, CDCl3): \(\delta\)H 0.88 (3H, t, \(J = 7.5\) Hz), 1.12 (3H, d, \(J = 6.5\) Hz), 1.36
1H, m), 1.61 (3H, s), 1.69 (3H, s), 1.74 (3H, s), 1.79 (1H, m), 2.10 (2H, m), 2.13 (2H, m), 3.68 (1H, m), 4.56 (2H, d, J = 6.5 Hz), 5.10 (1H, m), 5.44 (1H, br s), 5.50 (1H, m), 5.91 (1H, d, J = 2.0 Hz), 5.98 (1H, d, J = 2.0 Hz), 14.10 (1H, s); 13C NMR (125 MHz, CDCl3): δ: 11.9, 16.5, 16.6, 17.7, 25.7, 26.2, 26.9, 39.5, 46.2, 65.6, 91.5, 96.5, 105.9, 118.2, 122.6, 132.0, 142.4, 161.9, 162.6, 167.5, 210.4; HRMS (ESI) m/z: [M-H]– Calcd. for C21H29O4 345.2071; Found 345.2067.

4.2.5. Synthesis of compounds 7-15 from TBDMS-protected acylphloroglucinol 5

Compounds 7-15 were synthesised from TBDMS-protected acylphloroglucinol 5 using the procedure as described for 6 above but replacing the geranyl bromide with the appropriate alkyl bromide.

4.2.5.1. (S)-1-(2,4-Dihydroxy-6-(((3-methylbut-2-en-1-yl)oxy)phenyl)-2-methylbutan-1-one (7)

Synthesised using 0.259 mmol of 5 and 0.311 mmol of prenyl bromide. Isolated after SPE (SiGel; hexane/EtOAc 9:1) as a pale yellow oil (15 mg, 0.054 mmol, 21%). [α]D22 +5.2 (c 0.30, MeOH); UV (MeOH) λmax (log ε): 239 (4.36), 290 (3.97) nm; IR νmax (thin film) cm−1: 3423, 2968, 2914, 1630, 1595, 1560, 1420, 1350, 1200; 1H NMR (500 MHz, CDCl3): δH 0.88 (3H, t, J = 7.5 Hz), 1.13 (3H, d, J = 6.5 Hz), 1.33 (1H, m), 1.76 (3H, s), 1.78 (1H, m), 1.82 (3H, s), 3.66 (1H, m), 4.55 (2H, d, J = 6.5 Hz), 5.51 (1H, t, J = 6.5 Hz), 5.70 (1H, br s), 5.93 (1H, d, J = 2.0 Hz), 6.00 (1H, d, J = 2.0 Hz), 14.07 (1H, br s); 13C NMR (125 MHz, CDCl3): δC: 0.88 (3H, t, J = 7.5 Hz), 1.13 (3H, d, J = 6.5 Hz), 1.33 (1H, m), 1.76 (3H, s), 1.78 (1H, m), 1.82 (3H, s), 3.66 (1H, m), 4.55 (2H, d, J = 6.5 Hz), 5.51 (1H, t, J = 6.5 Hz), 5.70 (1H, br s), 5.93 (1H, d, J = 2.0 Hz), 6.00 (1H, d, J = 2.0 Hz), 14.07 (1H, br s); 13C NMR (125 MHz, CDCl3): δC: 12.0, 16.7, 16.4, 25.9, 27.1, 46.3, 65.8, 91.7, 96.7, 106.1, 118.6, 139.3, 162.3, 162.8, 167.7, 210.6; HRMS (ESI) m/z: [M-H]– Calcd. for C16H21O4 277.1445; Found 277.1453.

4.2.5.2. (S)-1-(2,4-Dihydroxy-6-(((2E,6E)-3,7,11-trimethylldodeca-2,6,10-trien-1-yl)oxy)phenyl)-2-methylbutan-1-one (8)

Synthesised using 0.925 mmol of 5 and 1.11 mmol of farnesyl bromide. Isolated after SPE (SiGel; hexane/EtOAc 92:8) as a yellow oil (25 mg, 0.060 mmol, 6.5%). [α]D22 +6.7 (c
0.51, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$): 239 (4.09), 285 (4.22) nm; IR $\nu_{\text{max}}$ (thin film) cm$^{-1}$: 314, 1529, 1442, 1292, 1166, 1074, 821; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 0.90 (3H, t, $J = 7.5$ Hz), 1.13 (3H, d, $J = 6.5$ Hz), 1.37 (1H, m), 1.61 (3H, s), 1.62 (3H, s), 1.69 (3H, s), 1.75 (3H, s), 1.80 (1H, m), 1.99 (2H, s), 2.07 (2H, s), 2.11 (2H, m), 2.13 (2H, m), 3.67 (1H, m), 4.57 (2H, d, $J = 6.5$ Hz), 5.08 (1H, m), 5.12 (1H, m), 5.52 (1H, m), 5.93 (1H, d, $J = 2.5$ Hz), 5.99 (1H, d, $J = 2.5$ Hz), 14.05 (1H, s); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta_C$ 12.1, 16.2, 16.8, 16.9, 17.9, 25.9, 26.5, 26.9, 27.0, 39.7, 39.9, 46.3, 65.9, 91.8, 96.7, 106.1, 118.4, 123.7, 124.5, 131.6, 135.9, 142.7, 162.3, 162.8, 167.7, 210.6; HRMS (ESI) m/z: [M-H]$^+$ Calcd. for C$_{29}$H$_{37}$O$_4$ 413.2698; Found 413.2697.

4.2.5.3.(2S)-1-(2-((3,7-Dimethyloctyl)oxy)-4,6-dihydroxyphenyl)-2-methylbutan-1-one (9)

Synthesised using 1.073 mmol of 5 and 1.289 mmol of 1-bromo-3,7-dimethyloctane. Isolated after SPE (SiGel; hexane/EtOAc 9:1) as a colourless oil (110 mg, 0.313 mmol, 29%).

$[\alpha]_D^{22} +4.4$ (c 0.40, CHCl$_3$); UV (CHCl$_3$) $\lambda_{\text{max}}$ (log $\varepsilon$): 239 (3.99), 290 (4.21) nm; IR $\nu_{\text{max}}$ (thin film) cm$^{-1}$: 3233, 2964, 2921, 1661, 1583, 1432, 1241, 1103, 1057; $^1$H NMR (CDCl$_3$): $\delta$ 0.88 (3H, t, $J = 7.5$ Hz), 0.89 (6H, d, $J = 6.5$ Hz), 0.97 (3H, d, $J = 6.5$ Hz), 1.15 (3H, d, $J = 6.5$ Hz), 1.18 (2H, m), 1.19 (1H, m), 1.35 (3H, m), 1.43 (1H, m), 1.54 (1H, m), 1.66 (3H, m), 1.82 (1H, m), 1.89 (1H, m), 3.72 (1H, m), 4.04 (2H, d, $J = 6.5$ Hz), 5.36 (1H, br s), 5.93 (1H, d, $J = 2.0$ Hz), 5.99 (1H, d, $J = 2.0$ Hz), 14.05 (1H, s); $^{13}$C NMR (CDCl$_3$): $\delta_C$ 11.7, 16.8, 19.6, 22.6, 24.6, 26.6, 28.0, 29.9, 36.0, 37.5, 39.2, 46.0, 66.7, 91.5, 96.5, 105.8, 162.4, 162.8, 167.4, 210.4; HRMS (ESI) m/z: [M+H]$^+$ Calcd. for C$_{32}$H$_{35}$O$_4$ 351.2531; Found 351.2529.

4.2.5.4.(S)-1-(2-(4-Dihydroxy-6-(isopentyl)oxy)phenyl)-2-methylbutan-1-one (10)

Synthesised using 0.579 mmol of 5 and 0.695 mmol of 1-bromo-3-dimethylbutane. Isolated after SPE (SiGel; hexane/EtOAc 9:1) as a yellow oil (5 mg, 0.018 mmol, 3.1%).

$[\alpha]_D^{22} +3.5$ (c 0.34, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$): 242 (4.39), 288 (4.09) nm; IR $\nu_{\text{max}}$ (thin film) cm$^{-1}$: 333, 1631, 1593, 1258, 1110; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 0.89 (3H, t, $J = 7.5$ Hz), 1.16 (3H, s), 1.18 (3H, s), 1.24 (3H, s), 1.53 (1H, m), 1.65 (1H, m), 2.08 (2H, m), 2.10 (2H, m), 3.68 (1H, m), 4.57 (2H, d, $J = 6.5$ Hz), 5.07 (1H, m), 5.12 (1H, m), 5.51 (1H, m), 5.93 (1H, d, $J = 2.5$ Hz), 5.99 (1H, d, $J = 2.5$ Hz), 14.05 (1H, s); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta_C$ 12.1, 16.2, 16.8, 16.9, 17.9, 25.9, 26.5, 26.9, 27.0, 39.7, 39.9, 46.3, 65.9, 91.8, 96.7, 106.1, 118.4, 123.7, 124.5, 131.6, 135.9, 142.7, 162.3, 162.8, 167.7, 210.6; HRMS (ESI) m/z: [M-H]$^+$ Calcd. for C$_{29}$H$_{37}$O$_4$ 413.2698; Found 413.2697.
Hz), 0.98 (6H, d, J = 6.5 Hz), 1.14 (3H, d, J = 6.5 Hz), 1.41 (1H, m), 1.73 (2H, m), 1.80 (1H, m), 1.81 (1H, m), 3.69 (1H, m), 4.02 (2H, d, J = 6.5 Hz), 5.40 (1H, br s), 5.92 (1H, d, J = 2.0 Hz), 5.99 (1H, d, J = 2.0 Hz), 14.17 (1H, s); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$: 11.7, 16.8, 22.5 (2C), 25.2, 26.6, 37.7, 46.0, 67.5, 91.5, 96.5, 105.8, 162.4, 162.8, 167.4, 210.4; HRMS (ESI) m/z: [M-H]$^-$ Calcd. for C$_{18}$H$_{23}$O$_4$ 279.1602; Found 279.1598.

4.2.5.5. (S)-1-(2-(Benzyloxy)-4,6-dihydroxyphenyl)-2-methylbutan-1-one (II)

Synthesised using 0.934 mmol of 5 and 1.121 mmol of benzyl bromide. Isolated after SPE (SiGel; hexane/EtOAc 8:2) as a pale yellow oil (85 mg, 0.283 mmol, 30%). $[\alpha]_{D}^{22} +4.2$ (c 0.32, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 241 (4.30), 289 (3.94) nm; IR $\nu_{max}$ (thin film) cm$^{-1}$: 3239, 2958, 1624, 1568, 1500, 1456, 1383, 1255, 1161, 1105, 751; $^1$H NMR (500 MHz, CDCl$_3$): $\delta_{H}$ 0.68 (3H, t, J = 6.5 Hz), 1.00 (3H, d, J = 6.5 Hz), 1.29 (1H, m), 1.71 (1H, m), 3.57 (1H, m), 5.08 (2H, s), 6.00 (1H, d, J = 2.0 Hz), 6.01 (1H, d, J = 2.0 Hz), 7.38-7.42 (5H, m), 14.00 (1H, s); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$: 11.7, 16.8, 26.9, 46.2, 71.6, 92.0, 97.1, 106.1, 128.4 (2C), 128.8, 129.0 (2C), 135.6, 162.2, 162.5, 167.8, 210.6; HRMS (ESI) m/z: [M+H]$^+$ Calcd. for C$_{18}$H$_{23}$O$_4$ 301.1440; Found 301.1430.

4.2.5.6. (S)-1-(2,4-Dihydroxy-6-(octyloxy)phenyl)-2-methylbutan-1-one (II)

Synthesised using 0.893 mmol of 5 and 1.931 mmol of 1-bromoocctane. Isolated after SPE (SiGel; hexane/EtOAc 92:8) as a pale yellow oil (82 mg, 0.255 mmol, 29%). $[\alpha]_{D}^{22} +6.1$ (c 0.45, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 239 (4.39), 288 (3.98) nm; IR $\nu_{max}$ (thin film) cm$^{-1}$: 3328, 2928, 2857, 1621, 1591, 1444, 1377, 1212, 1158, 1102, 826, 754; $^1$H NMR (500 MHz, CDCl$_3$): $\delta_{H}$ 0.89 (3H, t, J = 7.5 Hz), 0.90 (3H, t, J = 7.5 Hz), 1.16 (3H, d, J = 7.0 Hz), 1.26-1.40 (8H, m), 1.38 (1H, m), 1.46 (2H, m), 1.81 m (1H, m), 1.85 (2H, m), 3.72 (1H, q, J = 6.5 Hz), 4.00 (2H, t, J = 6.5 Hz), 5.42 (1H, br s), 5.91 (1H, d, J = 2.0 Hz), 5.99 (1H, d, J = 2.0 Hz), 14.06 (1H, s); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$: 12.0, 14.3, 17.1, 22.8, 26.5, 26.9,
29.3, 29.4, 29.5, 32.0, 46.3, 69.4, 91.5, 96.8, 106.2, 162.2, 163.0, 167.8, 210.5; HRMS (ESI) m/z: [M+H]^+ Calcd. for C_{19}H_{31}O_{4} 323.2222; Found 323.2211.

4.2.5.7. (S)-1-(2-(Decyloxy)-4,6-dihydroxyphenyl)-2-methylbutan-1-one (13)

Synthesised using 0.936 mmol of 5 and 1.123 mmol of 1-bromodecane. Isolated after SPE (SiGel; hexane/EtOAc 9:1) as a yellow oil (96 mg, 0.264 mmol, 28%). [α]_D^{22} +4.5 (c 0.25, MeOH); UV (MeOH) λ_{max} (log ε): 243 (4.33), 292 (3.99) nm; IR ν_{max} (thin film) cm\(^{-1}\): 3327, 2926, 2855, 1739, 1621, 1592, 1443, 1368, 1216, 1156, 1103, 827, 752; \(^{1}\)H NMR (500 MHz, CDCl\(_3\)): δ\(_{H}\) 0.88 (3H, t, J = 7.5 Hz), 0.89 (3H, t, J = 7.5 Hz), 1.17 (3H, d, J = 6.5 Hz), 1.28-1.31 (12H, m), 1.34 (1H, m), 1.46 (2H, m), 1.81 (1H, m), 1.84 (2H, m), 3.72 (1H, q, J = 6.5 Hz), 4.00 (2H, t, J = 6.5 Hz), 5.92 (1H, d, J = 2.0 Hz), 6.00 (1H, d, J = 2.0 Hz), 14.16 (1H, s); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): δ\(_{C}\) 12.0, 14.3, 17.3, 26.5, 26.6, 29.1, 29.4, 29.5, 29.6, 29.8, 29.9, 32.0 (8C), 46.3, 69.3, 91.6, 96.8, 106.2, 162.3, 163.0, 167.7, 210.6; HRMS (ESI) m/z: [M+H]^+ Calcd. for C\(_{22}\)H\(_{37}\)O\(_{5}\) 365.2692; Found 365.2699.

4.2.5.8. (S)-1-(2-(Dodecyloxy)-4,6-dihydroxyphenyl)-2-methylbutan-1-one (14)

Synthesised using 0.913 mmol of 5 and 1.096 mmol of 1-bromododecane. Isolated after SPE (SiGel; hexane/EtOAc 92:8) as a pale yellow oil (98 mg, 0.259 mmol, 28%). [α]_D^{22} +4.7 (c 0.30, MeOH); UV (MeOH) λ_{max} (log ε): 238 (4.32), 290 (3.99) nm; IR ν_{max} (thin film) cm\(^{-1}\): 3327, 2924, 2854, 1739, 1622, 1593, 1456, 1366, 1216, 1160, 1109, 827; \(^{1}\)H NMR (500 MHz, CDCl\(_3\)): δ\(_{H}\) 0.88 (3H, t, J = 7.5 Hz), 0.89 (3H, t, J = 7.5 Hz), 1.16 (3H, d, J = 6.5 Hz), 1.28-1.30 (16H, m), 1.35 (1H, m), 1.46 (2H, m), 1.83 m (1H, m), 1.86 (2H, m), 3.73 (1H, q, J = 6.5 Hz), 4.00 (2H, t, J = 6.5 Hz), 5.92 (1H, d, J = 2.0 Hz), 6.00 (1H, d, J = 2.0 Hz), 6.02 (1H, br s), 14.19 (1H, s); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): δ\(_{C}\) 12.0, 14.3, 17.1, 22.9, 26.9, 26.5, 29.3, 29.5, 29.6, 29.77 (2C), 29.8, 29.9, 32.1, 46.3, 69.4, 91.7, 96.8, 106.1, 162.5, 163.0, 167.7, 210.7; HRMS (ESI) m/z: [M+H]^+ Calcd. for C\(_{23}\)H\(_{39}\)O\(_{5}\) 379.2848; Found 379.2864.

4.2.5.9. (S)-1-(2,4-Dihydroxy-6-(octadecyloxy)phenyl)-2-methylbutan-1-one (15)
Synthesised using 0.9 mmol of 5 and 1.079 mmol of 1-bromooctadecane. Isolated after SPE (SiGel; hexane/EtOAc 92:8) as a colourless oil (118 mg, 0.255 mmol, 28%). $\left[\alpha\right]_D^{22} +4.2$ (c 0.35, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$): 244 (4.32), 289 (3.98) nm; IR $\nu_{\text{max}}$ (thin film) cm$^{-1}$: 3328, 2923, 2853, 1738, 1623, 1593, 1455, 1373, 1214, 1159, 1104, 774; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$H 0.89 (3H, t, $J = 7.5$ Hz), 0.91 (3H, t, $J = 7.5$ Hz), 1.16 (3H, d, $J = 6.5$ Hz), 1.27-1.33 (28H, m), 1.42 (1H, m), 1.46 (2H, m), 1.81 (1H, m), 1.85 (2H, m), 3.73 (1H, q, $J = 6.5$ Hz), 4.00 (2H, t, $J = 6.5$ Hz), 5.92 (1H, d, $J = 2.0$ Hz), 6.00 (1H, d, $J = 2.0$ Hz), 6.07 (1H, br s), 14.12 (1H, s); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$C 12.0, 14.3, 17.1, 22.9, 26.5, 26.9, 29.3, 29.6, 29.8-29.9 (11C), 32.2, 46.3, 69.4, 91.6, 96.8, 106.1, 162.3, 163.0, 167.8, 210.6; HRMS (ESI) m/z: [M+H]$^+$ Calcd for C$_{29}$H$_{51}$O$_4$ 463.3787; Found 463.3796.

4.2.6. Synthesis of compounds 18-24 from phloroglucinol

The first step in the synthesis of compounds 19-24 was the Friedel-Crafts acylation of phloroglucinol using the appropriate acyl (propionyl, butyryl, pentanoyl, decanoyl, octadecanoyl and benzoyl) chloride using the procedure described for 4 above. Compound 18 was synthesised starting from commercially available acetylphloroglucinol. TBDMS protection of each acylphloroglucinol 16 was achieved according to the procedure described for 5 above. Finally, the TBDMS-protected acylphloroglucinols 17 were simultaneously deprotected and geranylated at the ortho phenolic hydroxyl as described for compound 6 to produce compounds 18-24. Further details of the experimental procedures used for the synthesis of compounds 18-24, including full compound characterisation data for the corresponding precursor acylphloroglucinols and TBDMS-protected acylphloroglucinols, are included in the Supporting Information.

4.2.6.1. (E)-1-((2-((3,7-Dimethylocta-2,6-dien-1-yl)oxy)-4,6-dihydroxyphenyl)ethanone (18)

Isolated after SPE (SiGel; hexane/EtOAc 8:2) as a pale yellow oil (45 mg, 0.148 mmol, 4.3% over two steps starting from commercial acetylphloroglucinol). UV (MeOH)
\( \lambda_{\text{max}} \) (log \( c \)): 244 (4.36), 288 (3.97) nm; IR \( \nu_{\text{max}} \) (thin film) cm\(^{-1}\): 3136, 2909, 1737, 1621, 1561, 1464, 1373, 1284, 1259, 1222, 1165, 1104, 1071, 822, 756; \(^1\)H NMR (500 MHz, (CD\(_3\)_2)CO): \( \delta \)H 1.59 (3H, s), 1.64 (3H, s), 1.77 (3H, s), 2.11-2.16 (4H, m), 2.55 (3H, s), 4.64 (2H, d, \( J = 6.5 \) Hz), 5.11 (1H, m), 5.56 (1H, m), 5.93 (1H, d, \( J = 2.0 \) Hz), 56.03 (1H, d, \( J = 2.0 \) Hz), 13.91 (1H, s); \(^1^3\)C NMR (125 MHz, (CD\(_3\)_2)CO): \( \delta \)C: 16.7, 17.8, 25.9, 27.0, 33.2, 40.1, 66.4, 92.8, 96.6, 106.1, 119.9, 124.7, 132.2, 142.6, 164.0, 166.0, 168.3, 203.5; HRMS (ESI) m/z: [M+H]\(^+\) Calcd. for C\(_{18}\)H\(_{25}\)O\(_4\) 305.1753; Found 305.1766.

4.2.6.2. (E)-1-(2-((3,7-Dimethylocta-2,6-dien-1-yl)oxy)-4,6-dihydroxyphenyl)propan-1-one

(19)

Isolated after SPE (SiGel; hexane/EtOAc 9:1) as a yellow oil (50 mg, 0.157 mmol, 2.6% over three steps starting from 3). UV (MeOH) \( \lambda_{\text{max}} \) (log \( c \)): 243 (4.32), 289 (3.99) nm; IR \( \nu_{\text{max}} \) (thin film) cm\(^{-1}\): 3310, 2973, 2937, 1622, 1594, 1448, 1376, 1220, 1166, 1100, 828, 756; \(^1\)H NMR (500 MHz, CDCl\(_3\)): \( \delta \)H 1.55 (3H, t, \( J = 7.0 \) Hz), 1.61 (3H, s), 1.68 (3H, s), 1.74 (3H, s), 2.10 (2H, m), 2.13 (2H, m), 3.03 (2H, q, \( J = 7.0 \) Hz), 4.57 (2H, d, \( J = 6.5 \) Hz), 5.10 (1H, m), 5.51 (1H, br s), 5.91 (1H, d, \( J = 2.0 \) Hz), 5.98 (1H, d, \( J = 2.0 \) Hz), 14.05 (1H, br s); \(^1^3\)C NMR (125 MHz, CDCl\(_3\)): \( \delta \)C: 9.0, 16.9, 18.0, 25.9, 26.5, 37.8, 39.7, 66.0, 91.7, 96.6, 106.3, 118.6, 123.8, 132.3, 142.4, 162.4, 163.2, 167.4, 207.0; HRMS (ESI) m/z: [M+H]\(^+\) Calcd. for C\(_{19}\)H\(_{27}\)O\(_4\) 319.1909; Found 319.1917.

4.2.6.3. (E)-1-(2-((3,7-Dimethylocta-2,6-dien-1-yl)oxy)-4,6-dihydroxyphenyl)butan-1-one

(20)

Isolated after SPE (SiGel; hexane/EtOAc 94:6) as a pale yellow oil (110 mg, 0.331 mmol, 5.7% over three steps starting from 3). UV (MeOH) \( \lambda_{\text{max}} \) (log \( c \)): 243 (4.39), 290 (3.99) nm; IR \( \nu_{\text{max}} \) (thin film) cm\(^{-1}\): 3317, 2970, 1739, 1606, 1435, 1366, 1215, 745; \(^1\)H NMR (500 MHz, CDCl\(_3\)): \( \delta \)H 0.97 (3H, t, \( J = 6.5 \) Hz), 1.61 (3H, s), 1.69 (3H, s), 1.71 (2H, s), 1.74 (3H, s), 2.10 (2H, m), 2.13 (2H, m), 2.97 (2H, t, \( J = 7.0 \) Hz), 4.54 (2H, d, \( J = 6.5 \) Hz), 5.10
(1H, m), 5.50 (1H, t, J = 6.5 Hz), 5.91 (1H, s, HO4), 5.93 (1H, d, J = 2.0 Hz), 5.99 (1H, d, J = 2.0 Hz), 14.10 (1H, s); subjs. NMR (125 MHz, CDCl_3): δC 14.2, 16.9, 17.9, 18.9, 25.9, 26.5, 39.7, 46.5, 65.9, 92.1, 96.6, 106.0, 118.4, 123.8, 132.2, 142.7, 162.43, 163.4, 167.3, 206.9. HRMS (ESI) m/z: [M+H]^+ Calcd. for C_{20}H_{29}O_{4}333.2066; Found 333.2057.

4.2.6.4. (E)-1-(2-((3,7-Dimethylocta-2,6-dien-1-yl)oxy)-4,6-dihydroxyphenyl)pent-1-one

Isolated after SPE (SiGel; hexane/EtOAc 8:2) as a colourless oil (95 mg, 0.274 mmol, 2.2% over three steps starting from 3). UV (MeOH) λ_{max} (log ε): 239 (4.36), 290 (3.97) nm; IR ν_{max} (thin film) cm^{-1}: 3263, 2957, 2929, 1739, 1621, 1569, 1416, 1346, 1198, 1164, 1102, 827, 791; 1H NMR (500 MHz, CDCl_3): δH 0.92 (3H, t, J = 6.5 Hz), 1.37 (2H, q, J = 6.5 Hz), 1.61 (3H, s), 1.64 (2H, m), 1.69 (3H, s), 1.74 (3H, s), 2.10 (2H, m), 2.13 (2H, m), 3.00 (2H, m), 4.55 (2H, d, J = 6.5 Hz), 5.10 (1H, t, J = 6.5 Hz), 5.50 (1H, t, J = 6.5 Hz), 5.92 (1H, d, J = 2.0 Hz), 5.99 (1H, d, J = 2.0 Hz), 6.38 (1H, s), 14.04 (1H, s); 13C NMR (125 MHz): δC 14.2, 16.9, 19.7, 22.8, 27.4, 65.9, 91.9, 96.6, 106.1, 119.8, 123.8, 132.2, 142.6, 162.9, 163.1, 167.4, 206.8; HRMS (ESI) m/z: [M+H]^+ Calcd. for C_{21}H_{31}O_{4} 347.2222; Found 347.2223.

4.2.6.5. (E)-1-(2-((3,7-Dimethylocta-2,6-dien-1-yl)oxy)-4,6-dihydroxyphenyl)decan-1-one

Isolated after SPE (SiGel; hexane/EtOAc 96:4) as a colourless oil (85 mg, 0.204 mmol, 4.2% over three steps starting from 3). UV (MeOH) λ_{max} (log ε): 244 (4.39), 293 (3.98) nm; IR ν_{max} (thin film) cm^{-1}: 3309, 2924, 2854, 1738, 1620, 1594, 1445, 1376, 1206, 1167, 1097, 829, 754; 1H NMR (500 MHz, CDCl_3): δH 0.88 (3H, t, J = 6.5 Hz), 1.26 (12H, br s), 1.35 (2H, m), 1.61 (3H, s), 1.68 (3H, s), 1.74 (3H, s), 2.09 (2H, m), 2.13 (2H, m), 2.99 (2H, m), 4.56 (2H, d, J = 6.5 Hz), 5.11 (1H, t, J = 6.5 Hz), 5.51 (1H, t, J = 6.5 Hz), 5.92 (1H, d, J = 2.0 Hz), 5.99 (1H, d, J = 2.0 Hz), 14.12 (1H, s); 13C NMR (125 MHz, CDCl_3): δC 14.4,
16.9, 17.9, 22.9, 25., 25.9, 26.6, 29.6, 29.7-29.8 (3C), 32.2, 39.8, 44.7, 65.9, 91.8, 96.6, 106.1, 118.5, 123.8, 132.2, 142.6, 163.0, 163.1, 167.5, 206.5; HRMS (ESI) m/z: [M+H]^+ Calcd. for C_{26}H_{41}O_4 417.3005; Found 417.3001.

4.2.6.6. (E)-1-(2-((3,7-Dimethylocta-2,6-dien-1-yl)oxy)-4,6-dihydroxyphenyl)octadecan-1-one (23)

Isolated after SPE (SiGel; hexane/EtOAc 96:4) as a pale yellow oil (650 mg, 1.230 mmol, 19% over three steps starting from 3). UV (MeOH) \( \lambda_{\text{max}} \) (log \( \varepsilon \)): 237 (4.18), 286 (3.87) nm; IR \( \nu_{\text{max}} \) (thin film) cm\(^{-1}\): 3020, 2924, 2854, 1715, 1662, 1447, 1214, 1097, 829, 750; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \)\(_{\text{H}} \) 0.89 (3H, t, \( J = 6.8 \) Hz), 1.26-1.35 (34H, br s), 1.62 (3H, s), 1.69 (3H, s), 1.75 (3H, s), 2.10 (2H, m), 2.13 (2H, m), 2.99 (2H, m), 4.55 (2H, d, \( J = 6.5 \) Hz), 5.10 (1H, t, \( J = 6.5 \) Hz), 5.50 (1H, t, \( J = 6.5 \) Hz), 5.91 (1H, d, \( J = 2.0 \) Hz), 5.98 (1H, d, \( J = 2.0 \) Hz), 14.12 (1H, s); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \)\(_{\text{C}} \) 14.3, 16.9, 17.9, 22.9, 25.3, 25.9, 26.5, 29.6, 29.8-29.9 (15C'), 32.1, 39.7, 44.7, 65.9, 91.7, 96.6, 106.3, 118.5, 123.8, 132.2, 142.5, 162.6, 163.1, 167.5, 206.7; HRMS (ESI) m/z: [M+H]^+ Calcd. for C\(_{34}\)H\(_{57}\)O\(_4\) 529.4257; Found 529.3710.

4.2.6.7. (E)-2-((3,7-Dimethylocta-2,6-dien-1-yl)oxy)-4,6-dihydroxyphenyl)(phenyl) methanone (24)

Isolated after SPE (SiGel; hexane/EtOAc 9:1) as a pale yellow oil (96 mg, 0.262 mmol, 10% over three steps starting from 3); UV (MeOH) \( \lambda_{\text{max}} \) (log \( \varepsilon \)): 237 (4.45), 288 (3.99) nm; IR \( \nu_{\text{max}} \) (thin film) cm\(^{-1}\): 3308, 2970, 2923, 1738, 1621, 1590, 1446, 1376, 1271, 1154, 1095, 825, 752; \(^1\)H NMR (500 MHz, CDCl\(_3\)): \( \delta \)\(_{\text{H}} \) 1.52 (3H, s), 1.61 (3H, s), 1.71 (3H, s), 1.85 (2H, m), 1.94 (2H, m), 4.21 (2H, d, \( J = 6.5 \) Hz), 4.61 (1H, t, \( J = 6.5 \) Hz), 5.05 (1H, m), 5.89 (1H, d, \( J = 2.0 \) Hz), 6.07 (1H, d, \( J = 2.0 \) Hz), 7.36 (2H, dt, \( J = 8.0, 1.5 \) Hz), 7.42 (1H, d, \( J = 8.0 \) Hz), 7.49 (2H, dt, \( J = 8.0, 1.5 \) Hz), 12.27 (1H, s); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \( \delta \)\(_{\text{C}} \) 16.8, 17.9, 25.9, 26.4, 39.4, 65.5, 92.4, 96.5, 106.2, 118.1, 124.0, 127.6 (2C), 127.8, 130.7, 132.0,
140.7, 142.5, 162.4, 163.5, 165.9, 200.1; HRMS (ESI) m/z: [M+H]+ Calcd. for C_{23}H_{27}O_{4} 367.1909; Found 367.1893.

4.3. Antibacterial assay against Staphylococcus aureus

Unless otherwise stated, all chemicals were obtained from Sigma-Aldrich, Gillingham, UK. Cation-adjusted Mueller-Hinton broth (MHB) was obtained from Oxoid and was adjusted to contain 20 mg/L and 10 mg/L of Ca<sup>2+</sup> and Mg<sup>2+</sup>, respectively. The S. aureus strains used in this study included ATCC 25923, SA-1199B, RN4220, XU212, EMRSA-15 and EMRSA-16. A standard laboratory strain, ATCC 25923, which is sensitive to antibiotics like tetracycline [15], was also used in this study. SA-1199B over-expresses the NorA MDR efflux pump [16], RN4220 possesses the MsrA macrolide efflux protein [17], XU212 is a Kuwaiti hospital isolate which is an MRSA strain possessing the TetK tetracycline efflux pump [15], whilst the EMRSA-15 strain [18] and EMRSA-16 strain [19] are epidemic in the UK. These strains were the generous gift of Dr Paul Stapleton (UCL).

All S. aureus strains were cultured on nutrient agar (Oxoid) and incubated for 24 h at 37 °C prior to MIC determination. A stock solution of norfloxacin was prepared by dissolving the antibiotic (2 mg) in DMSO (244 μL; Sigma) and diluting 16-fold with MHB to obtain the desired starting concentration (512 mg/L) of antibiotic. Similarly, stock solutions of the test compounds were prepared by initial dissolution in DMSO followed by dilution with MHB to produce target concentrations of 512 mg/L. No significant precipitation was noted for any of the test compounds when stock solutions were prepared at this concentration. An inoculum density of 5 × 10<sup>5</sup> colony forming units (cfu/mL) of each bacterial strain was prepared in normal saline (9 g/L) by comparison with a 0.5 MacFarland turbidity standard.

During the experiment, MHB (125 μL) was added to each well of a 96-well plate, save for the final column which was left empty. 125 μL of the stock solution of the compound to be tested (or the control antibiotic norfloxacin) was then added to the MHB in
the first well. Using a multi-channel pipette, the contents of the first well were mixed thoroughly, followed by the transfer of 125 µL of the well contents to the second well. This two-fold serial dilution process was continued up until the tenth well, and the final 125 µL solution was added to the final (empty) well. The inoculum (125 µL) of each bacterium at a density of $5 \times 10^5$ cfu/mL was added to all wells except those in the final column. The final concentrations of each test compound thus ranged from 128 mg/L to 0.25 mg/L in the initial evaluation (similarly, the final concentrations of DMSO in the assays ranged from 1.56% v/v at the highest concentration of test compound to 0.0031% v/v at the lowest). The contents of the wells in columns 11 and 12 represented growth control (bacteria but no compound) and sterility control (compound but no bacteria) respectively. Every assay was performed in duplicate. The microtitre plates were then incubated at 37 °C for the appropriate incubation time. For the MIC determination, 20 µL of a 5 mg/mL methanolic solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each of the wells, followed by incubation for 20 min. Bacterial growth was indicated by a colour change from yellow to dark blue. The MIC was recorded as the lowest concentration at which no growth was observed [11]. If no growth was observed at any of the concentrations tested, the assay was repeated starting with a stock solution of lower concentration. If growth was observed at all of the concentrations tested, the assay was repeated starting with a stock solution of higher concentration.

**Acknowledgements**

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**Appendix A. Supplementary data**

Supplementary data related to this article can be found at
References


Fig. 1. (S)-(2-Methylbutanoyl)phloroglucinols with ortho alkyloxy variants
Fig. 2. ortho geranylated acylphloroglucinols with acyl variants

18 $R = \text{CH}_3$
19 $R = \text{CH}_2\text{CH}_3$
20 $R = (\text{CH}_2)_2\text{CH}_3$
21 $R = (\text{CH}_2)_3\text{CH}_3$
22 $R = (\text{CH}_2)_6\text{CH}_3$
23 $R = (\text{CH}_2)_{16}\text{CH}_3$
24 $R = \text{Ph}$
Scheme 1

Synthesis of compounds 6-15

1. Reaction of compound 1 with SOCl₂ to form compound 2 (93%)

2. Reaction of compound 2 with AlCl₃ and CS₂, PhNO₂ to form compound 4 (54%)

3. Reaction of compound 3 with TBDMSOT to form compound 5 (81%)

4. Reaction of compound 5 with R-Br, K₂CO₃ in DMF to form compound 6-15 (3-30%)
Scheme 2

Synthesis of compounds 18-24

\[ 3 + \text{Cl-} \overset{\text{AlCl}_3}{\longrightarrow} \overset{\text{CS}_2, \text{PhNO}_2}{\longrightarrow} \text{R} \]

16 (51-60%)

\[ \overset{\text{TBDMSI}, \text{imidazole}}{\text{TBDMSI}} \overset{\text{acetone}}{\longrightarrow} \text{R} \]

17 (40-82%)

\[ \overset{\text{K}_2\text{CO}_3, \text{DMF}}{\text{geranyl bromide}} \]

18-24 (6-46%)
Table 1

MICs (in mg/L) of compounds (8-24) against standard, multi-drug resistant (MDR) and methicillin-resistant strains of *Staphylococcus aureus*

<table>
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<th>Compound</th>
<th>SA1199B</th>
<th>XU212</th>
<th>ATCC25941</th>
<th>RN4220</th>
<th>EMRSA15</th>
<th>EMRSA16</th>
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<td>&gt;512</td>
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\(^a\)No inhibition of growth was apparent at the highest concentration tested