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Semisynthesis and biological evaluation of a focused library of unguinol derivatives as next-generation antibiotics†

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In this study, we report the semisynthesis and *in vitro* biological evaluation of thirty-four derivatives of the fungal depsidone antibiotic, unguinol. Initially, the semisynthetic modifications were focused on the two free hydroxy groups (3-OH and 8-OH), the three free aromatic positions (C-2, C-4 and C-7), the butenyl side chain and the depsidone ester linkage. Fifteen first-generation unguinol analogues were synthesised and screened against a panel of bacteria, fungi and mammalian cells to formulate a basic structure activity relationship (SAR) for the unguinol pharmacophore. Based on the SAR studies, we synthesised a further nineteen second-generation analogues, specifically aimed at improving the antibacterial potency of the pharmacophore. *In vitro* antibacterial activity testing of these compounds revealed that 3-*O*-(2-fluorobenzyl)unguinol and 3-*O*-(2,4-difluorobenzyl)unguinol showed potent activity against both methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* (MIC 0.25–1 μg mL^{-1}) and are promising candidates for further development *in vivo*.

Introduction

Infections caused by multidrug-resistant bacteria are a major threat to global health. In the clinical setting, methicillin-resistant *Staphylococcus aureus* (MRSA) continues to be a leading cause of infection-related mortality.[1] While vancomycin has been the treatment of choice for serious MRSA infections for decades, there have been increasing reports of vancomycin treatment failures due to the emergence and dissemination of resistant strains.[2,3] Concerningly, treatment failure with daptomycin, a drug of last resort for MRSA, can occur in more than 20% of cases.[3,4] Therefore, there is a pressing need for the discovery and development of new antibiotics with novel modes of action to combat these deadly superbugs.[5]

There are many approaches currently being explored to identify new classes of antibiotics, including *in situ* cultivation of uncultured microbes,[6,7] identification and prioritisation of novel organisms by chemotaxonomy[8–14] and activation of silent biosynthetic gene clusters (BGCs) using synthetic biology and bioinformatics tools.[15,16] An alternate strategy involves revisiting some of the old antibiotic scaffolds that were discovered many decades ago, during a time of plenty, that were abandoned in favour of more promising leads. Re-examining these neglected historic scaffolds through the lens of modern drug discovery platforms has proven to be an effective method of bringing new antibiotic classes to the market.[17] Notable antibiotic revivals include linezolid (2000), daptomycin (2003) and lefamulin (2019), which belong to chemical classes first reported in 1978,[18] 1987[19] and 1952,[20] respectively.

In our ongoing search for new antibiotic leads, we recently reported our work on expanding chemical space around the nidulin antibiotic pharmacophore.[21] Nidulin is a trichlorinated depsidone antibiotic, first identified in 1945 from the fungus *Aspergillus unguis*.[22] While nidulin has reported antibacterial activity against *Mycobacterium tuberculosis*[23] and MRSA,[24] the compound has received only modest attention since its initial discovery and the scaffold has not been systematically investigated as an antibiotic lead. In our recent study, manipulating the halide ion concentration in the cultivation medium of *A. unguis* led to the production of 12 previously unreported nidulin analogues, along with 11 known nidulin analogues. Biological testing of this small library revealed a number of interesting trends in potency and selectivity that warranted further investigation. In

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†Electronic supplementary information (ESI) available: NMR spectra, UV-vis spectra and HRMS spectra of all compounds. See DOI: 10.1039/d0ob02460k

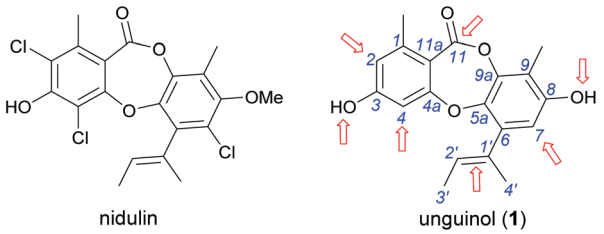


Fig. 1 Chemical structures of nidulin and unguinol (**1**) and the initial sites selected for semisynthetic modification of **1** (arrowed).

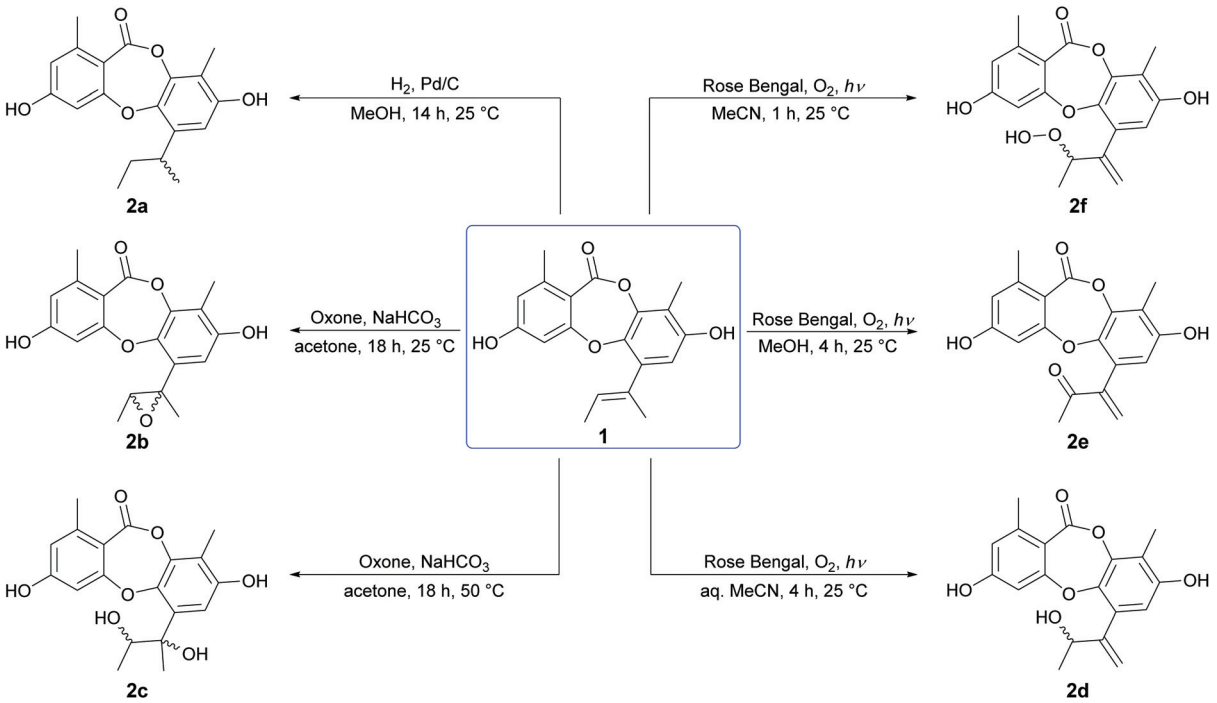
this study, we have employed a semisynthetic approach to expand the structure activity relationship (SAR) of the nidulin pharmacophore. Starting from the closely related metabolite unguinol, we have generated a library of 15 analogues by modifying 7 different locations around the unguinol core (Fig. 1). All semisynthetic analogues were screened for *in vitro* activity against a panel of bacteria, fungi and mammalian cell lines. *In vitro* antimicrobial testing revealed 3-*O*-benzylunguinol is fifteen times more potent than ampicillin against *S. aureus*. Further exploration of benzylation of unguinol with halogen-substituted benzyl bromide yielded more potent antibiotics, 3-*O*-(2-fluorobenzyl)unguinol and 3-*O*-(2,4-difluorobenzyl)unguinol.

Results and discussion

First-generation semisynthetic unguinol analogues

We initiated our semisynthetic program starting with unguinol (**1**), which is the major metabolite of *A. unguis* and could be isolated in reasonable quantities by large-scale cultivation of the organism. Compound **1** is a nonchlorinated analogue of nidulin, containing two free hydroxy groups (3-OH and 8-OH), three unsubstituted aromatic positions (H-2, H-4 and H-7), one double bond ($\Delta^{1',2'}$) in the butenyl side chain and one ester linkage. These 7 locations were selected as the initial sites for semisynthetic modification to assess the contributions of each group towards the antibiotic activity of the depsidone scaffold.

Oxidation and reduction. Initially, the $\Delta^{1',2'}$ double bond in the butenyl side chain of **1** was reduced by catalytic hydrogenation (H_2, Pd/C), to give a racemic mixture of 1′,2′-dihydrounguinol (**2a**) in quantitative yield (Scheme 1). Next, the $\Delta^{1',2'}$ double bond of **1** was oxidised at 25 °C with dimethyldioxirane (DMDO), which was generated *in situ* from basic acetone and Oxone, to give *cis*-1′,2′-epoxyunguinol (**2b**) as a racemic mixture in 53% yield. Repeating the DMDO oxidation at 50 °C yielded a small quantity of 1′,2′-dihydroxyunguinol (**2c**), formed from **2b** by nucleophilic ring-opening of the epoxide under the basic reaction conditions. LCMS analysis of crude reaction mixture revealed two diastereomers had formed in a 3 : 1 ratio, although only the major could be isolated in sufficient quantities for characterisation. Oxidation of the $\Delta^{1',2'}$ double bond of **1** by molecular oxygen in the presence of UV light (254 nm) and Rose Bengal photosensitizer in aqueous MeCN, MeOH and anhydrous MeCN yielded three unguinol derivatives, 2′-hydroxy-$\Delta^{1',4'}$-unguinol (**2d**), 2′-oxo-$\Delta^{1',4'}$-unguinol (**2e**) and 2′-hydroperoxy-$\Delta^{1',4'}$-unguinol (**2f**), respectively. The dye-sensitized photooxidation of alkenes has been studied extensively[25,26] and the distribution of products observed can

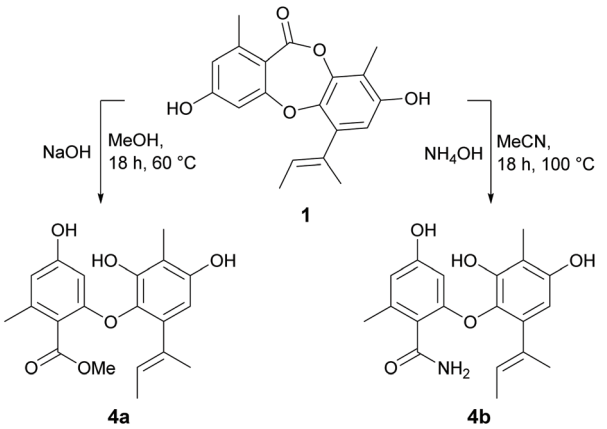


Scheme 1 Oxidation and reduction of the butenyl side chain of unguinol (**1**).

be rationalised by addition of singlet oxygen to the $\Delta^{1',2'}$ double bond of **1** to yield allylic hydroperoxide **2f** (Schenck ene reaction), followed by subsequent thermal decomposition to give alcohol **2d** or dehydration to give ketone **2e**.

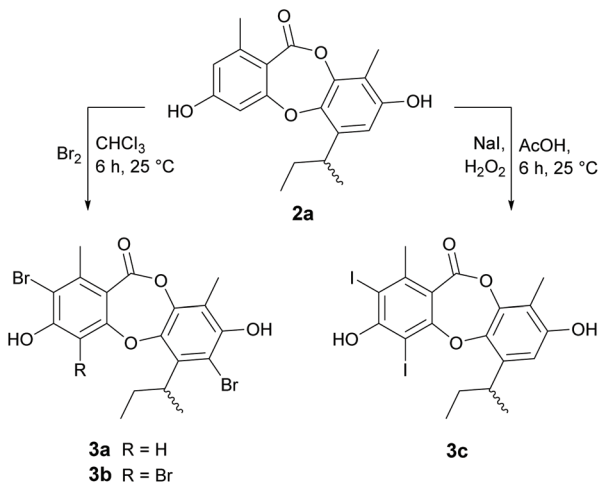
Halogenation. In our previous paper,[21] we reported a series of novel brominated depsidones (mono- and dibromounguinol), which were obtained by supplementing the culture media with potassium bromide. Significantly, we found that these brominated depsidones showed improved antibacterial activities against *Bacillus subtilis* and *S. aureus* when compared with unguinol. To explore this effect more fully, we synthesised several brominated and iodinated unguinol analogues, as shown in (Scheme 2). As reduction of the butenyl side chain of **1** did not have any significant effects on antibacterial activity, we conducted the halogenation reactions on hydrogenated analogue **2a** to avoid complicating side reactions. Treatment of **2a** with bromine in chloroform at 25 °C yielded a mixture of 2,7-dibromo-1′,2′-dihydrounguinol (**3a**) and 2,4,7-tribromo-1′,2′-dihydrounguinol (**3b**) in 16% and 19% yield, respectively. While LCMS analysis of the crude reaction mixture suggested that trace amounts of monobrominated products had formed under these reaction conditions, varying the reaction temperature, solvent and equivalents of bromine did not yield sufficient quantities of these products for isolation and characterisation. Similarly, treatment of **2a** with NaI and H_2O_2 in acetic acid at 25 °C yielded 2,4-diiodo-1′,2′-dihydrounguinol (**3c**) in 56% yield. LCMS analysis of the crude reaction mixture suggested trace amounts of monoiodinated products had formed, but there was no evidence for the formation of the triiodinated product, suggesting iodination at C-7 is not favoured under these conditions.

Nucleophilic ring opening. The depsidone scaffold contains a central seven-membered ring consisting of one ether and one ester linkage. Cleavage of the ether linkage yields depsides, while cleavage of the ester linkage yields diphenyl ethers. In our previous paper,[21] we reported several naturally occurring depsides and diphenyl ethers with moderate to weak antibacterial activities. Therefore, to gain further insights into the SAR of diphenyl ethers, we next explored cleaving the ester linkage of **1** with sodium hydroxide in methanol and ammonium hydroxide in water to yield methyl unguinolate (**4a**) and unguinolamide (**4b**), respectively (Scheme 3). The presence of an additional exchangeable resonance in the ^{1}H NMR spectra (δ_H 8.36 for **4a** and 9.38 for **4b**) attributable to 9a-OH confirmed that the depsidone ester linkages had been cleaved in both compounds. The presence of a methyl ester in **4a** was evident from signals at δ_C 167.9 and 51.6 in ^{13}C NMR spectrum, while the presence of a primary amide in **4b** was evident from two additional exchangeable signals at δ_H 7.71 and 7.94. The free acid was isolated and characterised in our previous work[21] as a natural product (unguinolic acid) and hence was not prepared synthetically in this work.

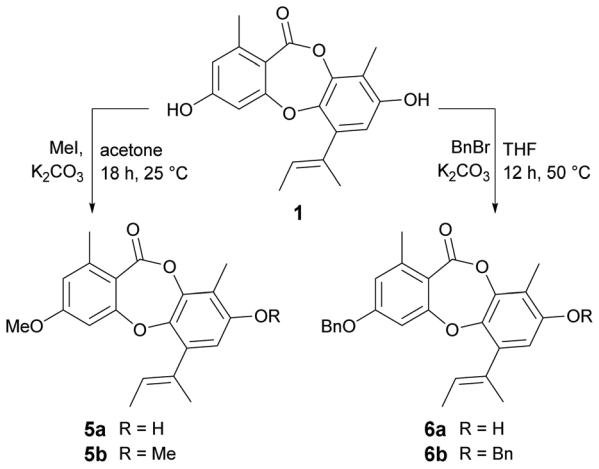


Scheme 3 Nucleophilic opening of the ester linkage of unguinol (**1**).

Methylation and benzylation. We next turned our attention to derivatising the two free hydroxy groups (3-OH and 8-OH) of **1** (Scheme 4). Treatment of **1** with methyl iodide (2 eq.) in K_2CO_3 resulted in a mixture of 3-*O*-methylunguinol (**5a**) and 3,8-di-*O*-methylunguinol (**5b**), but not the 8-*O*-monomethylated product. The structure of **5a** was confirmed by diagnostic ROESY NMR correlations between 3-OMe and aromatic protons H-2 and H-4. Attempts to access 8-*O*-methylunguinol by first protecting 3-OH as the *tert*-butyldimethylsilyl (TBDMS) ether were unsuccessful. This apparent regioselectivity is interesting given the preference for 8-*O*-methylation in Nature, with the only naturally occurring 3-*O*-methylated unguinol analogue reported to date being aspergillusidone B.[27,28] Next, benzylation of **1** was carried out using benzyl bromide and K_2CO_3 at 50 °C in THF (Scheme 4). Under these reaction conditions, a mixture of mono- and dibenzylated products was formed, with 3-*O*-benzylunguinol (**6a**) isolated as the major product. The position of benzyl group on 3-OH was confirmed by diagnostic HMBC correlations from the benzyl methylene protons to C-3 (δ_C 161.7) and a ROESY correlation from the methylene protons to H-2. Interestingly, repeating the benzylation reaction using either acetone or acetonitrile as the solvent yielded 3,8-di-*O*-dibenzylunguinol (**6b**) as the major product.



Scheme 2 Halogenation of 1′,2′-dihydrounguinol (**2a**).



Scheme 4 Methylation and benzylation of unguinol (**1**).

Bioassay of first-generation semisynthetic unguinol analogues

The fifteen first-generation semisynthetic unguinol analogues were tested for *in vitro* activity against the Gram-positive bacteria *B. subtilis* (ATCC 6633) and *S. aureus* (ATCC 25923), the Gram-negative bacterium *Escherichia coli* (ATCC 25922), the fungi *Candida albicans* (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 9763), and mouse NS-1 myeloma (ATCC TIB-18) cells (Table 1). The compounds exhibited a wide range of antibacterial activities against the Gram-positive bacteria, but no activity was observed for any of the analogues against *E. coli* or *C. albicans.*

Reduction of the butenyl side chain of **1** by catalytic hydrogenation yielded **2a**, which was equipotent against *B. subtilis* but two-fold less potent against *S. aureus*. Oxidation of the butenyl side chain of **1** yielded five oxygenated derivatives, **2b**–**2f**. While epoxidation (**2b**) and dihydroxylation (**2c**) of the double bond significantly reduced antibacterial activity, the allylic ketone (**2e**) and hydroperoxide (**2f**) derivatives showed similar activity against *B. subtilis* and up to eight-fold increased activity against *S. aureus*. This was also accompanied by significantly increased cytotoxicity against mammalian tumour cells, with activities more potent than the positive control 5-fluorouracil. Dibromination (**3a**) and tribromination (**3b**) of **2a** also significantly improved the antibacterial activity against both of the Gram-positive bacteria, but again was accompanied by increased cytotoxicity against NS-1 cells. Interestingly, **3a** also showed twelve-fold improved activity against *S. cerevisiae* compared to **2a**, while **3b** showed no antifungal activity. Di-iodination (**3c**) of **2a** yielded a similar improvement in antibacterial activity as dibromination, but with no increase in cytotoxicity. Nucleophilic opening of the ester linkage of **1** with NaOH/MeOH and NH_4OH/MeCN to give methyl unguinolate (**4a**) and unguinolamide (**4b**), respectively, resulted in a significant decrease in antibacterial activity against both of the Gram-positive bacteria, highlighting the importance of the seven-membered depsidone ring system.

Methylation of **1** at 3-OH (**5a**) resulted in a two-fold decrease in activity against *B. subtilis*, with no change in activity against *S. aureus*. However, methylation of **1** at both 3-OH and 8-OH (**5b**) abolished activity against both of the Gram-positive bacteria, suggesting at least one free hydroxy group is essential for activity. Benzylation of **1** at 3-OH (**6a**) resulted in a modest increase in activity against *B. subtilis*, but a very significant increase in activity against *S. aureus*. Indeed, **6a** (MIC 0.2 μg mL^{-1}) was found to be over thirty-fold more active than nidulin (MIC 6.3 μg mL^{-1}) and over sixty-fold more active than

Table 1 *In vitro* biological activities of first-generation semisynthetic unguinol analogues

	MIC (μg mL^{-1})			
Compounds	*B. subtilis* (ATCC 6633)	*S. aureus* (ATCC 25923)	*S. cerevisiae* (ATCC 9763)	NS-1 (ATCC TIB-18)
Nidulin	0.8	6.3	—	27.2
Unguinol (**1**)	3.1	12.5	50	25
1′,2′-Dihydrounguinol (**2a**)	3.1	25	50	25
cis-1′,2′-Epoxyunguinol (**2b**)	25	100	—	25
1′,2′-Dihydroxyunguinol (**2c**)	—	—	—	—
2′-Hydroxy-$\Delta^{1',4'}$-unguinol (**2d**)	50	100	—	50
2′-Oxo-$\Delta^{1',4'}$-unguinol (**2e**)	1.6	1.6	—	<0.1
2′-Hydroperoxy-$\Delta^{1',4'}$-unguinol (**2f**)	3.1	3.1	—	<0.1
2,7-Dibromo-1′,2′-dihydrounguinol (**3a**)	2.1	2.1	4.2	4.2
2,4,7-Tribromo-1′,2′-dihydrounguinol (**3b**)	1.2	4.6	—	9.2
2,4-Diiodo-1′,2′-dihydrounguinol (**3c**)	1.1	4.6	9.1	36.4
Methyl unguinolate (**4a**)	25	100	—	50
Unguinolamide (**4b**)	25	—	—	<0.1
3-*O*-Methylunguinol (**5a**)	6.3	12.5	12.5	25
3,8-Di-*O*-methylunguinol (**5b**)	—	—	—	16.1
3-*O*-Benzylunguinol (**6a**)	1.6	0.2	—	3.1
3,8-Di-*O*-benzylunguinol (**6b**)	50	—	—	[a]
Ampicillin	0.2	3.1	[a]	[a]
Clotrimazole	[a]	[a]	0.4	[a]
5-Fluorouracil	[a]	[a]	[a]	0.1

[a] Not tested; – no activity up to 100 μg mL^{-1}.

unguinol (MIC 12.5 μg mL^{-1}) against *S. aureus*. Benzylation of **1** at both 3-OH and 8-OH (**6b**) also abolished all antibacterial activity, as was observed for dimethylation.

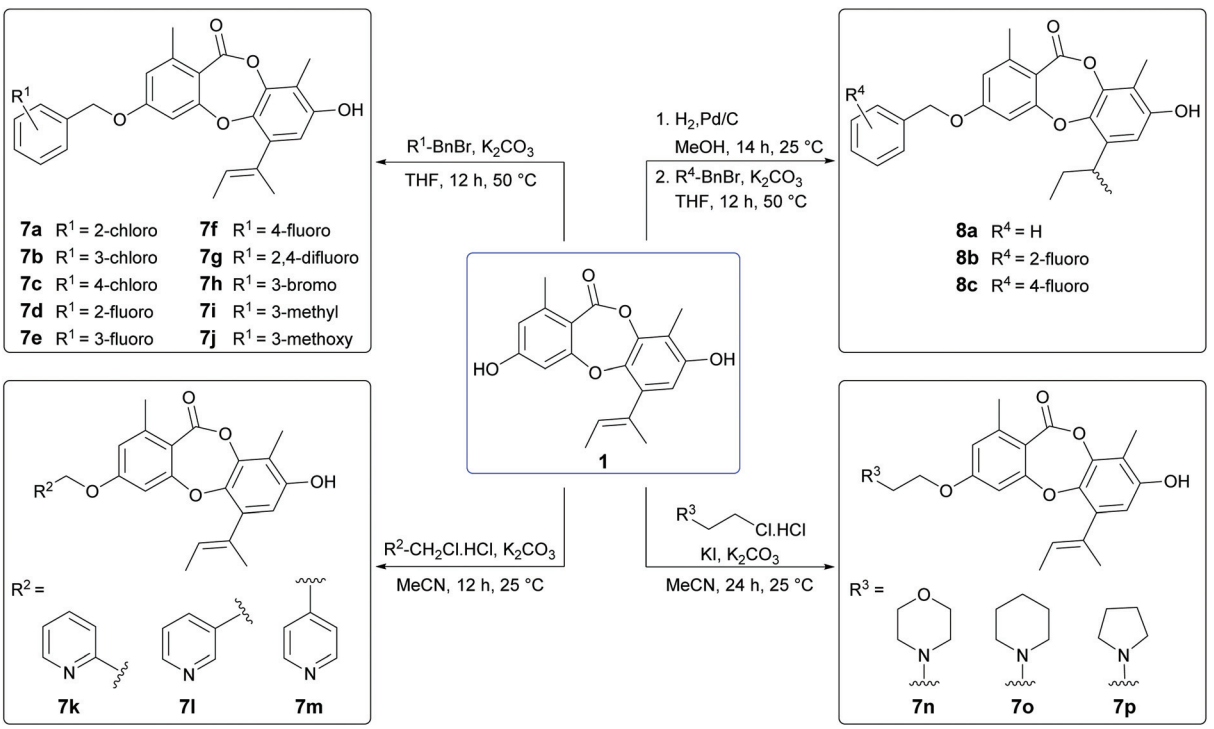
Second-generation semisynthetic unguinol analogues

From our preliminary SAR studies, it was evident that benzylation of the 3-OH group of **1** significantly improved antibacterial activity, particularly against *S. aureus*. Inspired by these initial results, we next explored benzylation of **1** with a range of *ortho*-, *meta*- and *para*-substituted benzyl bromides (Scheme 5). Reaction of **1** with five equivalents of each benzyl bromide in THF at 50 °C yielded ten second-generation 3-*O*-benzylated derivatives, **7a–7j** as the major products. Three picolyl derivatives (**7k–7m**) were also synthesised by reaction of **1** with 2-, 3-, and 4-picolyl chloride in MeCN at 25 °C. Alkylation of the 3-OH group of **1** by reaction with 4-(2-chloroethyl)morpholine, 1-(2-chloroethyl)piperidine and 1-(2-chloroethyl)pyrrolidine in MeCN at 25 °C yielded **7n–7p**. Finally, benzylation of the 3-OH of **2a** was performed using three different benzyl bromides, yielding **8a–8c**.

Bioassay of second-generation semisynthetic unguinol analogues

The nineteen second-generation unguinol congeners were initially tested for *in vitro* against the same panel of bacteria, fungi and mammalian cells as the first-generation analogues (Table 2). No activity was observed for any of the second-generation compounds against the tested Gram-negative bacterial or fungal species. The ten 3-*O*-benzylated unguinol analogues **7a–7j** exhibited significant antibacterial activity against Gram-positive bacteria, with MICs ranging from <0.1–6.3 μg mL^{-1}, and showed modest cytotoxicity against mouse NS-1 myeloma cells, with MICs ranging from 6.3–12.5 μg mL^{-1}. Significantly, all ten benzylated analogues showed potent activity against *S. aureus*, with MICs ranging from <0.1–0.8 μg mL^{-1}. The fluorobenzyl analogues **7d** and **7f** were the most promising leads, with activities superior to the antibiotic standards gentamicin (MIC 0.4 μg mL^{-1}) and ampicillin (MIC 3.1 μg mL^{-1}), and a selectivity index of 125. The 2-, 3- and 4-picolyl derivatives of **1** (**7k–7m**, respectively) showed 8- to 16-fold less activity against *S. aureus* compared to **6a**, with only **7k** retaining any activity against *B. subtilis* (MIC 6.3 μg mL^{-1}). The 3-*O*-alkylamino derivatives **7n–7p** also exhibited significantly reduced antibacterial activity compared to **6a**. Intriguingly, this was accompanied by a dramatic increase in cytotoxicity against mouse myeloma NS-1 cells (MIC 0.2 μg mL^{-1}), comparable in potency to the standard compound 5-fluorouracil (MIC 0.1 μg mL^{-1}). The appearance of this potent and selective mammalian cytotoxicity is indicative of a mode of action distinct from the antibacterial activity of the unguinol scaffold. It has been recently reported that 8-*O*-alkylation of nornidulin results in moderate cytotoxicity against African green monkey kidney (Vero) cells.[29] Similarly, the 1′,2′-dihydro analogues of **6a**, **7d** and **7f** (**8a–8c**, respectively) showed slightly decreased antibacterial activities accompanied by markedly increased cytotoxicity against NS-1 cells (MIC 0.8 μg mL^{-1}).

We next explored the activity of the nineteen second-generation unguinol analogues against MRSA (ATCC 33592). Encouragingly, all ten 3-*O*-benzylated unguinol analogues **7a–7j** retained equal potency against MRSA (MICs 0.1–0.8 μg



Scheme 5 Synthesis of second-generation unguinol analogues.

Table 2 *In vitro* biological activities of second-generation semisynthetic unguinol analogues

Compound	MIC (μg mL^{-1})			
	B. subtilis (ATCC 6633)	*S. aureus* (ATCC 25923)	MRSA (ATCC 33592)	NS-1 (ATCC TIB-18)
3-*O*-Benzylunguinol (**6a**)	1.6	0.2	0.4	3.1
3-*O*-(2-Chlorobenzyl)unguinol (**7a**)	3.1	0.2	0.2	12.5
3-*O*-(3-Chlorobenzyl)unguinol (**7b**)	3.1	0.8	0.4	6.3
3-*O*-(4-Chlorobenzyl)unguinol (**7c**)	1.6	0.2	0.2	12.5
3-*O*-(2-Fluorobenzyl)unguinol (**7d**)	0.8	0.1	0.1	12.5
3-*O*-(3-Fluorobenzyl)unguinol (**7e**)	1.6	0.4	0.4	6.3
3-*O*-(4-Fluorobenzyl)unguinol (**7f**)	1.6	<0.1	0.1	12.5
3-*O*-(2,4-Difluorobenzyl)unguinol (**7g**)	0.8	0.2	0.2	12.5
3-*O*-(3-Bromobenzyl)unguinol (**7h**)	6.3	1.6	1.6	6.3
3-*O*-(3-Methylbenzyl)unguinol (**7i**)	3.1	0.8	0.8	6.3
3-*O*-(3-Methoxybenzyl)unguinol (**7j**)	6.3	1.6	1.6	6.3
3-*O*-(2-Picolyl)unguinol (**7k**)	6.3	1.6	1.6	12.5
3-*O*-(3-Picolyl)unguinol (**7l**)	—	3.1	6.3	25
3-*O*-(4-Picolyl)unguinol (**7m**)	—	1.6	3.1	12.5
3-*O*-(4-Morpholinoethyl)unguinol (**7n**)	6.3	12.5	6.3	0.2
3-*O*-(1-Piperidinylethyl)unguinol (**7o**)	6.3	25	6.3	0.2
3-*O*-(1-Pyrrolidinylethyl)unguinol (**7p**)	12.5	100	50	0.2
3-*O*-Benzyl-1′,2′-dihydrounguinol (**8a**)	3.1	3.1	1.6	0.8
3-*O*-(2-Fluorobenzyl)-1′,2′-dihydrounguinol (**8b**)	3.1	3.1	1.6	0.8
3-*O*-(4-Fluorobenzyl)-1′,2′-dihydrounguinol (**8c**)	3.1	3.1	1.6	0.8
Ampicillin	0.2	3.1	—	[a]
Gentamicin	[a]	0.4	25	[a]
5-Fluorouracil	[a]	[a]	[a]	0.1

[a] Not tested; – no activity up to 100 μg mL^{-1}.

mL^{-1}) and were significantly more active than the standard gentamicin (MIC 25 μg mL^{-1}). The picolyl derivatives **7k–7m** also showed similar potencies against MRSA, while the alkylamino derivatives **7n–7p** and the hydrogenated benzyl derivatives **8a–8c** showed slightly improved potencies against MRSA. A subset of the 3-*O*-benzylated unguinol analogues (**6a**, **7a**, **7c**, **7d** and **7g**) was screened against one additional strain of MRSA (USA300), one additional strain of methicillin-sensitive *S. aureus* (MSSA; ATCC 49775) and two strains of *Enterococcus faecium* (ATCC 19434, E734) (Table 3), as well as two strains of *Pseudomonas aeruginosa* (PA01, ATCC 27853) and two additional strains of *E. coli* (ATCC 25322, ATCC 35218). No Gram-negative activity, or activity against either *E. faecium*, was detected for any of the compounds up to 16 μg mL^{-1}. The analogues all showed good activities against both MRSA and MSSA (MICs 0.5–2 μg mL^{-1}), comparable to the control compound daptomycin (MIC 0.5 μg mL^{-1}). It is noteworthy that the MICs for the 3-*O*-benzylated unguinol analogues increased 32-fold in the presence of 10% foetal calf serum.

Given these findings, further in-depth evaluation of the antibacterial activities of **7d** and **7g** was carried out with an expanded list of *S. aureus* isolates and strains to obtain a clearer picture of the potency and selectivity of these analogues. The results show potent activity for both compounds, returning MIC range, MIC_{50} and MIC_{90} values comparable to the daptomycin standard (Table 4). The potency of **7d** and **7g** was further investigated in a kinetic assay to measure the time- and concentration-dependent activity of the two compounds against two *S. aureus* ATCC strains using daptomycin as a comparator. The results show a time- and concentration-dependent inhibition of growth for **7d** and **7g**, consistent with features of bacteriostatic drugs. As expected, daptomycin displayed patterns of a bactericidal drug (Fig. 2).

A preliminary investigation into the suitability of **7d** and/or **7g** for administration as a drug was conducted by assessing

Table 3 *In vitro* antibacterial activity of benzylunguinol derivatives against MSSA, MRSA and *E. faecium*

Compound	MIC (μg mL^{-1})			
	MRSA[a]	MSSA[b]	*E. faecium*[c]	*E. faecium*[d]
1	8	8	>16	>16
6a	1	1	>16	>16
7a	1	2	>16	>16
7c	0.5	1	>16	>16
7d	0.5	1	>16	>16
7g	0.5	0.5	>16	>16
Daptomycin	0.5	0.5	>2	>2

[a] USA300. [b] ATCC 49775. [c] ATCC 19434. [d] E734.

Table 4 MIC range, MIC_{50} and MIC_{90} against *S. aureus* (20 MRSA and 3 MSSA)

Compound	MIC (μg mL^{-1})		
	MIC range	MIC_{50}	MIC_{90}
7d	0.25–1	0.5	0.5
7g	0.25–1	0.5	0.5
Daptomycin	0.25–1	0.5	0.5
Ampicillin	0.125–>16	>16	>16

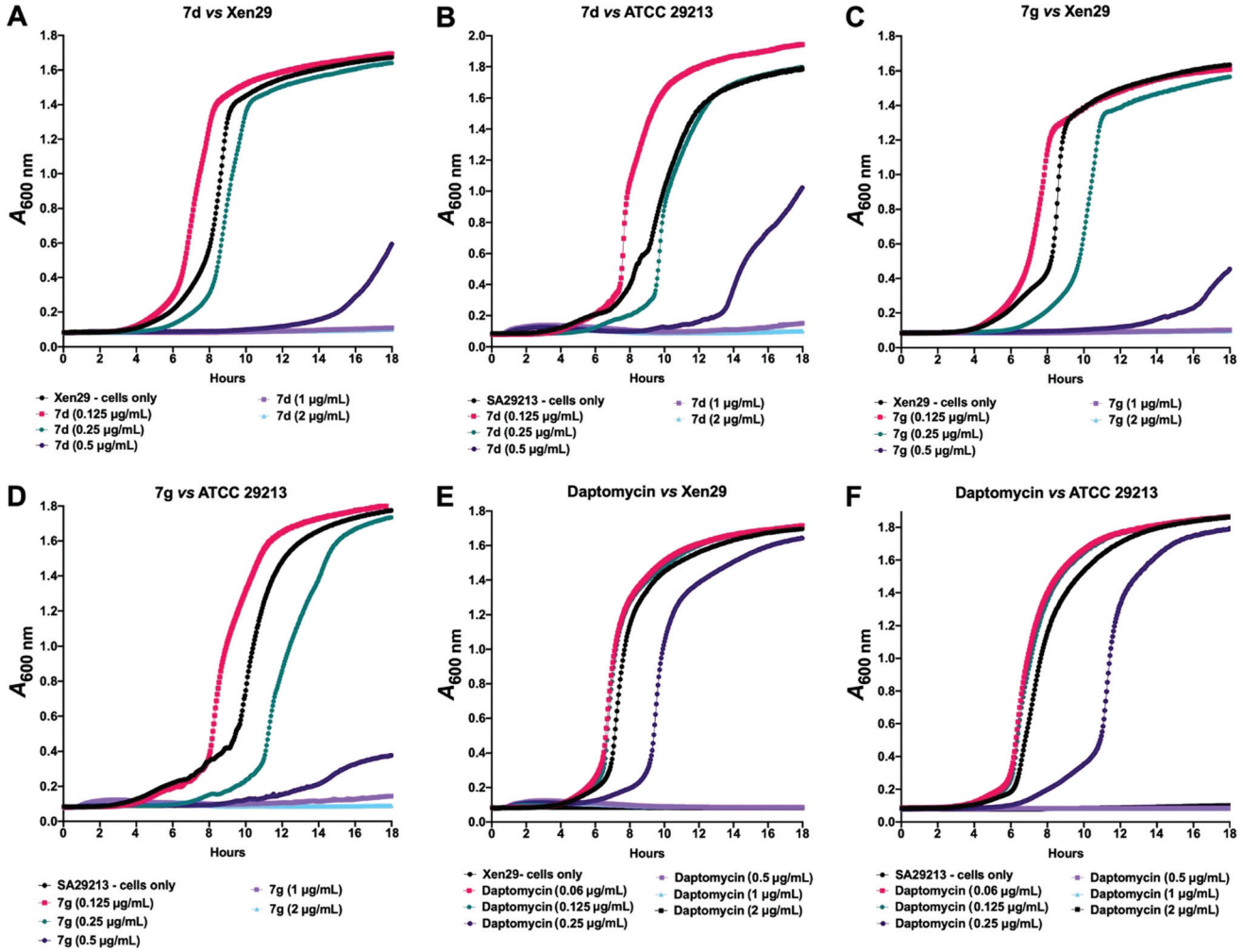


Fig. 2 Kinetic assay showing time- and concentration-dependent inhibition of *S. aureus* ATCC 12600 (Xen29; A, C and E) and ATCC 29213 (B, D and F) by **7d** and **7g**, with daptomycin as a comparator. Results show **7d** and **7g** are bacteriostatic, while daptomycin is bactericidal.

their cytotoxicity to mammalian cells in fresh human red blood cells (RBCs), human embryonic kidney (HEK293) cell line and human epithelial liver (Hep G2) cell line. At the highest concentration (128 μg mL^{-1}), **7d** and **7g** did not result in haemolysis of RBCs and both returned IC_{50} values of 32 μg mL^{-1} against the HEK293 and Hep G2 cell lines. These desirable cytotoxicity profiles promote exploration of **7d** and **7g** for *in vivo* safety and subsequent efficacy testing in relevant animal models.

Conclusions

In this study, we have completed the semisynthesis and *in vitro* biological evaluation of thirty-four derivatives of the fungal depsidone antibiotic, unguinol. Our SAR studies against a panel of microorganisms and mammalian cells revealed that at least one free hydroxy group is essential for antibacterial activity. We have demonstrated that by modification of the butenyl side chain of unguinol, we can target more potent and selective antitumor activity, while the introduction of bulkier halogens abolishes selectivity. Most notably, we have demonstrated that introduction of a 3-*O*-benzyl group exploits a putative additional hydrophobic pocket present in Gram-positive bacteria, affording a more potent and selective antibiotic class. The binding pocket appears to be optimal for fluorine-substituted benzyl analogues. Two of these analogues, 3-*O*-(2-fluorobenzyl)unguinol and 3-*O*-(2,4-difluorobenzyl)unguinol, demonstrated potent activity against MSSA and MRSA at concentrations comparable to those of a leading clinically effective Gram-positive antibiotic, daptomycin. Our assays also show these two compounds appear to be bacteriostatic and exhibit desirable mammalian cytotoxicity profiles, supporting their consideration for *in vivo* safety and drug efficacy testing in animal models of disease. While the mode of action of unguinol and its analogues remains unknown, our SAR results suggest this family of depsidones may act by binding to a

target shared by prokaryotes, lower eukaryotes and higher eukaryotes. It is noteworthy that many lichen symbionts use variants of the depsidone scaffold to similar effect.[30–32] This broad chemotherapeutic specificity represents an efficient use of resources, enabling the fungus to ward off a wide taxonomic framework of competitors. In Nature, potency and selectivity are implicit to each metabolite, which is an inverse template of its site of action. Employing a cohort of bioassays to help illuminate previously unrecognised aspects of potency and selectivity is an effective strategy for reviving existing chemical classes as potential new drugs.

Experimental

General experimental details

UV–vis spectra were acquired in MeOH on a Varian Cary 4000 spectrophotometer or a Jasco V-760 spectrophotometer in a 10 × 10 mm quartz cuvette. IR spectra were recorded on a Jasco FT/IR-6000 FTIR (ATR) spectrometer. Photooxidation reactions were performed using a Philips TUV PL-S 11 W/2P UVC light. ^{1}H NMR and ^{13}C NMR spectra were recorded in 5 mm Pyrex tubes (Wilmad, USA) on either a Bruker Avance II DRX-600K 600 MHz or Bruker Avance III HD 500 MHz spectrometer. All NMR spectra were obtained at 25 °C, processed using Bruker Topspin 3.5 software and referenced to residual solvent signals (DMSO-d_6 δ_H 2.49/δ_C 39.5 ppm). High resolution electrospray ionisation mass spectra (HRESIMS) were obtained on a Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) by direct infusion. Electrospray ionisation mass spectra (ESIMS) were acquired on an Agilent 1260 UHPLC coupled to an Agilent 6130B single quadrupole mass detector. Analytical HPLC was performed on a gradient Agilent 1260 Infinity quaternary HPLC system equipped with a G4212B diode array detector. The column was an Agilent Poroshell 120 EC-C_{18} (4.6 × 50 mm, 2.7 μm) eluted with a 1 mL min^{-1} gradient of 10–100% MeCN/water (0.01% TFA) over 8.33 min. Semipreparative HPLC was performed on a gradient Agilent 1260 Infinity quaternary HPLC system coupled to a G4212B diode array detector. The column used in the purification of the compounds was an Agilent Zorbax SB-C_{18} (9.4 × 250 mm, 5 μm) eluted isocratically at 4.18 mL min^{-1}. Preparative HPLC was performed on a gradient Shimadzu HPLC system comprising two LC-8A preparative liquid pumps with static mixer, SPD-M10AVP diode array detector and SCL-10AVP system controller with standard Rheodyne injection port. The columns used were a Grace Discovery Hypersil C_{18} spring column (150 × 50 nm, 5 μm) eluted isocratically at 60 mL min^{-1}, an Agilent Zorbax SB-C_{18} column (150 × 50 nm, 5 μm) eluted isocratically at 60 mL min^{-1} and an Agilent Zorbax SB-C_{18} column (2 × 250 mm, 5 μm) eluted isocratically at 20 mL min^{-1}. Silica flash chromatography was performed on a Biotage Isolera Four system coupled with a variable UV (200–400 nm) detector.

Isolation and purification of unguinol

A. unguis MST-FP511 was grown on pearl barley, which had been boiled in distilled water for 12 min and sterilised (120 °C for 40 min), in 60 × 250 mL Erlenmeyer flasks, with each flask containing 50 g of barley. Agar squares from a 7-day-old Petri plate of *A. unguis* were used as the inoculum for the flasks. The cultures were incubated for 21 days at 24 °C, then the grains were pooled and extracted with acetone (2 × 4 L) and the combined extracts were evaporated under vacuum to produce an aqueous slurry (2 L). The slurry was partitioned against ethyl acetate (2 × 2 L) and the ethyl acetate was reduced *in vacuo* to give the crude extract (55.6 g). The crude extract was redissolved in 90% MeOH/H_2O (500 mL) and partitioned against hexane (2 × 500 mL) to remove lipids, yielding an enriched extract (35.7 g). The enriched extract was adsorbed onto silica gel (40 g), which was then loaded onto a silica gel column (100 g, 300 × 50 mm). The column was washed once with hexane (500 mL), then eluted with 50% hexane/$CHCl_3$ (500 mL), 25% hexane/$CHCl_3$ and $CHCl_3$ (500 mL), followed by a stepwise gradient of 1, 2, 4, 8, 16, 32 and 100% MeOH/$CHCl_3$ (500 mL each step), to yield 11 fractions (Fr. 1–11). Fraction 6 (2.1 g) was purified by isocratic preparative HPLC (Hypersil C_{18}, isocratic 60% MeCN/H_2O containing 0.01% TFA, 60 mL min^{-1}) to yield **1** (t_R 14.62 min; 384 mg).

Semisynthesis of unguinol analogues

1′,2′-Dihydrounguinol (2a). Unguinol (**1**; 50 mg, 0.15 mmol) was dissolved in methanol (5 mL) and 10% palladium on carbon catalyst (3.2 mg) was added. The reaction mixture was stirred overnight under an atmosphere of H_2 (balloon) at 25 °C. The reaction mixture was filtered through Celite and the solvent was removed *in vacuo* to yield 1′,2′-dihydrounguinol (**2a**; 50 mg, quant.) as a colourless solid, which was used without further purification. UV (MeOH) λ_{max} (log ε) 203 (5.08), 223 (4.78), 265 (4.43) nm; IR (ATR) ν_{max} 3310, 2963, 1698, 1608, 1576, 1427, 1336, 1256, 1210, 1151, 1108, 1086 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.61 (s, 1H), 9.49 (s, 1H), 6.57 (dd, J = 2.4, 0.8 Hz, 1H), 6.54 (d, J = 2.4 Hz, 1H), 6.49, (s, 1H), 3.25 (m, 1H), 2.33 (s, 3H), 2.03 (s, 3H), 1.51 (m, 2H), 1.11 (d, J = 7.0 Hz, 3H), 0.79 (t, J = 7.3 Hz, 3H), ^{13}C NMR (150 MHz, DMSO-d_6): δ 163.1, 162.5, 161.8, 152.9, 144.7, 142.8, 141.0, 136.1, 115.6, 113.6, 111.6, 108.0, 104.4, 32.4, 29.8, 21.4, 20.7, 12.1, 9.1. HRESI(+)MS m/z 329.1381 $[M + H]^+$ (calculated for $C_{19}H_{21}O_5^+$ 329.1384).

***cis*-1′,2′-Epoxyunguinol (2b).** To a stirred mixture of unguinol (**1**; 15 mg, 46 μmol), $NaHCO_3$ (23 mg, 0.27 mmol) and acetone (5 mL) at 0 °C, a solution of Oxone (84 mg, 0.27 mmol) in water (3 mL) was added dropwise. The resulting mixture was stirred at 25 °C for 18 h and then extracted with ethyl acetate (3 × 3 mL). The organic layer was dried over $MgSO_4$ and the solvent was evaporated under nitrogen. The residue (13.8 mg) was purified by semipreparative C_{18} HPLC with isocratic 80% MeCN/H_2O (4.18 mL min^{-1}), yielding a racemic mixture of *cis*-1′,2′-epoxyunguinol (**2b**; t_R = 17.5 min; 8.0 mg, 53%) as white amorphous solid. UV (MeOH) λ_{max} (log ε) 202 (4.92), 221

(4.57), 267 (4.23) nm; IR (ATR) ν_{max} 3461, 2989, 1699, 1619, 1579, 1424, 1384, 1381, 1254, 1217, 1188, 1160, 1104 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 10.76 (br s, 1H), 9.72 (br s, 1H), 6.58 (s, 1H), 6.57 (d, J = 2.4 Hz, 1H), 6.46 (d, J = 2.4 Hz, 1H), 2.85 (q, J = 5.4 Hz, 1H), 2.33 (s, 3H), 2.03 (s, 3H), 1.56 (s, 3H), 1.47 (d, J = 5.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.6, 162.2, 162.1, 152.8, 144.9, 142.9, 140.2, 132.7, 115.9, 115.4, 111.1, 108.3, 104.4, 59.4, 59.0, 20.7, 18.8, 13.8, 9.2. HRESI(−)MS m/z 341.1032 $[M - H]^-$ (calculated for $C_{19}H_{17}O_6^-$, 341.1031).

1′,2′-Dihydroxyunguinol (2c). To a stirred solution of unguinol (**1**; 15 mg, 46 μmol) in acetone (5 mL) was added $NaHCO_3$ (38 mg) and a solution of Oxone (141 mg, 0.46 mmol) in water (2 mL). The reaction mixture was stirred at 50 °C for 18 h and then extracted with ethyl acetate (3 × 3 mL). The organic layer was dried over anhydrous $MgSO_4$, and reduced to dryness under nitrogen. The residue (12.5 mg) was purified by semipreparative HPLC with isocratic 80% MeCN/H_2O plus 0.01% TFA (4.18 mL min^{-1}), yielding 1′,2′-dihydroxyunguinol (**2c**; t_R = 15.8 min; 2.2 mg, 14.7%) as a colourless solid. UV (MeOH) λ_{max} (log ε) 203 (4.91), 222 (4.59), 268 (4.28) nm; IR (ATR) ν_{max} 3674, 2985, 2901, 1393, 1251, 1066 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6): δ 10.56 (s, 1H), 9.44 (s, 1H), 6.88 (s, 1H), 6.73 (d, J = 2.4 Hz, 1H), 6.56 (d, J = 2.4 Hz, 1H), 4.23 (p, J = 6.4 Hz, 1H), 2.36 (s, 3H), 2.05 (s, 3H), 1.43 (s, 3H), 1.02 (d, J = 6.4 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 163.3, 162.5, 161.5, 151.7, 145.0, 143.4, 141.1, 136.7, 115.5, 114.6, 111.3, 110.6, 105.7, 75.8, 70.9, 24.5, 21.1, 17.7, 9.3. HRESI(−)MS m/z 359.1135 $[M - H]^-$ (calculated for $C_{19}H_{19}O_7^-$, 359.1136).

2′-Hydroxy-$\Delta^{1',4'}$-unguinol (2d). Unguinol (**1**; 40 mg, 123 μmol) was dissolved in 95% aqueous MeCN (10 mL) and Rose Bengal (4 mg, 4 μmol) was added. The reaction mixture was irradiated with UV light (254 nm) for 4 h then reduced to dryness under nitrogen. The residue was redissolved in MeOH (1 mL) and purified by preparative HPLC running with isocratic 60% MeCN/H_2O, 20 mL min^{-1} yielding 2′-hydroxy-$\Delta^{1',4'}$-unguinol (**2d**; t_R = 5.75 min, 3.5 mg, 8.75%) as a colourless solid. UV (MeOH) λ_{max} (log ε) 206 (5.11), 243 (4.62) nm; IR (ATR) ν_{max} 2976, 1724, 1607, 1576, 1422, 1332, 1247, 1213, 1153, 1104 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 10.55 (s, 1H), 9.63 (s, 1H), 6.54 (d, J = 2.4 Hz, 1H), 6.49 (s, 1H), 6.44 (d, J = 2.4 Hz, 1H), 5.52 (s, 1H), 5.12 (d, J = 4.7 Hz, 1H), 4.95 (s, 1H), 4.59 (p, J = 6.5 Hz, 1H), 2.32 (s, 3H), 2.05 (s, 3H), 1.05 (d, J = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 163.0, 162.3, 161.7, 152.4, 150.3, 144.4, 143.2, 140.2, 131.1, 115.6, 115.3, 113.2, 111.4, 111.3, 104.9, 67.9, 22.7, 20.6, 9.23. HRESI(−)MS m/z 341.1032 $[M - H]^-$ (calculated for $C_{19}H_{17}O_6^-$, 341.1031).

2′-Oxo-$\Delta^{1',4'}$-unguinol (2e). Unguinol (**1**; 60 mg, 184 μmol) was dissolved in MeOH (10 mL) and Rose Bengal (20 mg, 20 μmol) was added. The reaction mixture was transferred to glass reaction chamber and exposed to UV light (254 nm) for 4 h. The solution was then reduced to dryness under nitrogen and purified *via* preparative HPLC running with an isocratic gradient 60% MeCN/H_2O (60 mL min^{-1}) yielding 2′-oxo-$\Delta^{1',4'}$-unguinol (**2e**; t_R = 6.35 min, 1.8 mg, 3.0%) as a colourless solid. UV (MeOH) λ_{max} (log ε) 204 (4.71), 222 (4.39), 263 (4.08) nm; IR (ATR) ν_{max} 3398, 2920, 2851, 1698, 1673, 1618, 1575, 1427, 1352, 1328, 1256, 1197, 1157, 1105 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6): δ 6.51 (d, J = 2.1 Hz, 1H) 6.47 (s, 1H), 6.32 (s, 1H), 6.17 (d, J = 2.1 Hz, 1H), 5.85 (s, 1H), 2.40 (s, 3H), 2.30 (s, 3H), 2.08 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 198.3, 162.5, 162.1, 152.7, 145.5, 144.6, 142.9, 140.1, 128.0, 127.5, 116.4, 115.9, 111.7, 110.6, 104.3, 26.9, 20.7, 9.3. HRESI(−)MS m/z 339.0871 $[M - H]^-$ (calculated for $C_{19}H_{15}O_6^-$, 339.0874).

2′-Hydroperoxy-$\Delta^{1',4'}$-unguinol (2f). Unguinol (**1**; 65 mg, 199 μmol) was dissolved in MeCN (10 mL) and Rose Bengal (0.5 mg, 0.5 μmol) was added. The reaction mixture was transferred to glass UV reaction chamber and exposed to UV light (254 nm) for 1 h. The solution was reduced to dryness under nitrogen, redissolved in MeCN (1 mL) and purified by preparative HPLC running with an isocratic gradient running 50% MeCN/H_2O (20 mL min^{-1}), yielding 2′-hydroperoxy-$\Delta^{1',4'}$-unguinol (**2f**; t_R = 8.02 min, 9.4 mg, 14.5%) as a colourless solid. UV (MeOH) λ_{max} (log ε) 205 (4.95), 226 (4.66), 264 (4.32) nm; IR (ATR) ν_{max} 3166, 2984, 1726, 1608, 1576, 1424, 1331, 1284, 1214, 1153, 1105 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6): δ 11.66 (br s, 1H), 9.97 (br s, 1H), 6.54 (s, 1H), 6.51 (d, J = 2.3 Hz, 1H), 6.42 (d, J = 2.3 Hz, 1H), 5.53 (dq, J = 1.6, 1.2 Hz, 1H), 5.13 (d, J = 1.6 Hz, 1H), 4.81 (q, J = 6.5 Hz, 1H), 2.31 (s, 3H), 2.06 (s, 3H), 1.16 (d, J = 6.5 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 163.1, 162.6, 162.3, 152.4, 145.2, 144.4, 143.2, 140.3, 130.2, 116.5, 115.8, 115.6, 111.6, 110.7, 104.9, 81.9, 20.6, 17.9, 9.24. ESI; HRESI(−)MS m/z 357.0980 $[M - H]^-$ (calculated for $C_{19}H_{17}O_7^-$, 357.0980).

2,7-Dibromo-1′,2′-dihydrounguinol (3a) and 2,4,7-tribromo-1′,2′-dihydrounguinol (3b). 1′,2′-Dihydrounguinol (**2a**; 10 mg, 30 μmol) was dissolved in chloroform (3 mL) and a solution of bromine in chloroform (2.1 M; 33 μL, 70 μmol) was added. The reaction mixture was stirred at 25 °C for 6 h. The crude reaction mixture was washed with brine (3 × 3 mL) and the organic layer was dried over $MgSO_4$, filtered and reduced to dryness under nitrogen. The residue (9.1 mg) was purified by semipreparative HPLC with isocratic 60% MeCN/H_2O plus 0.01% TFA (4.18 mL min^{-1}). After separation, 2,7-dibromo-1′,2′-dihydrounguinol (**3a**; t_R = 22.8 min; 1.64 mg, 16.4%) and 2,4,7-tribromo-1′,2′-dihydrounguinol (**3b**; t_R = 23.5 min; 1.9 mg, 19%) were isolated. Compound **3a** was isolated as white solid; UV (MeOH) λ_{max} (log ε) 203 (4.74), 221 (4.58), 283 (4.05), 322 (4.10) nm; IR (ATR) ν_{max} 2965, 1732, 1596, 1567, 1418, 1338, 1226, 1179 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6): δ 11.63 (s, 1H), 9.23 (s, 1H), 6.86 (s, 1H), 3.74 (br s, 1H), 2.44 (s, 3H), 2.16 (s, 3H), 1.99 (m, 1H), 1.81 (m, 1H), 1.34 (d, J = 7.2 Hz, 3H), 0.82 (br s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 161.7, 161.3, 158.6, 149.9, 143.5, 142.3, 134.0, 116.7, 113.2, 111.3, 104.6, 34.1, 26.2, 21.6, 18.0, 12.7, 10.7. HRESI(−)MS m/z 482.9443 $[M - H]^-$ (calculated for $C_{19}H_{17}{}^{79}Br_2O_5^-$, 482.9448). Compound **3b** was isolated as white solid; UV (MeOH) λ_{max} (log ε) 205 (4.54), 223 (4.36), 251 (4.07), 322 (4.14) nm; IR (ATR) ν_{max} 2963, 1732, 1560, 1419, 1363, 1338, 1289, 1227 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6): δ 11.15 (s, 1H), 9.30 (s, 1H), 4.44 (br s, 1H), 2.41 (s, 3H), 2.19 (s, 3H), 2.00 (m, 1H), 1.76 (m, 1H),

1.30 (d, J = 7.2 Hz, 3H), 0.70 (t, J = 7.3 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 161.3, 158.7, 156.0, 150.4, 143.4, 142.2, 141.8, 134.2, 116.7, 114.1, 113.0, 108.4, 100.4, 33.6, 25.7, 22.3, 18.0, 12.3, 10.7. HRESI(−)MS m/z 560.8553 [M − H]$^-$ (calculated for $C_{19}H_{16}{}^{79}Br_3O_5^-$, 560.8553).

2,4-Diiodo-1′,2′-dihydrounguinol (3c). 1′,2′-Dihydrounguinol (**2a**; 10 mg, 30 μmol) was dissolved in acetic acid (3 mL) and NaI (130 mg, 88 μmol) was added. An aqueous solution of H_2O_2 (30%; 0.5 mL, 0.15 mmol) was added dropwise to the well-stirred solution, and the reaction mixture was stirred at 25 °C for 6 h. The mixture was treated with an aqueous sodium thiosulfate solution (2 × 3 mL) and extracted with ethyl acetate (3 × 3 mL). The organic layer was dried over anhydrous $MgSO_4$, and reduced to dryness under nitrogen. The residue was purified by silica flash chromatography using a SNAP-10 g cartridge. The whole reaction product was dissolved in 1 mL n-hexane/ethyl acetate (50 : 50) and separated with n-hexane and ethyl acetate gradient system (7% to 60%), 12 mL min^{-1}, yielding 2,4-diiodo-1′,2′-dihydrounguinol (**3c**; t_R = 12.5 min, 5.6 mg, 56%) as a colourless solid. UV (MeOH) λ_{max} (log ε) 205 (4.85), 231 (4.41), 281 (3.88) nm; IR (ATR) ν_{max} 2961, 1727, 1590, 1560, 1420, 1334, 1226, 1177 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.47, (br s, 1H), 9.61 (s, 1H), 6.52 (s, 1H), 3.98 (m, 1H), 2.45 (s, 3H), 2.06 (s, 3H), 1.49 (m, 2H), 1.05 (d, J = 6.9 Hz, 3H), 0.79 (t, J = 7.3 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 163.0, 162.1, 160.4, 153.4, 146.3, 142.5, 142.1, 136.5, 113.9, 108.3, 92.3, 76.9, 32.9, 29.8, 28.2, 21.6, 11.7, 9.1. HRESI(−)MS m/z 578.9172 [M − H]$^-$ (calculated for $C_{19}H_{17}I_2O_5^-$, 578.9171).

Methyl unguinolate (4a). Unguinol (**1**; 50 mg, 153.0 μmol) was dissolved in 2 M KOH in MeOH (10 mL) and transferred to clear glass scintillation vial and the reaction mixture was heated to 60 °C for 18 h. The reaction was quenched by diluting with H_2O (50 mL) and recovered with ethyl acetate (50 mL). The organic layer was dried over Na_2SO_4 and reduced to dryness *in vacuo* followed by purification with preparative HPLC (Alltima, 20 mL min^{-1}, 0.01% TFA) with an isocratic gradient running 50% MeCN/H_2O, yielding methyl unguinolate (**4a**; t_R = 23.89 min, 8.7 mg, 11.9%) as white residue. UV (MeOH) λ_{max} (log ε) 215 (4.48), 248 (4.00), 285 (3.37) nm; IR (ATR) ν_{max} 3231, 2918, 1695, 1605, 1429, 1327, 1265, 1208, 1152 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.56 (s, 1H), 9.09 (s, 1H), 8.36 (s, 1H), 6.19 (s, 1H), 6.19 (d, J = 2.2 Hz, 1H), 5.73 (d, J = 2.2 Hz, 1H), 5.41 (qq, J = 6.8, 1.4 Hz, 1H), 3.77 (s, 3H), 2.17 (s, 3H), 1.96 (s, 3H), 1.75 (dq, J = 1.4, 1.1 Hz, 3H), 1.55 (dq, J = 6.8, 1.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 167.9, 159.0, 157.7, 152.6, 148.0, 137.9, 136.0, 133.2, 131.8, 123.5, 113.4, 110.7, 110.0, 105.8, 98.8, 51.6, 19.7, 16.6, 13.7, 9.0. HRESI(−)MS m/z 357.1340 [M − H]$^-$ (calculated for $C_{20}H_{21}O_6^-$, 357.1343).

Unguinolamide (4b). Unguinol (**1**; 60 mg, μmol) was dissolved in MeCN (10 mL) and transferred to clear glass scintillation vial. Concentrated aqueous ammonia (25%; 8 mL) was added and reaction mixture was heated to 100 °C for 18 h. The reaction mixture was neutralised with HCl, diluted with H_2O (50 mL) and extracted with ethyl acetate (50 mL). The organic layer was dried over Na_2SO_4 and reduced to dryness *in vacuo*. The residue purified *via* preparative HPLC (Alltima, 20 mL min^{-1}, 0.01% TFA) with an isocratic gradient running 60% MeCN/H_2O, yielding unguinolamide (**4b**; t_R = 11.99 min, 7.1 mg, 12.1%) as white residue. UV (MeOH) λ_{max} (log ε) 208 (4.62), 244 (4.09), 283 (3.49) nm; IR (ATR) ν_{max} 3172, 1648, 1588, 1425, 1378, 1320, 1267, 1209, 1157, 1104 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.43 (s, 1H), 9.38 (s, 1H), 9.03 (s, 1H), 7.94 (s, 1H), 7.71 (s, 1H), 6.22 (d, J = 2.2 Hz, 1H) 6.17 (s, 1H), 5.82 (d, J = 2.2 Hz, 1H), 5.54 (qq, J = 6.8, 1.5 Hz, 1H), 2.22 (s, 3H), 1.91 (s, 3H), 1.79 (dq, J = 1.5, 1.1 Hz, 3H), 1.59, (dq, J = 6.8, 1.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 170.2, 158.0, 156.5, 152.6, 148.4, 136.7, 135.8, 133.5, 132.5, 123.7, 118.5, 110.6, 110.2, 105.0, 100.0, 19.6, 16.6, 13.8, 8.8. HRESI(−)MS m/z 342.1341 [M − H]$^-$ (calculated for $C_{19}H_{20}NO_5^-$, 342.1347).

3-*O*-Methylunguinol (5a) and 3,8-di-*O*-methylunguinol (5b). Unguinol (**1**; 15 mg, 46 μmol) was dissolved in acetone (5 mL) and iodomethane (6 μL, 0.09 mmol) and excess K_2CO_3 (5 mg) were added. The reaction mixture was stirred at 25 °C for 12 hours then filtered. The filtrate was reduced to dryness under nitrogen. The residue was purified by silica chromatography using a SNAP-10 g cartridge. The whole reaction product was dissolved in 1 mL n-hexane/ethyl acetate (50 : 50). The mixture was separated with n-hexane and ethyl acetate gradient system (2% to 20%), 12 mL min^{-1}, yielding 3-*O*-methylunguinol (**5a**; 3.6 mg, t_R = 23.5 min, 24%) and 3,8-di-*O*-methylunguinol (**5b**; 3.0 mg, t_R = 17.8 min, 20%) as solid white powder. **3-*O*-Methylunguinol (5a)**: UV (MeOH) λ_{max} (log ε) 205 (4.81), 225 (4.55), 263 (4.20) nm; IR (ATR) ν_{max} 3385, 2927, 2353, 1710, 1602, 1424, 1333, 1253, 1205, 1144, 1101, 102.3 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.62 (s, 1H), 6.77 (d, J = 2.5 Hz, 1H), 6.42 (s, 1H), 6.39 (d, J = 2.5 Hz, 1H), 5.45 (qq, J = 6.8, 1.5 Hz, 1H) 3.76 (s, 3H), 2.38 (s, 3H), 2.04 (s, 3H), 2.00 (dq, J = 1.5, 1.2 Hz, 3H), 1.78 (dq, J = 6.8, 1.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.8, 162.6, 162.2, 152.6, 144.4, 143.0, 140.1, 135.3, 132.4, 125.0, 114.6, 114.2, 113.2, 110.7, 103.0, 55.6, 20.5, 17.6, 13.6, 9.1. HRESI(−)MS m/z 339.1237 [M − H]$^-$ (calculated for $C_{20}H_{19}O_5^-$, 339.1238). **3,8-Di-*O*-methylunguinol (5b)**: UV (MeOH) λ_{max} (log ε) 205 (4.81), 225 (4.55), 263 (4.20) nm; IR (ATR) ν_{max} 2934, 1731, 1607, 1573, 1484, 1445, 1415, 1378, 1324, 1296, 1235, 1216, 1198, 1147, 1126 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 6.78 (d, J = 2.5 Hz, 1H), 6.57 (s, 1H), 6.42 (d, J = 2.5 Hz, 1H), 5.53 (qq, J = 6.8, 1.5 Hz, 1H) 3.77 (s, 3H), 3.76 (s, 3H), 2.38 (s, 3H), 2.07 (s, 3H), 2.04 (dq, J = 1.5, 1.2 Hz, 3H), 1.80 (dq, J = 6.8, 1.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.7, 162.6, 162.0, 154.2, 144.6, 142.8, 141.2, 135.5, 132.4, 125.6, 116.3, 114.3, 113.0, 107.2, 103.0, 56.0, 55.7, 20.5, 17.7, 13.7, 9.0. HRESI(+)MS m/z 355.1535 [M + H]$^+$ (calculated for $C_{21}H_{23}O_5^+$, 355.1540).

General procedure for benzylation of unguinol. A mixture of unguinol (**1**; 15 mg, 46 μmol), benzyl bromide (0.43 mmol) and excess K_2CO_3 (5 mg) in tetrahydrofuran (5 mL) was stirred at 50 °C for 12 h. The crude reaction mixture was filtered and the filtrate was reduced to dryness under nitrogen. The crude reaction mixture was purified by silica chromatography using a

SNAP-10 g cartridge with a *n*-hexane/ethyl acetate gradient system (1% to 20%), 12 mL min^{-1}, yielding a mixture of 3-*O*-benzylated unguinol and 3,8-di-*O*-benzylated unguinol.

3-*O*-Benzylunguinol (6a). Isolated as white powder, t_R = 22.3 min, 6.0 mg, 40%. UV (MeOH) λ_{max} (log ε) 206 (5.08), 262 (4.50) nm; IR (ATR) ν_{max} 2923, 2360, 1728, 1605, 1569, 1420, 1378, 1323, 1291, 1252, 1215, 1176, 1145, 1103 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.62 (br s, 1H), 7.39, (m, 2H), 7.38 (m, 2H), 7.32 (m, 1H), 6.87 (d, J = 2.6 Hz, 1H), 6.46 (d, J = 2.6 Hz, 1H) 6.41 (s, 1H), 5.45 (qq, J = 6.7, 1.5 Hz, 1H), 5.14, (s, 2H), 2.32 (s, 3H), 2.04 (s, 3H), 1.93 (dq, J = 1.5, 1.1 Hz, 3H), 1.75, (dq, J = 6.7, 1.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.8, 162.2, 161.7, 152.6, 144.4, 143.0, 140.1, 136.0, 135.2, 132.2, 128.5, 128.0, 127.5, 125.1, 115.2, 114.6, 113.4, 110.7, 103.7, 69.6, 20.5, 17.5, 13.7, 9.1. HRESI(+)MS *m*/*z* 417.1689 $[M + H]^+$ (calculated for $C_{26}H_{25}O_5^+$, 417.1696).

3,8-Di-*O*-benzylunguinol (6b). Isolated as white powder, t_R = 12.8 min, 4.0 mg, 27%. UV (MeOH) λ_{max} (log ε) 206 (4.94), 260 (4.34) nm; IR (ATR) ν_{max} 3673, 2924, 2360, 2339, 1733, 1650, 1607, 1573, 1484, 1452, 1417, 1377, 1324, 1252, 1218, 1146, 1120 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.39 (m, 2H), 7.38 (m, 4H), 7.33 (m, 1H), 7.41 (m, 2H), 7.31 (m, 1H), 6.88, (d, J = 2.4 Hz, 1H), 6.68 (s, 1H), 6.48, (d, J = 2.4 Hz, 1H), 5.51 (qq, J = 6.8, 1.4 Hz, 1H), 5.15 (s, 2H), 5.08, (s, 2H), 2.38 (s, 3H), 2.13 (s, 3H), 1.96 (dq, J = 1.4, 1.1 Hz, 3H), 1.77, (dq, J = 6.8, 1.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.6, 162.0, 161.8, 153.3, 144.6, 142.9, 141.4, 137.0, 136.1, 135.3, 132.1, 128.5, 128.4, 128.1, 127.8, 127.5, 127.3, 125.7, 116.9, 115.4, 113.3, 108.8, 103.9, 69.9, 69.6, 20.6, 17.6, 13.8, 9.2. HRESI(+)MS *m*/*z* 507.2162 $[M + H]^+$ (calculated for $C_{33}H_{31}O_5^+$, 507.2166).

3-*O*-(2-Chlorobenzyl)unguinol (7a). Isolated as white powder, t_R = 21.5 min, 7.8 mg, 52%. UV (MeOH) λ_{max} (log ε) 204 (4.82), 258 (4.25) nm; IR (ATR) ν_{max} 3673, 3364, 2972, 2360, 2339, 1701, 1606, 1570, 1420, 1380, 1326, 1253, 1216, 1147, 1101 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.62 (br s, 1H), 7.50, (m, 2H), 7.39 (m, 1H), 7.36 (m, 1H), 6.90 (d, J = 2.6 Hz, 1H), 6.45 (d, J = 2.6 Hz, 1H) 6.41 (s, 1H), 5.45 (qq, J = 6.8, 1.4 Hz, 1H), 5.19, (s, 2H), 2.39 (s, 3H), 2.04 (s, 3H), 1.93 (dq, J = 1.4, 1.2 Hz, 3H), 1.73, (dq, J = 6.8, 1.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.8, 162.2, 161.4, 152.6, 144.5, 143.0, 140.1, 135.2, 133.3, 132.4, 132.1, 130.0, 129.8, 129.4, 127.4, 125.1, 115.2, 114.7, 113.7, 110.7, 103.4, 69.1, 20.5, 17.5, 13.6, 9.1. HRESI(+)MS *m*/*z* 451.1306 $[M + H]^+$ (calculated for $C_{26}H_{24}{}^{35}ClO_5^+$, 451.1306).

3-*O*-(3-Chlorobenzyl)unguinol (7b). Isolated as white powder, t_R = 21.7 min, 7.7 mg, 51.3%. UV (MeOH) λ_{max} (log ε) 203 (4.62), 263 (4.01) nm; IR (ATR) ν_{max} 3356, 2926, 2360, 1696, 1610, 1567, 1478, 1417, 1378, 1366, 1323, 1293, 1265, 1215, 1197, 1146 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.63 (br s, 1H), 7.45 (m, 1H), 7.41 (m, 1H), 7.40 (m, 1H), 7.34 (m, 1H), 6.89 (d, J = 2.6 Hz, 1H), 6.45 (d, J = 2.5 Hz, 1H), 6.41 (s, 1H), 5.45 (qq, J = 6.7, 1.2 Hz, 1H), 5.17 (s, 2H), 2.38 (s, 3H), 2.04 (s, 3H), 1.91 (dq, J = 1.2, 1.0 Hz, 3H), 1.75 (dq, J = 6.7, 1.0 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.8, 162.2, 161.4, 152.6, 144.5, 143.0, 140.1, 138.7, 135.2, 133.2, 132.1, 130.4, 127.9, 127.0, 125.9, 125.1, 115.2, 114.7, 113.6, 110.7, 103.7, 68.6, 20.5, 17.5, 13.7, 9.1. HRESI(−)MS *m*/*z* 449.1162 $[M - H]^-$ (calculated for $C_{26}H_{22}{}^{35}ClO_5^-$, 449.1161).

3-*O*-(4-Chlorobenzyl)unguinol (7c). Isolated as white powder, t_R = 21.4 min; 5.3 mg, 35.3%. UV (MeOH) λ_{max} (log ε) 202 (4.91), 258 (4.37) nm; IR (ATR) ν_{max} 3672, 2971, 2901, 2360, 1730, 1607, 1570, 1491, 1421, 1379, 1324, 1255, 1218, 1148, 1102 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.61 (br s, 1H), 7.44, (m, 2H), 7.40 (m, 2H), 6.87 (d, J = 2.6 Hz, 1H), 6.43 (d, J = 2.6 Hz, 1H) 6.41 (s, 1H), 5.45 (qq, J = 6.8, 1.4 Hz, 1H), 5.15 (s, 2H), 2.38 (s, 3H), 2.04 (s, 3H), 1.92 (dq, J = 1.4, 1.0 Hz, 3H), 1.74, (dq, J = 6.8, 1.0 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.8, 162.2, 161.5, 152.6, 144.5, 143.0, 140.1, 135.2, 135.1, 132.6, 132.1, 129.2, 128.5, 125.1, 115.2, 114.7, 113.5, 110.7, 103.8, 68.7, 20.5, 17.5, 13.7, 9.1. HRESI(−)MS *m*/*z* 449.1164 $[M - H]^-$ (calculated for $C_{26}H_{22}{}^{35}ClO_5^-$, 449.1161).

3-*O*-(2-Fluorobenzyl)unguinol (7d). Isolated as white powder, t_R = 22.1 min; 6.5 mg, 43.3%. UV (MeOH) λ_{max} (log ε) 205 (4.87), 263 (4.31) nm; IR (ATR) ν_{max} 3672, 2971, 2360, 1729, 1606, 1570, 1493, 1421, 1380, 1325, 1253, 1216, 1181, 1146, 1104, cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.62 (br s, 1H), 7.48 (ddd, J = 7.5, 7.5, 1.7 Hz, 1H), 7.42 (m, 1H), 7.25 (ddd, J = 10.9, 8.2, 1.1 Hz, 1H), 7.22 (ddd, J = 7.5, 7.5, 1.1 Hz, 1H), 6.89 (d, J = 2.5 Hz, 1H) 6.48 (d, J = 2.5 Hz, 1H), 6.42 (s, 1H), 5.46 (qq, J = 6.8, 1.4 Hz, 1H), 5.18 (s, 2H), 2.38 (s, 3H), 2.04 (s, 3H), 1.96 (dq, J = 1.4, 1.0 Hz, 3H), 1.75 (dq, J = 6.8, 1.0 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.8, 162.2, 161.4, 160.2, 152.6, 144.5, 143.0, 140.1, 135.2, 132.2, 130.6, 130.4, 125.1, 124.6, 122.9, 115.4, 115.1, 114.7, 113.6, 110.7, 103.6, 63.9, 20.5, 17.5, 13.6, 9.1. HRESI(+)MS *m*/*z* 435.1603 $[M + H]^+$ (calculated for $C_{26}H_{24}FO_5^+$, 435.1602).

3-*O*-(3-Fluorobenzyl)unguinol (7e). Isolated as white powder, t_R = 22.8 min; 6.3 mg, 42.0%. UV (MeOH) λ_{max} (log ε) 207 (4.75), 262 (4.23) nm; IR (ATR) ν_{max} 2919, 2360, 2340, 1719, 1608, 1568, 1488, 1475, 1416, 1376, 1347, 1324, 1290, 1254, 1238, 1218, 1147, 1128 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.62 (br s, 1H), 7.42 (m, 1H), 7.22 (m, 2H), 7.15 (dddd, J = 9.2, 7.5, 2.5, 1.0 Hz, 1H), 6.88 (d, J = 2.4 Hz, 1H) 6.45 (d, J = 2.4 Hz, 1H), 6.41 (s, 1H), 5.44 (qq, J = 6.7, 1.3 Hz, 1H), 5.18 (s, 2H), 2.38 (s, 3H), 2.04 (s, 3H), 1.92 (dq, J = 1.3, 1.0 Hz, 3H), 1.74 (dq, J = 6.7, 1.0 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.8, 162.2, 162.1, 161.2, 152.6, 144.5, 143.0, 140.1, 139.0, 135.2, 132.1, 130.6, 125.1, 123.3, 115.2, 114.8, 114.7, 114.0, 113.6, 110.7, 103.8, 68.7, 20.5, 17.5, 13.7, 9.1. HRESI(−)MS *m*/*z* 433.1454 $[M - H]^-$ (calculated for $C_{26}H_{22}FO_5^-$, 433.1456).

3-*O*-(4-Fluorobenzyl)unguinol (7f). Isolated as white powder, t_R = 23.4 min; 7.1 mg, 47.3%. UV (MeOH) λ_{max} (log ε) 205 (4.82), 262 (4.29) nm; IR (ATR) ν_{max} 3673, 2971, 2901, 2360, 1730, 1607, 1571, 1511, 1420, 1379, 1325, 1254, 1222, 1147, 1103 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.61 (br s, 1H), 7.44, (m, 2H), 7.21 (m, 2H), 6.87 (d, J = 2.5 Hz, 1H) 6.45 (d, J = 2.5 Hz, 1H), 6.41, (s, 1H), 5.46 (qq, J = 6.7, 1.4 Hz, 1H), 5.12 (s, 2H), 2.38 (s, 3H), 2.04 (s, 3H), 1.94 (dq, J = 1.4, 1.0 Hz, 3H), 1.75, (dq, J = 6.7, 1.0 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.8, 162.2, 161.8, 161.6, 152.5, 144.5, 143.0, 140.1, 135.2, 132.3, 132.2, 129.8, 125.1, 115.3, 115.1, 114.7, 113.4, 110.7,

103.8, 68.9, 20.5, 17.5, 13.7, 9.1. HRESI(−)MS *m*/*z* 433.1456 [M − H]$^-$ (calculated for $C_{26}H_{22}FO_5^-$, 433.1457).

3-*O*-(2,4-Difluorobenzyl)unguinol (7g). Isolated as white powder, t_R = 23.1 min; 8.4 mg, 56%. UV (MeOH) λ_{max} (log ε) 205 (5.00), 262 (4.45) nm; IR (ATR) ν_{max} 3673, 3377, 2971, 2901, 2360, 2339, 1702, 1606, 1571, 1506, 1421, 1381, 1327, 1255, 1217, 1147, 1100 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.62 (br s, 1H), 7.56 (m, 1H), 7.31 (ddd, *J* = 10.6, 9.3, 2.5 Hz, 1H), 7.12 (m, 1H), 6.89 (d, *J* = 2.5 Hz, 1H) 6.47 (d, *J* = 2.5 Hz, 1H), 6.42 (s, 1H), 5.46 (qq, *J* = 6.8, 1.4 Hz, 1H), 5.14 (s, 2H), 2.38 (s, 3H), 2.04 (s, 3H), 1.97 (dq, *J* = 1.4, 1.1 Hz, 3H), 1.76 (dq, *J* = 6.8, 1.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.8, 162.4, 162.2, 161.3, 160.5, 152.6, 144.5, 143.0, 140.1, 135.2, 132.2, 131.9, 125.1, 119.4, 115.4, 115.0, 114.7, 113.7, 111.7, 110.7, 104.1, 103.6, 63.5, 20.5, 17.5, 13.6, 9.1. HRESI(−)MS *m*/*z* 451.1363 [M − H]$^-$ (calculated for $C_{26}H_{21}F_2O_5^-$, 451.1363).

3-*O*-(3-Bromobenzyl)unguinol (7h). Isolated as white powder, t_R = 22.5 min; 6.8 mg, 45.3%. UV (MeOH) λ_{max} (log ε) 203 (4.76), 260 (4.17) nm; IR (ATR) ν_{max} 2922, 2360, 2340, 1727, 1606, 1575, 1510, 1416, 1383, 1346, 1317, 1295, 1263, 1217, 1142, 1107 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.62 (br s, 1H), 7.59 (dd, *J* = 1.6, 1.6 Hz, 1H), 7.52 (ddd, *J* = 7.7, 1.6, 1.3 Hz, 1H), 7.39 (ddd, *J* = 7.7, 1.6, 1.3 Hz, 1H), 7.34 (t, *J* = 7.7 Hz, 1H), 6.88 (d, *J* = 2.5 Hz, 1H), 6.44 (d, *J* = 2.5 Hz, 1H) 6.41 (s, 1H), 5.45 (qq, *J* = 6.8, 1.2 Hz, 1H), 5.16 (s, 2H), 2.38 (s, 3H), 2.04 (s, 3H), 1.91 (dq, *J* = 1.2, 1.1 Hz, 3H), 1.75 (dq, *J* = 6.8, 1.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.8, 162.2, 161.4, 152.6, 144.5, 143.0, 140.1, 138.9, 135.2, 132.1, 130.8, 130.7, 129.9, 126.3, 125.1, 121.7, 115.2, 114.7, 113.6, 110.7, 103.7, 68.5, 20.5, 17.5, 13.7, 9.1. HRESI(−)MS *m*/*z* 493.0656 [M − H]$^-$ (calculated for $C_{26}H_{22}{}^{79}BrO_5^-$, 493.0656).

3-*O*-(3-Methylbenzyl)unguinol (7i). Isolated as white powder, t_R = 22.1 min; 6.5 mg, 43.3%. UV (MeOH) λ_{max} (log ε) 205 (4.88), 262 (4.30) nm; IR (ATR) ν_{max} 2920, 2360, 2340, 1723, 1608, 1586, 1568, 1493, 1422, 1383, 1348, 1327, 1288, 1257, 1238, 1225, 1217, 1188, 1149 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.62 (br s, 1H), 7.26 (t, *J* = 7.4 Hz, 1H), 7.19, (m, 1H), 7.16 (m, 1H), 7.14 (m, 1H), 6.86 (d, *J* = 2.5 Hz, 1H), 6.44 (d, *J* = 2.5 Hz, 1H), 6.41 (s, 1H), 5.45 (qq, *J* = 6.8, 1.4 Hz, 1H), 5.09 (s, 2H), 2.37 (s, 3H), 2.29 (s, 3H), 2.04 (s, 3H), 1.93 (dq, *J* = 1.4, 1.0 Hz, 3H), 1.75 (dq, *J* = 6.8, 1.0 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.8, 162.2, 161.7, 152.6, 144.4, 143.0, 140.1, 137.7, 136.0, 135.2, 132.2, 128.7, 128.4, 128.0, 125.1, 124.5, 115.2, 114.7, 113.4, 110.7, 103.7, 69.6, 20.9, 20.5, 17.5, 13.7, 9.1. HRESI(−)MS *m*/*z* 429.1706 [M − H]$^-$ (calculated for $C_{27}H_{25}O_5^-$, 429.1707).

3-*O*-(3-Methoxybenzyl)unguinol (7j). Isolated as white powder, t_R = 20.8 min; 5.7 mg, 38.0%. UV (MeOH) λ_{max} (log ε) 203 (4.80), 263 (4.23), 281 (4.08) nm; IR (ATR) ν_{max} 3399, 2926, 2360, 2340, 1708, 1608, 1570, 1503, 1477, 1419, 1395, 1347, 1324, 1290, 1274, 1242, 1221, 1184, 1145, 1130 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.62 (br s, 1H), 7.29 (t, *J* = 8.1 Hz, 1H), 6.94 (m, 2H), 6.89 (m, 1H), 6.88 (d, *J* = 2.5, 1H), 6.44 (d, *J* = 2.5, 1H), 6.41 (s, 1H), 5.45 (qq, *J* = 6.7, 1.1, 1H), 5.16 (s, 2H), 3.73 (s, 3H), 2.38 (s, 3H), 2.04 (s, 3H), 1.91 (dq, *J* = 1.2, 1.1 Hz, 3H), 1.75 (dq, *J* = 6.7, 1.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.8, 162.2, 161.7, 159.3, 152.6, 144.4, 143.0, 140.1, 137.6, 135.2, 132.1, 129.6, 125.1, 119.4, 115.2, 114.7, 113.4, 113.4, 112.9, 110.7 103.8, 69.4, 55.0, 20.5, 17.5, 13.7, 9.1. HRESI(−)MS *m*/*z* 445.1656 [M − H]$^-$ (calculated for $C_{27}H_{25}O_6^-$, 445.1656).

3-*O*-(2-Picolyl)unguinol (7k). A mixture of unguinol (15 mg, 46 μmol), 2-(bromomethyl)pyridine (23 mg, 2 eq.) and excess K_2CO_3 (5 mg) in MeCN (5 mL) was stirred for 12 h at 25 °C. The crude reaction mixture was filtered and the filtrate was reduced to dryness under nitrogen. The residue was purified by semipreparative HPLC with isocratic 40% MeCN/H_2O plus 0.01% TFA (4.18 mL min^{-1}), yielding 3-*O*-(2-picolyl)unguinol (**7k**; t_R = 17.8 min; 4.1 mg, 27.3%). UV (MeOH) λ_{max} (log ε) 205 (4.92), 263 (4.44) nm; IR (ATR) ν_{max} 3673, 2985, 2901, 2360, 2339, 1730, 1608, 1572, 1420, 1326, 1252, 1215, 1150, 1105 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.61 (s, 1H), 8.57 (d, *J* = 4.4 Hz, 1H), 7.81 (td, *J* = 7.7, 1.7 Hz, 1H), 7.42 (d, *J* = 7.7 Hz, 1H), 7.34 (ddd, *J* = 7.7, 4.4, 1.0 Hz, 1H), 6.89 (d, *J* = 2.5 Hz, 1H) 6.46 (d, *J* = 2.5 Hz, 1H), 6.41 (s, 1H), 5.43 (qq, *J* = 6.7, 1.4 Hz, 1H), 5.21 (s, 2H), 2.38 (s, 3H), 2.04 (s, 3H), 1.91 (dq, *J* = 1.4, 1.0 Hz, 3H), 1.73 (dq, *J* = 6.7, 1.0 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.8, 162.2, 161.5, 155.6, 152.5, 149.2, 144.4, 143.0, 140.1, 137.0, 135.2, 132.1, 125.1, 123.1, 121.5, 115.3, 114.7, 113.6, 110.7, 103.6, 70.5, 20.5, 17.5, 13.6, 9.1. HRESI(+)MS *m*/*z* 418.1644 [M + H]$^+$ (calculated for $C_{25}H_{24}NO_5^+$, 418.1649).

3-*O*-(3-Picolyl)unguinol (7l). A mixture of unguinol (15 mg, 46 μmol), 3-(bromomethyl)pyridine (46 mg, 4 eq.) and excess K_2CO_3 (5 mg) in MeCN (5 mL) was stirred at 25 °C for 12 h. The crude reaction mixture was filtered and the filtrate was reduced to dryness under nitrogen and purified by semipreparative HPLC with isocratic 30% MeCN/H_2O plus 0.01% TFA (4.18 mL min^{-1}), yielding 3-*O*-(3-picolyl)unguinol (**7l**; t_R = 19.4 min; 1.2 mg, 8.0%). UV (MeOH) λ_{max} (log ε) 204 (4.48), 260 (4.00) nm; IR (ATR) ν_{max} 3673, 2985, 2901, 2360, 2339, 1732, 1606, 1407, 1251, 1148 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.61 (br s, 1H), 8.69 (br s, 2H), 7.83 (d, *J* = 7.7 Hz, 1H), 7.47 (br s, 1H), 6.90 (d, *J* = 2.5 Hz, 1H), 6.48 (d, *J* = 2.5 Hz, 1H), 6.41 (s, 1H), 5.45 (qq, *J* = 6.7, 1.4 Hz, 1H), 5.21 (s, 2H), 2.38 (s, 3H), 2.04 (s, 3H), 1.94 (dq, *J* = 1.4, 1.1 Hz, 3H), 1.76, (dq, *J* = 6.7, 1.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.8, 162.2, 161.4, 152.6, 149.1, 148.7, 144.5, 143.0, 140.1, 135.6, 135.2, 132.2, 132.2, 125.1, 124.1, 115.1, 114.7, 113.6, 110.7, 103.8, 67.3, 20.5, 17.5, 13.7, 9.1. HRESI(+)MS *m*/*z* 418.1643 [M + H]$^+$ (calculated for $C_{25}H_{24}NO_5^+$, 418.1649).

3-*O*-(4-Picolyl)unguinol (7m). A mixture of unguinol (**1**; 15 mg, 46 μmol), 4-(bromomethyl)pyridine (23 mg, 4 eq.) and excess K_2CO_3 (5 mg) in MeCN (5 mL) was stirred at 25 °C for 12 h. The crude reaction mixture was filtered and the filtrate was reduced to dryness under nitrogen and purified by semipreparative HPLC with isocratic 35% MeCN/H_2O plus 0.01% TFA (4.18 mL min^{-1}), yielding 3-*O*-(4-picolyl)unguinol (**7m**; t_R = 22.1 min; 4.6 mg, 30.7%). UV (MeOH) λ_{max} (log ε) 204 (4.84), 256 (4.33) nm; IR (ATR) ν_{max} 3671, 3203, 2986, 2901, 2359, 2090, 1611, 1517, 1410, 1253, 1150 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.62 (s, 1H), 8.68 (br s, 2H), 7.41 (br s, 2H), 6.89 (d, *J* = 2.5 Hz, 1H) 6.44 (d, *J* = 2.5 Hz, 1H), 6.40 (s, 1H), 5.42

(qq, J = 6.7, 1.4 Hz, 1H), 5.24 (s, 2H), 2.38, (s, 3H), 2.04 (s, 3H), 1.89 (dq, J = 1.4, 1.1 Hz, 3H), 1.71 (dq, J = 6.7, 1.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.8, 162.2, 161.2, 152.6, 149.6, 145.4, 144.6, 143.0, 140.1, 135.2, 132.0, 125.1, 121.8, 115.2, 114.7, 113.8, 110.7, 103.7, 67.8, 20.5, 17.5, 13.7, 9.1. HRESI(+) MS m/z 418.1643 $[M + H]^+$ (calculated for $C_{25}H_{24}NO_5^+$, 418.1649).

3-*O*-(4-Morpholinoethyl)unguinol (7n). A mixture of unguinol (**1**; 20 mg, 61 μmol), 4-(2-chloroethyl)morpholine (11.4 mg, 1.0 eq.), K_2CO_3 (8.5 mg, 1.0 eq.) and KI (7.64 mg, 1.0 eq.) in MeCN was stirred for 72 h at 25 °C. The crude reaction mixture was filtered and the filtrate was reduced to dryness under nitrogen and purified by semipreparative HPLC with isocratic 15% MeCN/H_2O plus 0.01% TFA (4.18 mL min^{-1}), yielding 3-*O*-(4-morpholinoethyl)unguinol (**7n**; t_R = 17.1 min; 5.3 mg, 34.6%). UV (MeOH) λ_{max} (log ε) 205 (5.05), 263 (4.51) nm; IR (ATR) ν_{max} 2927, 1728, 1605, 1570, 1420, 1323, 1251, 1214, 1151, 1106 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.61 (s, 1H), 6.79 (d, J = 2.5 Hz, 1H) 6.42 (s, 1H), 6.40 (d, J = 2.5 Hz, 1H), 5.45 (qq, J = 6.7, 1.5 Hz, 1H), 4.11 (br s, 1H), 3.55 (br s, 4H), 2.66 (br s, 1H), 2.43 (br s, 2H), 2.37, (s, 3H), 2.04 (s, 3H), 2.00 (dq, J = 1.5, 1.1 Hz, 3H), 1.78 (dq, J = 6.7, 1.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.8, 162.2, 161.8, 152.6, 144.4, 143.0, 140.2, 135.3, 132.5, 125.0, 114.8, 114.7, 113.2, 110.7, 103.4, 66.0, 65.8, 56.4, 53.4, 20.5, 17.6, 13.7, 9.1. HRESI(+)MS m/z 440.2059 $[M + H]^+$ (calculated for $C_{25}H_{30}NO_6^+$, 440.2068).

3-*O*-(1-Piperidinylethyl)unguinol (7o). A mixture of unguinol (**1**; 15 mg, 46 μmol), 1-(2-chloroethyl)piperidine (8.5 mg, 1.0 eq.) K_2CO_3 (9.53 mg, 1.5 eq.) and KI (7.64 mg, 1.0 eq.) in MeCN was stirred at 25 °C for 24 h. The crude reaction mixture was filtered and the filtrate was reduced to dryness under nitrogen and purified by semipreparative HPLC with isocratic 15% MeCN/H_2O plus 0.01% TFA (4.18 mL min^{-1}), yielding 3-*O*-(1-piperidinylethyl)unguinol (**7o**; t_R = 18.8 min; 3.4 mg, 22.7%). UV (MeOH) λ_{max} (log ε) 205 (4.97), 261 (4.45) nm; IR (ATR) ν_{max} 2969, 2360, 2339, 1728, 1673, 1606, 1573, 1420, 1324, 1253, 1199, 1153, 1106 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.64 (s, 1H), 6.82 (s, 1H), 6.42 (s, 2H), 4.30 (br s, 2H), 5.46 (qq, J = 6.7, 1.3 Hz, 1H), 2.38 (s, 3H), 2.05 (s, 3H), 2.00 (dq, J = 1.3, 1.1 Hz, 3H), 1.78 (dq, J = 6.7, 1.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.8, 162.2, 152.6, 144.5, 143.0, 140.1, 135.3, 132.4, 125.0, 114.7, 114.7, 110.8, 103.7, 20.5, 17.6, 13.7, 9.1. HRESI(+)MS m/z 438.2272 $[M + H]^+$ (calculated for $C_{26}H_{32}NO_5^+$, 438.2275).

3-*O*-(1-Pyrrolidinylethyl)unguinol (7p). A mixture of unguinol (**1**; 15 mg, 46 μmol), 1-(2-chloroethyl)pyrrolidine (7.8 mg, 1.0 eq.) K_2CO_3 (9.53 mg, 1.5 eq.) and KI (7.64 mg, 1.0 eq.) was stirred at 25 °C for 24 h. The crude reaction mixture was filtered and the filtrate was reduced to dryness under nitrogen and purified by semipreparative HPLC with isocratic 15% MeCN/H_2O plus 0.01% TFA (4.18 mL min^{-1}), yielding 3-*O*-(1-pyrrolidinylethyl)unguinol (**7p**; t_R = 19.5 min; 1.3 mg, 8.7%). UV (MeOH) λ_{max} (log ε) 205 (4.84), 226 (4.54), 266 (4.21) nm; IR (ATR) ν_{max} 3673, 2971, 2901, 2360, 2339, 1730, 1675, 1607, 1418, 1253, 1201, 1156, 1105 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.66 (s, 1H), 6.85 (d, J = 2.4 Hz, 1H), 6.44 (d, J = 2.4 Hz, 1H), 6.43 (s, 1H), 5.46 (qq, J = 6.8, 1.5 Hz, 1H), 4.32 (br s, 2H), 3.57 (br s, 4H), 3.07 (br s, 2H), 2.40 (s, 3H), 2.05 (s, 3H), 2.00 (dq, J = 1.5, 1.0 Hz, 3H), 1.79 (dq, J = 6.8, 1.0 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.8, 162.2, 152.6, 144.6, 143.0, 140.1, 135.3, 132.4, 125.0, 114.7, 110.8, 103.8, 63.6, 53.8, 52.5, 22.4, 20.5, 17.6, 13.7, 9.1. HRESI(+)MS m/z 424.2111 $[M + H]^+$ (calculated for $C_{25}H_{30}NO_5^+$, 424.2118).

3-*O*-Benzyl-1′,2′-dihydrounguinol (8a). Isolated as white powder, t_R = 20.5 min; 5.1 mg, 34%. UV (MeOH) λ_{max} (log ε) 203 (5.09), 265 (4.45) nm; IR (ATR) ν_{max} 3356, 2962, 2929, 1728, 1607, 1570, 1428, 1378, 1336, 1256, 1214, 1149, 1112 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.50 (br s, 1H), 7.41 (m, 2H), 7.37 (m, 2H), 7.32 (m, 1H), 6.88 (d, J = 2.5 Hz, 1H) 6.83 (d, J = 2.5 Hz, 1H), 6.48 (s, 1H), 5.19 (d, J = 12.0 Hz, 2H) 3.32 (m, 1H), 2.39 (s, 3H), 2.03 (s, 3H), 1.48 (m, 2H), 1.05 (d, J = 6.8 Hz, 3H), 0.75 (t, J = 7.3 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.9, 162.3, 161.8, 152.9, 144.6, 142.7, 140.9, 136.2, 136.1, 128.4, 128.0, 127.5, 115.3, 113.6, 113.5, 108.1, 103.9, 69.7, 32.2, 29.8, 21.5, 20.6, 12.0, 9.1. HRESI(+)MS m/z 419.1848 $[M + H]^+$ (calculated for $C_{26}H_{27}O_5^+$, 419.1853).

3-*O*-(2-Fluorobenzyl)-1′,2′-dihydrounguinol (8b). Isolated as white powder, t_R = 19.8 min; 5.7 mg, 38%. UV (MeOH) λ_{max} (log ε) 203 (5.08), 263 (4.44) nm; IR (ATR) ν_{max} 3341, 2962, 2360, 2339, 1727, 1606, 1570, 1493, 1427, 1379, 1335, 1256, 1214, 1148, 1111 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.51 (br s, 1H), 7.52 (ddd, J = 7.6, 7.6, 1.7 Hz, 1H), 7.42 (m, 1H), 7.25 (ddd, J = 10.9, 8.2, 1.1 Hz, 1H), 7.21 (ddd, J = 7.6, 7.6, 1.0 Hz, 1H), 6.90 (d, J = 2.5 Hz, 1H) 6.88 (d, J = 2.5 Hz, 1H), 6.49, (s, 1H), 5.22 (d, J = 12.0 Hz, 2H) 3.32 (m, 1H), 2.39 (s, 3H), 2.04 (s, 3H), 1.49 (m, 2H), 1.07 (d, J = 6.8 Hz, 3H), 0.76 (t, J = 7.3 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.9, 162.3, 161.6, 160.3, 152.9, 144.7, 142.7, 140.9, 136.2, 130.6, 124.5, 122.9, 115.4, 115.2, 113.7, 113.6, 108.1, 103.8, 64.1, 32.2, 29.8, 21.5, 20.6, 11.9, 9.1. HRESI(+)MS m/z 437.1756 $[M + H]^+$ (calculated for $C_{26}H_{26}FO_5^+$, 437.1758).

3-*O*-(4-Fluorobenzyl)-1′,2′-dihydrounguinol (8c). Isolated as white powder, t_R = 22.9 min; 6.4 mg, 42.6%. UV (MeOH) λ_{max} (log ε) 203 (5.10), 265 (4.43) nm; IR (ATR) ν_{max} 3673, 3378, 2966, 2360, 2339, 1728, 1606, 1570, 1511, 1427, 1378, 1336, 1256, 1219 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.50 (br s, 1H), 7.47 (m, 2H), 7.20 (m, 2H), 6.87 (d, J = 2.5 Hz, 1H) 6.82 (d, J = 2.5 Hz, 1H), 6.48, (s, 1H), 5.17 (d, J = 12.2 Hz, 2H) 3.28, (m, 1H), 2.39 (s, 3H), 2.03 (s, 3H), 1.48 (m, 2H), 1.05, (d, J = 6.8 Hz, 3H), 0.74 (t, J = 7.3 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.9, 162.3, 161.8, 161.7, 152.9, 144.6, 142.7, 140.9, 136.2, 132.4, 129.8, 115.3, 115.3, 113.6, 113.5, 108.1, 103.8, 68.9, 32.2, 29.8, 21.5, 20.6, 11.9, 9.1. HRESI(+)MS m/z 437.1756 $[M + H]^+$ (calculated for $C_{26}H_{26}FO_5^+$, 437.1758).

Biological screening

Purified metabolites were dissolved in DMSO to provide stock solutions (10 000 μg mL^{-1} or 1000 μg mL^{-1} depending on the amount of material available). An aliquot of each stock solution was transferred to the first lane of Rows B to G in a 96-well microtitre plate and two-fold serially diluted with DMSO across the 12 lanes of the plate to provide a 2048-fold concentration gradient. Bioassay medium was added to an

aliquot of each test solution to provide a 100-fold dilution into the final bioassay, thus yielding a test range of 100 to 0.05 μg mL^{-1} in 1% DMSO. Row A contained no test compound (as a reference for no inhibition) and Row H was uninoculated (as a reference for complete inhibition).

MIC determination and time-dependent inhibition assays. *B. subtilis* (ATCC 6633), five *S. aureus* (ATCC12600, ATCC 25923, ATCC 33592, ATCC 49775 and USA300), two *E. faecium* (ATCC 19434 and E734), two *P. aeruginosa* (PAO1 and ATCC 27853) and three *E. coli* (ATCC 25322, ATCC 25922 and ATCC 35218) strains were used as indicative species for antibacterial activity. A bacterial suspension (50 mL in 250 mL flask) was prepared in nutrient media by cultivation at 28 °C for 24 h with shaking at 250 rpm. The suspension was diluted to an absorbance of 0.01 absorbance units per mL, and 10 μL aliquots were added to the wells of a 96-well microtitre plate containing the test compounds dispersed in nutrient broth (Amyl) with resazurin (12.5 μg mL^{-1}). The plates were incubated at 28 °C for 48 h, during which time the control wells with no test compound changed colour from a blue to light pink colour. MIC end points were determined visually. The absorbance was measured using Spectromax plate reader (Molecular Devices) at 605 nm and the IC_{50} values determined graphically. Our preliminary testing clearly showed **7d** and **7g** exhibited potent activity against the selected *S. aureus* strains. Therefore, these two compounds were subjected to MIC testing against an expanded panel of 20 clinical MRSA isolates plus 3 ATCC *S. aureus* strains in a standard plate microdilution MIC assay, using the Clinical Laboratory Standards Institute guidelines (CLSI 2017),[33] essentially as described previously.[6,34] The time- and concentration-dependent activities of **7d** and **7g** against two *S. aureus* ATCC strains were also determined in a kinetics assay by optical density ($A_{600\ nm}$) measurements on a Cytation 5 Multimode reader (BioTek).

Antifungal assay. The yeasts *C. albicans* (ATCC 10231) and *S. cerevisiae* (ATCC 9763) were used as indicative species for antifungal activity. A yeast suspension (50 mL in 250 mL flask) was prepared in 1% malt extract broth by cultivation for 24 h at 250 rpm, 24 °C. The suspension was diluted to an absorbance of 0.005 and 0.03 absorbance units per mL for *C. albicans* and *S. cerevisiae*, respectively. Aliquots (20 μL and 30 μL) of *C. albicans* and *S. cerevisiae*, respectively were applied to the wells of a 96-well microtitre plate, which contained the test compounds dispersed in malt extract agar containing bromocresol green (50 μg mL^{-1}). The plates were incubated at 24 °C for 48 h during which time the control wells containing no test compound change colour from a blue to yellow colour. MIC end points were determined visually. The absorbance was measured using Spectromax plate reader (Molecular Devices) at 620 nm and the IC_{50} determined graphically.

Cytotoxicity assay. NS-1 (ATCC TIB-18) mouse myeloma cells were inoculated in 96-well microtitre plates (190 μL) at 50 000 cells per mL in DMEM (Dulbecco's Modified Eagle Medium + 10% fetal bovine serum (FBS) + 1% penicillin/streptomycin (10 000 U mL^{-1}/10 000 μg mL^{-1}, Life Technologies Cat. no. 15140122), together with resazurin (250 μg mL^{-1}; 10 μL) and incubated in 37 °C (5% CO_2) incubator. The plates were incubated for 72 h during which time the control wells containing no test compound changed colour from a blue to pink colour. The absorbance of each well was measured at 605 nm using a Spectromax plate reader (Molecular Devices).

Haemolysis and real-time *in vitro* cytotoxicity assays. Haemolysis assays for **7d** and **7g** were performed using fresh donor human red blood cells (RBCs), essentially as described previously.[6,34] The toxicity profiles of the 2 compounds were also evaluated by measuring viability of HEK293 kidney and Hep G2 liver cell lines over a 20 h period in the presence of two-fold increasing concentrations of the compounds (2 μg mL^{-1}–64 μg mL^{-1}) on a Cytation 5 Cell Imaging Multi-Mode Reader (BioTek) using the RealTime-Glo™ MT Cell Viability Assay reagent (Promega), as described previously.[34]

Conflicts of interest

There are no conflicts to declare.

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