DOI: 10.1002/chem.201303119

Inspiration from Old Dyes: Tris(stilbene) Compounds as Potent Gram-Positive Antibacterial Agents

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Abstract: Herein we describe the preparation and structure-activity relationship studies on range of stilbene based compounds and their antibacterial activity. Two related compounds, each bearing carboxylic acid moieties, exhibit good activity against several bacterial strains, including methicillin-resistant *Staphylococcus aureus* MRSA (ATCC 33592 and NCTC 10442). Compound **10** was most active against *Moraxella catarrhalis* with minimum inhibitory concentrations (MICs) of 0.12–0.25 $\mu g\,mL^{-1}$ and against *Staphylococcus spp.* with MICs ranging from 2–4 $\mu g\,mL^{-1}$. The derivative **17** showed increased activity with MICs of 0.06–0.25 $\mu g\,mL^{-1}$ against *M. catarrhalis* and 0.12–1 against *Staphylococcus spp*. This level of activity is similar to that reported for *S. aureus* for antibiotics, such as vancomycin, with MICs of $\leq 2.0\ \mu g\,mL^{-1}$ and clindamycin with MICs of $\leq 0.5\ \mu g\,mL^{-1}$. As an indicator of toxicity, **17** was tested for its ability to lyse sheep erythrocytes, and showed low haemolytic activity. Such results highlight the value of tris(stilbene) compounds as antibacterial agents providing suitable properties for further development.

Keywords: antibacterial · biological activity · Heck cross-coupling · MRSA · stilbenes

Introduction

The growth in prevalence of antibacterial resistance is a major public health problem that our society is currently facing.[1] The search for original compounds and novel molecular scaffolds with antimicrobial properties has attracted great interest in recent times due to the regular use of broad spectrum antibiotics leading to the increased occurrence of bacterial strains resistant to current antimicrobial formulations.[2] A decline in pharmaceutical endeavours and discoveries in the area of antibacterial drug discovery has been also observed.[3] Alarmingly, there has also been an increase in the prevalence of infections caused by some Gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile*, resulting in serious or fatal diseases. Despite several new drugs in late-stage development, the current reduced commitment to antimicrobial research and lack of development pipelines has, inexorably, led to a steady decline in the treatment choices for more serious bacterial infections.[4]

In the search for unexplored molecular scaffolds that possess antibacterial properties and new cellular targets for such molecules, we had shown that mechanosensitive (MS) channels could be exploited in this way.[5,6] In silico modelling studies had shown that eriochrome cynanine a triphenyl methyl (TPM) dye bound to the MscL channel (Large-Conductance Mechanosensitive Channels) and this result was confirmed experimentally with patch-clamp and ESR experiments.[7] A range of commercial dyes with reported antibacterial properties were used as simple molecular templates for novel analogues. In particular, we were interested

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Supporting information on for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201303119. It contains ^{1}H and ^{13}C spectra.

in the spatial arrangement of the phenyl ring systems in the triphenyl methyl (TPM) dyes, such as malachite green (**1**), brilliant green, gentian violet, methyl violet and aluminon (**2**), and the variation in the functional groups contained within of each of these dyes (Figure 1). Reports of brilliant green having antibacterial activity against Gram-positive bacteria are common in the literature,[8,9] whereas gentian violet, has served as both an antibacterial agent and a histological stain for many years.[10,11]

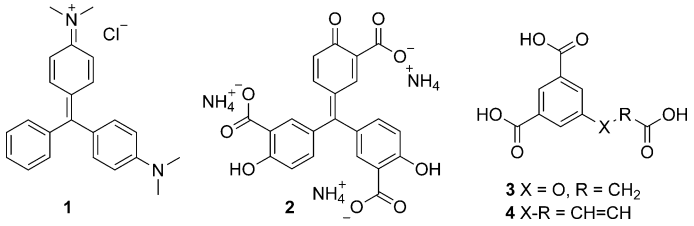


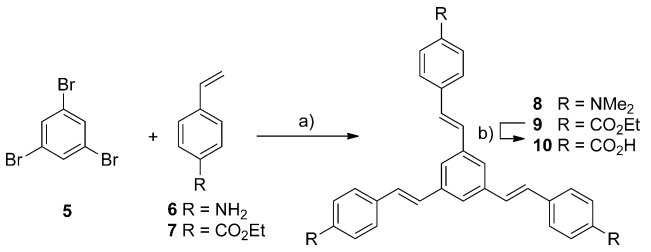
Figure 1. Antibacterial dyes and simple aromatic compounds with reported antibacterial properties.

Aluminon (**2**), or more so its equivalent protonated aurintricarboxylic acid, has been reported to show activity against *E. coli* through two pathways; inhibition of the attachment of natural mRNA to ribosomes and topoisomerase II inhibition.[12–15] Likewise structures containing multiple carboxylic acid groups, such as **3** and **4** have been highlighted as chorismate synthase inhibitors, used against the Gram-positive bacterium *S. aureus*. These two compounds make up a larger group of compounds, including derivatives of various chain lengths, protected by the pharmaceutical company PanTherix. Initial trials of these compounds indicated activity against *Streptococcus pneumoniae*.[16] Natural and synthetically derived stilbenes and related phenols have also attracted attention due to their antibacterial properties.[17–21] Even simpler compounds containing a central aromatic core such as atromentin and leucomelone have been reported as enoyl-ACP reductase (FabK) inhibitors of *S. pneumonia*; however, this is possibly due to their phenolic residues.[22]

Given the nature of these dyes and simpler aromatic derivatives, we decided to explore the antibacterial properties of a range of similar compounds containing benzoic acids and anilines tethered around a central aromatic core. The core was conceived to be tethered to a range of aromatic arms either through conjugation (styrene type) or through a flexible linker. In silico modelling experiments had predicted that such molecules would bind to the MscL channel.[23,24]

Results and Discussion

A Heck–Mizoroki-based approach, realised through the cross-coupling of the commercially available bromobenzene **5** and appropriate styrene precursor (**6** or **7**) was used in the preparation of the unsaturated derivatives **8**–**10**. This work was initially conceived through the pioneering work by de Meijere by using Jefferey's phosphine-free conditions; however, our group and others have reported methods for the construction of tri- and tetrastyrene benzene derivatives by using more recently reported active catalysts.[25–27] When using $[Pd_2(dba)_3]\cdot CHCl_3$ (dba = dibenzylideacetone) and P-(tBu)$_3$.BF_4 a good yield of the desired triamine derivative **8** and an excellent yield of the corresponding ester derivative **9** was achieved through this tandem process (Scheme 1).[28–30] By following saponification of the ester **9**, the first three simple test substrates based on compounds **1**–**4** and previous reports on stilbenes were prepared.



Scheme 1. a) $[Pd_2(dba)_3]\cdot CH_3/[(tBu)_3PH]BF_4$, THF, reflux, 17 h (82–97%); b) LiOH, H_2O/EtOH, reflux, 3 h (70–89%).

To investigate the hypothesis that these compounds would have antibacterial properties, they were screened against several Gram-negative and -positive bacterial strains by using disc diffusion assays. This first method indicated that both the dimethylamine derivative **8** and the ester **9** were either inactive or only mild inhibitors of these bacteria.

Conversely, the triacid styrene derivative **10** showed good activity in this initial series of disc diffusion assays and thus was screened for activity against a larger group of 38 isolates comprised of 23 Gram-negative bacteria, 14 Gram-positive bacteria and a yeast. This collection included the anaerobes *C. difficile*, *C. perfringens* (both Gram-positive) and *Bacteroides fragilis* (Gram-negative). This group of test organisms was selected to represent a broad range of clinically relevant pathogenic microorganisms. Data for the susceptible bacteria are shown in Table 1. Compound **10** did not inhibit the yeast *Candida albicans* and, in general, did not inhibit the growth of Gram-negative bacteria with the exception of *Moraxella catarrhalis*. However, triacid **10** did inhibit the growth of many of the Gram-positive bacteria tested by the disc diffusion assay (Table 1) but derivative **8** and **9** were inactive.

Compound **10** (at 10 mg mL^{-1}) was most efficacious against *M. catarrhalis* ATCC 25238, *Micrococcus luteus* ATCC 10240, *S. aureus* NCTC 6571, *S. aureus* (MRSA) NCTC 10442 and *S. epidermidis* ATCC 12228 with inhibition zones of 17–26 mm. Of particular interest was the inhibition of *M. catarrhalis*, which typically produces beta-lactamases (the enzymes capable of opening the critical beta-lactam ring of penicillin and cephalosporin-type drugs), and MRSA. Activity against *C. difficile* NCTC 43593 was moderate; however, importantly **10** could act as a lead compound

Table 1. Disc diffusion inhibition zone diameters (mm) for compound **10** (10 mg mL^{-1}).[a]

Organism[b]	Inhibition zones [mm]
Bacillus cereus ATCC 13061	10
Bacillus subtilis ATCC 6633	8
Clostridium difficile NCTC 43593	10
Clostridium perfringens ATCC 2734	12
Enterococcus faecalis NCTC 775	10
Enterococcus faecium ATCC 19434	11
Listeria monocytogenes NCTC 7973	9
Micrococcus luteus ATCC 10240	26
Moraxella catarrhalis ATCC 25238*	25
Staphylococcus aureus NCTC 6571	20
Staphylococcus aureus NCTC 10442	18
Staphylococcus epidermidis ATCC 12228	22
Staphylococcus xylosus ATCC 29971	17
Streptococcus pneumoniae ATCC 49619	16
Streptococcus pyogenes NCTC 8191	14

[a] NI = no inhibition; * = Gram negative; [b] Results are from two independent tests.

for development of more efficacious substances for treatment of this gut bacterium that causes antibiotic-associated diarrhoea.[31] These initial results were confirmed by determining the minimum inhibitory concentrations (MICs) of triacid **10** against the more susceptible bacterial species that had inhibition zones of 14 mm or greater (Table 2). The MIC of compound **10** for the two MRSA strains (ATCC 33592 and NCTC 10442) was respectable at 4 μg mL^{-1}. The minimum bactericidal concentrations (MBCs) suggest that compound **10** is not bactericidal but

Table 2. MICs and MBCs (μg mL^{-1}) of compound **10**.

Organism	MIC	MBC
Moraxella catarrhalis ATCC 25238	0.25	>32
Moraxella catarrhalis NCTC 3622	0.12	32
Staphylococcus aureus NCTC 6571	2	>32
Staphylococcus aureus ATCC 29213	4	>32
Staphylococcus aureus NCTC 10442	4	>32
Staphylococcus aureus ATCC 33592	4	>32
Staphylococcus epidermidis ATCC 12228	4	>32
Staphylococcus xylosus ATCC 29971	16	16
Streptococcus pneumoniae ATCC 49619	32	>32
Streptococcus pyogenes NCTC 8191	>32	>32
Streptococcus pyogenes NCTC 8302	>32	>32

acts in a bacteriostatic fashion. The remaining *S. aureus* strains and *S. epidermidis* also had low MICs of 2–4 μg mL^{-1} and the triacid **10** was bactericidal for *S. xylosus* ATCC 29971. In contrast, compound **10** showed only moderate activity against *Streptococcus spp.* with MICs of 32 μg mL^{-1} and above.

Given the promising activity of triacid **10** against a range of Gram-positive bacteria further pharmacological evaluations were carried out to ascertain its potential as an antibacterial drug. To test for the potential for chemically induced mutations, an Ames test for compound **10** was carried out (Table 3).[32,33] In the three *Salmonella typhimurium* test strains (TA98, TA100 and TA1535) compound **10** did not exceed the measured negative control by any significant amount at any of the concentrations (100, 300 or 1000 μg/plate) providing evidence that it is non-mutagenic. Haemolysis experiments indicated that even at the highest test concentration of 500 μg mL^{-1} compound **10** showed low haemolytic activity with only 5.05% of sheep erythrocytes lysed (Table 4). A cytotoxicity assay was performed on L929 cells, at the same concentrations as the haemolysis assay (Table 4). Cytotoxicity was quantified by using the neutral red uptake assay with a ratio of ≤0.5, which indicated a cytostatic effect. At concentrations of 0.5–50 μg mL^{-1} , the compound was not considered toxic; however, at 500 μg mL^{-1} there was a cytostatic effect.

Table 3. Mutagenicity of compound **10** was determined by the Ames test with three *Salmonella* strains.[a]

Strain	Control		Compound [μg/plate]		
	negative	positive	100	300	1000
TA98	13	292	14	11	3
TA100	104	355	102	107	88
TA1535	28	600	23	20	0

[a] Values represent mean numbers of colonies per agar plate.

Table 4. Cytotoxicity of compound **10**.[a]

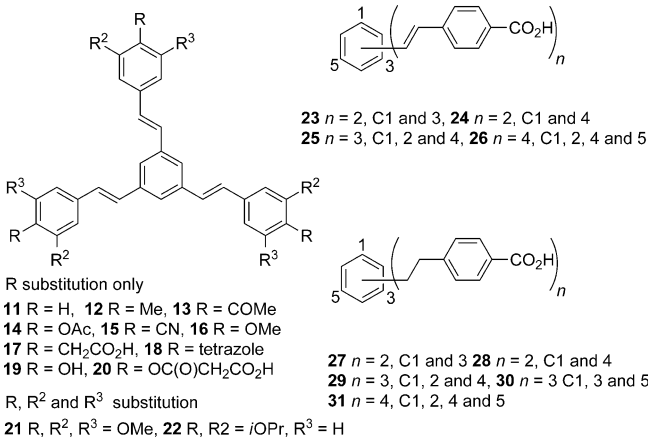
Assay	Concentration of compound [μg mL^{-1}]				
	0	0.5	5	50	500
L929 cytotoxicity[b]	1.0	0.85 (0.28)	0.88 (0.02)	0.87 (0.03)	0.38 (0.01)
haemolysis [%]	0.0	−2.32 (0.69)	2.17 (0.10)	2.32 (0.10)	5.05 (0.59)

[a] Values represent the mean (standard deviation). [b] Values represent the ratio of the optical density (OD) of treated cells compared to the OD of the negative control. Ratios of ≤0.5 indicate a cytostatic effect.

To gauge the pharmacokinetic compatibility of triacid **10**, the potential for protein to interfere with activity was examined by determining MICs in the presence of horse serum. In many reported examples, a reduced efficacy of compounds containing carboxylic acid motifs has been linked to human serum albumin (HSA) binding proving problematic for eventual human trials.[34,35] Compound **10** also was prone to diminished activity in the presence of serum. Specifically, triacid **10** had an MIC of 2 μg mL^{-1} against *S. aureus* NCTC 6571; however, in the presence of 5% horse serum the MIC was increased to 32 μg mL^{-1}. To increase the potential for compound **10** to be used in in vivo assays, we required a reduction in this level of protein binding. It is widely reported that other benzoic acid based drugs have a high incidence of HSA binding, for example ibuprofen and warfarin.[34,36] Several techniques of combating the serum albumin binding through drug formulation were considered. For example, when using a cyclodextrin as a drug-delivery tool similar to other antibiotics (Cefotiam and Itraconazole)[37] or using serum albumin as a drug carrier.[38] However, we were at-

tracted to structural modification because of the ease of synthesis of our lead compound **10**.

In designing the second iteration of type of compounds, we considered several aspects including, the spatial orientation of the acid groups, the degree of saturation of the molecule and introducing other functionalities that would perform as isosteres of the tris(benzoic acid) **10**. Compounds such as **11** and **12** (Figure 2) were chosen as targets to provide information on the antibacterial properties of the carbocyclic core of our system, whereas other derivatives were chosen because they were considered isosteric replacements to the initial series of derivatives (Scheme 1) or because as a group they contained a range of electronic properties. The target compound **17** was conceived through following the anthranilic acid derived class of antibacterial agents that suggested that the p*K*a of the carboxylic acid functional group plays a role in the potential for interaction with HSA.[39,40] Similar to the methodology described earlier, compounds **11**–**31** (Figure 2) were prepared through a multiple intermolecular Heck cross-coupling reaction protocol from the respective aryl halides and styrenes (see the Experimental Section and Supporting Information).[28]



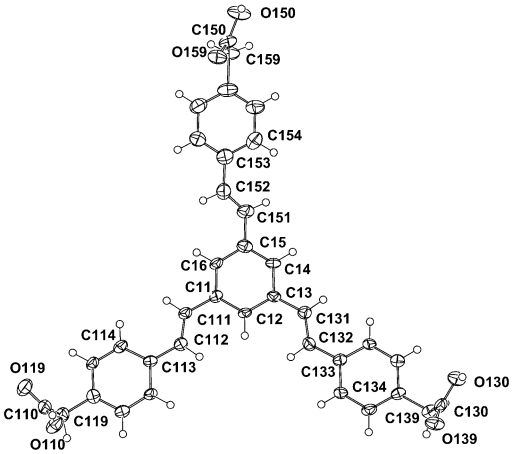


Figure 2. Series of prepared compounds as derivatives of lead triacid compound **10** and the structure of tris(acid) **17**.[41]

Initially, as a definitive test of the unsaturated tristilbene core of the active compound **10** analogues **11**–**20** were screened for activity against *S. aureus* NCTC 6571 by using the disc diffusion assay (Table 5). This data indicates that the presence of a carboxylic acid moiety was essential for retaining activity against *S. aureus*. The tetrazole bioisoteric replacement was also deemed a good replacement for the acid group. Unfortunately, the stability of the Heck product **18** resulting from the reaction between tribromide **5** and the aryl azide was poor and MIC results for this substrate were inconsistent.

Table 5. Disc diffusion inhibition zone diameters [mm] of compounds **17**, **25**, **26**, and **29** (10 mg mL^{-1}).[a]

Organism	Inhibition zones [mm] for compounds[b]			
	17	**25**	**26**	**29**
Bacillus cereus ATCC 13061	12	NI	NI	NI
Bacillus subtilis ATCC 6633	10	NI	NI	NI
Clostridium difficile NCTC 43593	9	7	NI	11
Clostridium perfringens ATCC 2734	10	NI	NI	10
Enterococcus faecalis NCTC 775	9	NI	NI	NI
Enterococcus faecium ATCC 19434	NI	NI	NI	NI
Listeria monocytogenes NCTC 7973	NI	12	NI	15
Micrococcus luteus ATCC 10240	22	2	NI	8
Moraxella catarrhalis ATCC 25238*	20	32	18	32
Staphylococcus aureus NCTC 6571	29	14	9	8
Staphylococcus aureus NCTC 10442	30	12	NI	NI
Staphylococcus epidermidis ATCC 12228	30	11	NI	NI
Staphylococcus xylosus ATCC 29971	20	NI	NI	NI
Streptococcus pneumoniae ATCC 49619	26	16	9	23
Streptococcus pyogenes NCTC 8191	22	14	8	20

[a] NI = no inhibition; * = Gram negative. [b] Results are from two independent tests.

A range of similarly functionalised compounds was also prepared. Compounds **23**–**26** were chosen to investigate the effects of the number and spatial arrangement of the carboxylic acid groups. Alternatively, compounds **27**–**31** were chosen to investigate the effect of introducing additional degrees of freedom to the core-to-periphery linker, by way of the alkene reduction to the alkane (Figure 2). Unfortunately, the linear unsaturated acid derivatives (compounds **23** and **24**) along with saturated compounds **27**, **28**, **30** and **31** did not show any inhibitory activity with *S. aureus* NCTC 6571 (Table 5) by disc diffusion (10 mg mL^{-1}). However, the derivatives **25**, **26** and **29** had complementary activity while compound **17** indicated more potent antibacterial activity using this assay. Compounds **17**, **25**, **26** and **29** were therefore screened for activity against a subset of the original screening set comprising all 14 Gram-positive bacteria, 11 of the Gram-negative bacterial strains and *C. albicans*.

These compounds showed little activity against the yeast and Gram-negative bacteria but were generally active against Gram-positive bacteria. The exception to this trend was the potent activity against *M. catarrhalis* NCTC 3625. Of the derivatives assayed, compound **17** was vastly more antimicrobially active with exceptional inhibition of several *S. aureus*, other Staphylococci and *M. catarrhalis* bacterial

strains (Table 5). The increased flexibility in the orientation introduced in compounds **27**, **28**, **30** and **31** resulted in a loss of this activity and thus we believe that the structural rigidity imparted by the conjugation in the central core linking the tethers was required for potency. The exception to this rule was compound **29**, which showed reasonable activity against *S. pyogenes* NCTC 8191 and *S. pneumoniae* ATCC 49619. In summary, the second series of compounds has indicated that the acid moiety was essential for antibacterial activity and, as expected, the spatial arrangement of the carboxylic acid groups appears to be critical to specific antibacterial activity. Even though the carboxylic acid derivatives **25**, **26** and **29** showed moderate activity, the acid derivative **17** was the most promising and was further examined in MIC and MBC assays (Table 6). Compound **17** was more potent than compound **10** against the majority of test organisms. However, when compound **17** was tested in the presence of 5, 10 and 30% of horse serum the MIC's for *S. aureus* NCTC 6571 were 2, 16 and 32 $\mu g\,mL^{-1}$, respectively, which indicated a decrease of activity in the presence of serum. This increase in MIC suggests that activity is possibly affected by serum in a manner similar to compound **10** (Table 2).

Table 6. MICs and MBCs ($\mu g\,mL^{-1}$) of compound **17**.

Organism	MIC	MBC
Moraxella catarrhalis ATCC 25238	0.25	4
Moraxella catarrhalis NCTC 3622	0.06	0.5
Staphylococcus aureus NCTC 6571	1	>32
Staphylococcus aureus ATCC 29213	0.25	>32
Staphylococcus aureus NCTC 10442	0.125	>32
Staphylococcus aureus ATCC 33592	0.125	>32
Staphylococcus epidermidis ATCC 12228	0.5	>32
Staphylococcus xylosus ATCC 29971	1	>32
Streptococcus pneumoniae ATCC 49619	32	>32
Streptococcus pyogenes NCTC 8191	32	>32
Streptococcus pyogenes NCTC 8302	32	>32

Comparison of the above results to other antibacterial agents shows that the level of activity is generally similar. For example, typical MICs for *S. aureus* are 2 $\mu g\,mL^{-1}$ for vancomycin, 4 $\mu g\,mL^{-1}$ for linezolid, 1 $\mu g\,mL^{-1}$ for daptomycin and 4 $\mu g\,mL^{-1}$ for oxacillin.[42] The tristilbene-based compounds described in this paper are clearly most active against Gram-positive bacteria and have little activity against Gram-negative bacteria. This difference in activity may indicate that compounds **10** and **17** cannot penetrate the Gram negative outer membrane, with the obvious exception of *M. catarrhalis*. The basis for the susceptibility of *M. catarrhalis* to these novel compounds remains to be determined.

When the Ames mutagenicity assay was repeated for compound **17** (Table 7) results were similar to compound **10** (Table 3) in that there was no evidence of mutagenicity. In addition, no haemolysis of sheep erythrocytes was evident at concentrations up to and including 50 $\mu g\,mL^{-1}$, but 13.01% of erythrocytes were lysed at 500 $\mu g\,mL^{-1}$ (Table 8).

Table 7. Mutagenicity of compound **17** determined by the Ames test with three *Salmonella* strains.[a]

Strain	Control		Compound [μg/plate]		
	negative	positive	100	300	1000
TA98	19	1293	23	19	12
TA100	120	1119	126	103	97
TA1535	27	2037	23	23	25

[a] Values represent mean numbers of colonies per agar plate.

Table 8. Cytotoxicity of compound **17**.[a]

Assay	Concentration of compound [$\mu g\,mL^{-1}$]				
	0	0.5	5	50	500
haemolysis [%]	0.0	0.14 (0.50)	−0.30 (0.19)	0.03 (0.19)	13.01 (1.67)

[a] Values represent the mean (standard deviation).

Conclusion

This study has illustrated the synthesis of novel tristilbene compounds, a potentially new class of antibacterial agents. Two particular compounds each bearing carboxylic acid moieties, exhibit good activity against several bacterial strains, including MRSA at concentrations comparable to frontline antibiotics. Compound **10** proved highly active against *M. catarrhalis* with MIC's of 0.12–0.25 $\mu g\,mL^{-1}$ and against *Staphylococcus* spp. with MICs ranging from 2 to 4 $\mu g\,mL^{-1}$. The acid derivative **17** showed more activity with MICs of 0.06–0.25 $\mu g\,mL^{-1}$ against *M. catarrhalis* and 0.12–1 against *Staphylococcus* spp. This level of activity is similar to that reported for *S. aureus* for antibiotics such as vancomycin, with MICs of $\leq 2\ \mu g\,mL^{-1}$ and clindamycin with MICs of $\leq 0.5\ \mu g\,mL^{-1}$. Similarly, *M. catarrhalis* is susceptible to antibiotics including erythromycin and ciprofloxacin at $\leq 0.25\ \mu g\,mL^{-1}$. These comparisons indicate that the novel tristilbene compounds synthesised in this study may have therapeutic potential although clearly further research is required.

Experimental Section

Chemistry experimental

General protocol: Starting materials and reagents were purchased from Sigma–Aldrich or Merck chemical companies. *N*-Methyldicyclohexylamine was distilled under reduced pressure and stored under argon. $[Pd_2(dba)_3]\cdot CHCl_3$,[43] were prepared as previously described. All reactions were performed under argon and at ambient temperature unless stated otherwise. All solvents used in reactions were anhydrous unless noted otherwise. Anhydrous solvents were distilled over the appropriate drying agent or acquired from a Pure Solv 5-Mid Solvent Purification System (Innovative Technology Inc.). ^1H and ^{13}C NMR spectra were acquired on a Varian 300, Varian 400, Bruker AV500 or a Bruker AV600 spectrometer and all signals δ are reported in parts per million (ppm). ^1H and ^{13}C assignments where indicated were made with the aid of DEPT, COSY, HSQC and HMBC sequences. Chemical shifts were referenced to the residual (partially) undeuterated solvents and reported in parts per million (ppm). IR samples were prepared by using the KBr disc method and samples acquired on a Perkin–Elmer Spectrum One spectrometer at 2 cm^{-1} resolution. Melting points were recorded on a Reichart heated-

stage microscope. The reported retention factors (R_f) were acquired via Thin Layer Chromatography (TLC) performed on Merck silica gel 60 F254 pre-coated aluminium sheets. Column chromatography was performed by using silica gel 60 (0.04–0.063) supplied by Merck. Bulk Chromatography solvents were distilled prior to use.

General protocol for Heck cross-coupling reactions: Halobenzene (1 equiv), $[Pd_2(dba)_3]\cdot CHCl_3$ (2–15 mol%) and $[(t\mathrm{Bu})_3PH]BF_4$ (10–60 mol%) were added to a flame-dried Schlenk flask, which was subsequently dried under vacuum for 15 min before being dissolved in dry THF. *N*-Methyldicyclohexylamine (4 equiv) and functionalised styrene (3.3 equiv) were added via syringe. Progress of the reaction was monitored by TLC (neat CH_2Cl_2) analysis. On completion of the reaction, the residual THF was removed under vacuum and the crude material re-dissolved in CH_2Cl_2, filtered to remove any insoluble material before being absorbed onto fine silica and eluting with a mobile phase in a range of 0:100 to 2:98 MeOH/CH_2Cl_2.

General protocol for saponification reactions: The ester (1 equiv) and LiOH (2 equiv per ester) were dissolved in 9:1 H_2O/MeOH or H_2O/EtOH, depending upon the ester, and refluxed overnight. After cooling to room temperature, the solvent was removed under reduced pressure, and the remaining solution diluted with H_2O, cooled in an ice-bath and the pH adjusted to 3 by the addition of HCl (1 M). The precipitate was collected, filtered and product dried under vacuum.

4,4′,4″-(1*E*,1′*E*,1″*E*)-2,2′,2″-(Benzene-1,3,5-triyl)tris(ethene-2,1-diyl)-tris(*N*,*N*-dimethylaniline) (8): Prepared as per the standard Heck cross-coupling procedure. ^{1}H NMR (500.1 MHz, $CDCl_3$): $\delta = 2.99$ (s, 18H; CH_3), 6.74 (app d, 6H; Ar-AA′-BB′), 6.95 (d, $J = 16.4$ Hz, 3H; CH=CH), 7.14 (d, $J = 16.4$ Hz, 3H; CH=CH), 7.48 (s, 3H), 7.46 ppm (app d, 6H; Ar-AA′-BB′); ^{13}C NMR ($CDCl_3$): $\delta = 40.5$, 112.5, 122.3, 124.5, 125.9, 127.6, 128.8, 138.5, 150.1 ppm; MS (70 ev; FAB): *m*/*z*: 514.9 (13%) $[MI+H]^+$, 513 (59%) $[MI]^+$, 512 (100%) $[MI-H]^+$, 326.9 (10%), 207.0; HRMS-EI$^+$: calcd for $C_{36}H_{39}N_3$: 513.3144; found: 513.3114; HRMS (EI$^+$): calcd: 512.3065 $[MI+H]^+$; found: 512.3050.

1,3,5-Tris[(1*E*)-2′-(ethyl 4″-benzoate)vinyl]benzene (9): Prepared as per the standard Heck cross-coupling procedure by using 1,3,5-tribromobenzene (**8**) (1010 mg, 3.21 mmol), $Pd_2(dba)_3.CHCl_3$ (882 mg, 0.85 mmol), ($t\mathrm{Bu}_3$)$PHBF_4$ (560 mg, 1.93 mmol), *N*,*N*-dicyclohexylmethylamine (3.0 mL), ethyl 4-vinylbenzoate (1870 mg, 10.61 mmol) and THF (40 mL). The product was eluted with 2:98 MeOH/CH_2Cl_2 and recrystallized from CH_2Cl_2/EtOH to give **10** as an off-white powder, (1.86 g, 97%). This compound matched that previously reported by this group.[28]

1,3,5-Tris[(1*E*)-2′-(4″-benzoic acid)vinyl]benzene (10): By using the standard saponification procedure, triester **9** (252.1 mg, 0.42 mmol), LiOH.H_2O (112.0 mg, 2.7 mmol) and 1:9 H_2O/EtOH (20 mL) gave a gelatinous precipitate that was collected and recrystallized from THF/H_2O and dried to give the triacid **10** as a pale-brown powder (209 mg, 95%). This compound matched that previously reported by this group.[28]

1,3,5-Tristyrylbenzene (11), 1,3,5-tris(4-methylstyryl)benzene (12) and 1,1′,1″-[4,4′,4″-(1*E*,1′*E*,1″*E*)-2,2′,2″-(benzene-1,3,5-triyl)tris(ethene-2,1-diyl)tris(benzene-4,1-diyl)]triethanone (13): All compounds were prepared according to the as per the standard Heck cross-coupling procedure. The spectral data for this compound matched those reported in the literature.[44]

4,4′,4″-(1*E*,1′*E*,1″*E*)-2,2′,2″-(Benzene-1,3,5-triyl)tris(ethene-2,1-diyl)tris(-benzene-4,1-diyl)triacetate (14): Reaction completed as indicated in the general Heck protocol section on 1,3,5-tribromobezene **5** (200 mg, 0.63 mmol) to produce triacetate **14** as a bright-yellow solid (188 mg, 58%). M.p. 105–125 °C; $R_f = 0.2$ (1:4 ethyl acetate/hexane); ^{1}H NMR (500.1 MHz, $CDCl_3$): $\delta = 2.32$ (s, 9H; CH_3), 7.09 (d, $J = 16.2$ Hz, 3H; CH=CH), 7.11 (d, 6H; Ar-AA′-BB′), 7.18 (d, $J = 16.2$ Hz, 3H; CH=CH), 7.54 (s, 3H), 7.55 ppm (d, 6H; Ar-AA′-BB′); ^{13}C NMR ($CDCl_3$): $\delta =$ 169.6 (C9′), 150.3 (C6′), 138.1 (C1), 135.1 (C3′), 128.7 (C1′), 128.5 (C2′), 127.7 (C4′/C8′), 124.1 (C2), 122.0 (C5′/C7′), 21.3 ppm (C10′); IR (neat): $\tilde{\nu} = 3436$, 1757 (C=O), 1505, 1370, 1215, 1197, 1165 cm^{-1}; HRMS (ESI): *m*/*z*: calcd for $C_{36}H_{30}O_6K$: 597.1679; found: 597.1663.

4,4′,4″-(1*E*,1′*E*,1″*E*)-2,2′,2″-(Benzene-1,3,5-triyl)tris(ethene-2,1-diyl)tribenzonitrile (15): Reaction completed as indicated in the general Heck protocol section on 1,3,5-tribromobezene (**5**). The spectral data for this compound matched those reported in the literature.[45]

1,3,5-Tris(4-methoxystyryl)benzene (16): Reaction completed as indicated in the general Heck protocol section on 1,3,5-tribromobezene (**5**). ^{1}H NMR (500.1 MHz, $CDCl_3$): $\delta = 3.84$ (s, 9H; OMe), 6.93 (d, $J = 8.6$ Hz, 6H), 7.01 (d, $J = 16.3$ Hz, 3H; CH=CH), 7.15 (d, $J = 16.3$ Hz, 3H; CH=CH), 7.50 ppm (app d, 9H); ^{13}C NMR ($CDCl_3$): $\delta = 55.4$, 114.3, 123.3, 126.6, 127.9, 128.7, 130.3, 138.5, 159.5 ppm. The spectral assignment matched those reported in the literature.[45,46]

Methyl 2-(4-vinylphenyl)acetate: 1,8-Diazabicycloundec-7-ene (1.707 g, 7.69 mmol) was added to a magnetically stirred solution of 2-(4-vinylphenyl)acetic acid (0.95 g, 5.92 mmol) in THF (10 mL) at 0 °C. The ensuing solution was treated in one portion with MeI (0.47 mL, 1.09 g, 7.69 mmol) and the mixture was stirred at room temperature for 3 h before being diluted with diethyl ether (20 mL). The mixture was then washed with H_2O (10 mL), HCl (2 N, 10 mL), NaOH (2 N, 10 mL), HCl (2 N, 10 mL), and H_2O (10 mL). The organic phase was then dried (Na_2SO_4), filtered and concentrated under reduced pressure to afford the desired product as a white solid (59.8 mg, 58%). The spectral data matched those reported in the literature. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.37$ (d, $J = 7.3$ Hz, 2H; H4/H6), 7.24 (d, $J = 7.3$ Hz, 2H; H3/H7), 6.70 (dd, $J = 17.6$, 12.0 Hz, 1H; H8), 5.73(d, $J = 17.6$ Hz, 1H; H9), 5.23 (d, $J =$ 12.0 Hz, 1H; H10), 3.69 (s, 3H; H11), 3.62 (s, 2H; H1).

2,2′,2″-{[(1*E*,1′*E*,1″*E*)-benzene-1,3,5-triyltris(ethene-2,1-diyl)]tris(benzene-4,1-diyl)}triacetic acid- (17): Prepared through a Heck cross-coupling reaction with between methyl 2-(4-vinylphenyl)acetate and 1,3,5-tribromobenzene (**8**) followed by saponification. Trimethyl 2,2′,2″-{[(1*E*,1′*E*,1″*E*)-benzene-1,3,5-triyltris(ethene-2,1-diyl)]tris(benzene-4,1-diyl)}triacetate: Reaction completed as indicated in the general protocol section on 1,3,5-tribromobezene (**5**) (200 mg, 0.63 mmol) and methyl 2-(4-vinylphenyl)acetate (368 mg, 2.09 mmol) to produce triacetate as a bright-yellow oil (190 mg, 50%). $R_f = 0.17$ (20% ethyl acetate/hexane); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.45$ (s, 3H; H2), 7.43 (d, $J = 8.8$ Hz, 6H; H5′/H7′), 7.22 (d, $J = 7.6$ Hz, 6H; H4′/H8′), 7.09 (d, $J = 16.2$ Hz, 3H; H2′), 7.03 (d, $J = 16.2$ Hz, 3H; H1′), 3.63 (s, 9H; H11′), 3.57 ppm (s, 6H; H9′); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 172.1$ (C10′), 138.2 (C3′), 136.3 (C1), 133.6 (C6′), 129.8 (C5′/C7′), 129.0 (C2′), 128.5 (C1′), 126.9 (C4′/C8′), 124.1 (C2), 52.3 (C11′), 41.1 ppm (C9′); IR (neat): $\tilde{\nu} = 3026$, 2950, 1730 (C=O), 1585, 1512, 1434, 1153 cm^{-1}; HRMS (API): calcd for CHO: 601.2590 $[M+H]^+$; found: 601.2604; triacetate from above (454.8 mg, 0.77 mmol) and LiOH·H_2O (292.7 mg, 6.98 mmol) were dissolved in EtOH/H_2O (1:9) (30 mL) and magnetically stirred at refluxed for 17 h. The reaction mixture was then cooled, the solvent was removed under reduced pressure, and the remaining mixture was diluted with water (10 mL). After cooling to 0 °C, HCl (1 M) was added to the mixture until the pH was adjusted to 3 and a grey precipitate resulted. The residue was collected by vacuum filtration and was recrystallised from THF/H_2O to afford a light-brown solid (241 mg, 57%). M.p. 100–108 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.43$ (d, $J = 8$ Hz, 6H; H4′/H8′), 7.39 (s, 3H; H2), 7.23 (d, $J = 7.6$ Hz, 6H; H5′/H7′), 7.08 (d, $J = 16.4$ Hz, 3H; H2′), 6.99 (d, $J = 16.4$ Hz, 3H; H1′), 3.57 ppm (s, 6H, H9′); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 175.3$ (C10′), 139.3 (C3′), 137.3 (C1), 135.3 (C6′), 130.5 (C5′/C7′), 129.7 (C2′), 129.1 (C1′), 127.5 (C4′/C8′), 124.7 (C2), 41.5 ppm (C9′); IR (neat): $\tilde{\nu} = 3026$ (O−H), 1701 (C=O) cm^{-1}; HRMS (ESI): *m*/*z*: calcd for $C_{36}H_{31}O_6$: 559.2121 $[M+H]^+$; found: 559.2120.

4,4′,4″-[(1*E*,1′*E*,1″*E*)-Benzene-1,3,5-triyltris(ethene-2,1-diyl)]triphenol (19): Triacetate **14** (150 mg, 0.31 mmol) and K_2CO_3 (387 mg, 2.8 mmol) were dissolved in MeOH/H_2O (6 mL, 1:1) and magnetically stirred at reflux for 20 h. After cooling, H_2O (5 mL) was added and the mixture was extracted with EtOAc (3×10 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4) and filtered. The solvent was removed under reduced pressure to afford a crude oil that was then purified by column chromatography (100% ethyl acetate) to afford a brown solid (92 mg, 83%). M.p. 135–140 °C); $R_f = 0.47$ (100% ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.52$ (s, 3H; H_2), 7.45 (d, $J = 8.4$ Hz, 6H; H_5/$H_{7'}$), 7.19 (d, $J = 16.4$ Hz, 3H; $H_{2'}$), 7.03 (d, $J = 16.4$ Hz, 3H; $H_{1'}$), 6.80 ppm (d, $J = 8.8$ Hz, 6H; H_4/$H_{8'}$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 158.3 ($C_{6'}$), 139.6 (C_1), 130.5 (C_4/$C_{8'}$), 129.7 ($C_{3'}$), 128.9 ($C_{1'}$), 126.7 ($C_{2'}$),

123.8 (C_2), 116.5 ($C_{5'}/C_{7'}$); IR (neat): $\tilde{\nu}$=3299 (OH), 3023, 1606, 1582, 1508, 1233, 1169 cm^{-1}; HRMS (API): calcd for $C_{30}H_{25}O_3$: calcd for 433.1804 $[M+H]^+$; found: 433.1823.

3,3′,3″-({[(1*E*,1′*E*,1″*E*)-Benzene-1,3,5-triyltris(ethene-2,1-diyl)]tris(benzene-4,1-diyl)}tris(oxy))tris(3-oxopropanoic acid) (20): Triphenol **19** (70 mg, 0.162 mmol) was magnetically stirred with 2,2-dimethyl-1,3-dioxane-4,6-dione (77 mg, 0.53 mmol) in toluene (2 mL) and the resulting solution heated at reflux for16 h. Upon cooling, the desired product was precipitated and was collected by vacuum filtration as a yellow/brown solid (91.9 mg, 82%). M.p. 110–120°C; ^{1}H NMR (400 MHz, MeOD): δ= 7.42 (s, 3H; H_2), 7.37 (d, J=7.6 Hz, 6H; $H_4/H_{8'}$), 7.09 (d, J=16 Hz, 3H; $H_{1'}$), 6.93 (d, J=16 Hz, 3H; $H_{2'}$), 6.76 (d, J=7.6 Hz, 6H; $H_{5'}/H_{7'}$) 3.67 ppm ($H_{10'}$); ^{13}C NMR (100 MHz, MeOD): δ=170.9 ($C_{9'}/C_{11'}$), 158.5 ($C_{6'}$), 139.9 (C_1), 130.7 ($C_{3'}$), 130.0 (C), 129.2 ($C_4/C_{8'}$), 126.9 ($C_{2'}$), 124.0 (C_2), 116.7 ($C_{5'}/C_{7'}$), 42.0 ppm ($C_{10'}$); IR (neat): $\tilde{\nu}$=3023 (O−H), 1701 (C=O) cm^{-1}; HRMS (ESI): m/z: calcd for $C_{39}H_{31}O_{12}$: 691.1816 $[M+H]^+$; found: 691.1816.

1,3,5-Tris(3,4,5-trimethoxystyryl)benzene (21): The reaction completed as indicated in the general Heck protocol section on 1,3,5-tribromobezene **5** (200 mg, 0.63 mmol) to produce **21** as a light-brown solid (120 mg, 29%). M.p. 170–182°C; R_f=0.31 (1:9 ethyl acetate/toluene); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.55 (s, 3H; H_1), 7.14 (d, J=16.1 Hz, 6H; $H_{2'}$), 7.05 (d, J=16.1 Hz, 6H; $H_{1'}$), 6.78 (s, 6H; $H_4/H_{8'}$), 3.93 (s, 18H; $H_{10'}/H_{11'}$), 3.89 ppm (s, 9H; $H_{9'}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ=153.6 ($C_{5'}/C_{7'}$), 138.3 ($C_{6'}$), 138.1 ($C_{3'}$), 133.0 (C_1), 129.4 ($C_{2'}$), 127.9 ($C_{1'}$), 123.9 (C_2), 103.8 ($C_4/C_{8'}$), 61.1 ($C_{9'}$), 56.3 ppm ($C_{10'}/C_{11'}$); IR (neat): $\tilde{\nu}$=2933, 2835, 1580, 1505, 1452, 1416, 1334, 1236, 1122, 1040, 1003 cm^{-1}; HRMS (API): calcd for $C_{39}H_{43}O_9$: 655.2907 $[M+H]^+$; found: 655.2915.

1,3,5-Tris(3,4-diisopropoxystyryl)benzene (22): Prepared as per the standard Heck cross-coupling procedure. ^{1}H NMR (600 MHz, $CDCl_3$): δ= 1.35 (d, J=6 Hz, 3H; CH_3), 1.37 (d, J=6 Hz, 3H; CH_3), 4.49–4.57 (m, 6H; CH), 6.91 (d, J=8.3 Hz, 3H; ArH), 6.99 (d, J=16.3 Hz, 3H; *CH*=CH), 7.07–7.13 (m, 6H; ArH, *CH*=CH), 7.14 (d, J=2.0 Hz, 3H; ArH), 7.49 ppm (s, 3H; ArH); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ=22.4, 22.5, 72.3, 72.6, 116.6, 117.5, 118.0, 120.9, 123.4, 126.8, 129.0, 131.3, 138.4, 149.3, 149.5 ppm.

Compounds **23**–**31** were prepared in good yields through the procedure outlined in our previous publication through a series of Heck cross-couplings, hydrogenation, and saponification reactions.[28]

Microbiology experimental

Micro-organisms: Bacteria and fungi were obtained from the culture collection of the School of Pathology and Laboratory Medicine at The University of Western Australia (UWA). Of the test organisms listed below, those marked with an asterisk were used in the initial screening of compound **10** and were not used for the subsequent screening of compounds **25**, **26**, **29**, and **17**; *Acinetobacter baumannii* ATCC 15308, *A. baumannii* ATCC 19606*, *Aeromonas hydrophila* NCTC 8049, *Bacillus cereus* ATCC 13061, *B. subtilis* ATCC 6633, *Bacteroides fragilis* ATCC 23745*, *Burkholderia cepacia* ATCC 25416*, *Candida albicans* ATCC 90028, *Citrobacter freundii* NCTC 9750, *Clostridium difficile* ATCC 43593, *C. perfringens* ATCC 27324, *Enterobacter aerogenes* ATCC 13048*, *E. cloacae* NCTC 10005*, *Enterococcus faecalis* NCTC 775, *E. faecium* ATCC 19434, *Escherichia coli* NCTC 10538, *Klebsiella edwardsii* NCTC 10896, *K. pneumoniae* ATCC 13883*, *Listeria monocytogenes* NCTC 7973, *Micrococcus luteus* ATCC 10240, *Moraxella catarrhalis* NCTC 3622, *M. catarrhalis* ATCC 25238, *Morganella morganii* NCTC 235*, *Proteus mirabilis* NCTC 10975, *P. vulgaris* NCTC 4635*, *Pseudomonas aeruginosa* NCTC 10662, *Salmonella enterica subsp. enterica serovar Typhimurium* ATCC 13311, *Serratia marcescens* NCTC 1377*, *Shigella flexneri* NCTC 8192, *Staphylococcus aureus* NCTC 6571, *S. aureus* (MRSA) NCTC 10442, *S. epidermidis* ATCC 12228, *S. xylosus* ATCC 29971, *Stenotrophomonas maltophilia* ATCC 13637, *Streptococcus pneumoniae* ATCC 49619, *S. pyogenes* NCTC 8191, *Vibrio cholerae (non-toxigenic)* M3695 and *Yersinia enterocolitica* ATCC 9610.

Antimicrobial agents: Stock solutions of all novel compounds were made by dissolving each in dimethyl sulfoxide (DMSO) at 10 $mg\,mL^{-1}$.

Disc diffusion method: The disc diffusion method was based on that recommended by the Clinical and Laboratory Standards Institute (CLSI, 2012a).[47] Briefly, inocula were prepared by suspending growth from fresh cultures on blood agar in 0.85% saline and adjusting to a cell concentration of approximately 10^8 colony forming units (cfu) per mL (0.5 McFarland turbidity). Cell suspensions were then swab-inoculated onto Mueller-Hinton (MH) agar plates (Oxoid Ltd., Hampshire, England). A blank 6 mm AA paper disc (Whatman Ltd., England) was placed in the centre of each plate and 20 μL of the antimicrobial agent (10 $mg\,mL^{-1}$) was pipetted onto the disc. Cultures were then incubated at 37°C for 24 h under the appropriate conditions. After incubation, zones of inhibition were measured to the nearest millimetre. Assays were repeated twice per organism/derivative combination.

Broth micro-dilution method: Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined by using methods recommended by the Clinical and Laboratory Standards Institute (CLSI 2012b).[48] Briefly, each compound was serially diluted two-fold in 100 μL volumes of Mueller Hinton broth in a 96-well microtitre tray (Falcon, Becton Dickinson, USA). Inocula were prepared by adjusting cell suspensions prepared from overnight blood agar cultures to 0.5 McFarland turbidity standard and then diluting 1 in 100 to give a cell suspension of approximately 1×10^6 $cfu\,mL^{-1}$. Each microtiter well was inoculated with 100 μL of a bacterial inoculum to result in final inocula concentrations of 5×10^5 $cfu\,mL^{-1}$ and the trays were incubated at 37°C for 24 h under the appropriate conditions. MICs were then determined visually as the lowest concentration of antimicrobial preventing growth. MBCs were determined by subculturing 10 μL volumes from non-turbid wells onto blood agar and incubating overnight. The lowest concentration of an antimicrobial with no resultant growth was determined as the MBC. This assay was repeated twice per organism/derivative combination. Where results differed the higher value was selected as the final result.

Haemolytic activity assay: As an indicator of cytotoxicity, the haemolysis of sheep erythrocytes by compounds **10** and **17** was investigated. Master stock solutions of compound **10** (10 $mg\,mL^{-1}$) were made by dissolving 20 mg of the dehydrated compound in 2 mL 100% DMSO. They were stored in foil-covered glass bottles at −20°C. Solutions stored in this way retained full antimicrobial activity for a minimum period of 6 weeks (results not shown). Serial 10-fold dilutions of compounds **10** and **17** were performed in phosphate-buffered saline (PBS) to make solutions of 1000, 100, 10 and 1 $\mu g\,mL^{-1}$. In microcentrifuge tubes, 500 μL of each dilution was combined with 480 μL PBS and 20 μL washed sheep erythrocytes (100%) so that the final concentration of erythrocytes was 2% and the final concentrations of compound were 500, 50, 5 and 0.5 $\mu g\,mL^{-1}$. Dilutions of DMSO alone were prepared and tested as above to check for haemolysis due to DMSO. A positive control (100% haemolysis) was prepared with 980 μL water and 20 μL erythrocytes. A negative control was prepared with 980 μL PBS and 20 μL erythrocyte suspension. Tubes were incubated at 37°C for 2 h on a rocker then centrifuged at 12,000 g for 5 min. The optical density of the supernatant was determined by transferring 100 μL volumes of each reaction to a microtitre tray then reading the OD at 540 nm. Percentage haemolysis was determined by blanking the OD against that of the negative control and presenting the resulting OD as a proportion of the OD of the positive control (blanked with water). All dilutions and controls were prepared in duplicate and the entire assay was repeated twice on separate occasions.

Cytotoxicity assay: The cytotoxocity of compound **10** was assessed by using an in vitro cytotoxicity assay. Mammalian fibroblast L929 cells grown to approximately 80% confluency in HGM-M, were washed with Hanks, to detach the cells from the flask, then diluted to 105 cells/mL in HGM-M. Volumes of 200 μL were used to inoculate the wells of a 96-well microtitre tray. After incubation for 24 h at 37°C, adherent cells were washed with Hanks and then 100 μL of HGM-M was added to the wells. Compound **10** (10 $mg\,mL^{-1}$ in DMSO) was serially 10-fold diluted in HGM-M to make solutions of 1000, 100, 10 and 1 $\mu g\,mL^{-1}$. Volumes of 100 μL of each solution were then added to the wells of the microtitre tray giving the final concentrations of 500, 50, 5 and 0.5 $\mu g\,mL^{-1}$. Equivalent dilutions of DMSO were prepared and tested to check that cytotoxic

activity was not due to the DMSO. A negative control was prepared containing only HGM-M, and a positive control was prepared by adding 100 μL of carboplatin (10 mg mL^{-1}; Mayne Pharma Pty Ltd, Australia) to wells containing 100 μL HGM-M. Controls and dilutions of compound **10** and DMSO were prepared in duplicate. After 24 h incubation at 37 °C with shaking, cytotoxicity was quantified using the neutral red assay. The cells were washed with Hanks then 200 μL HGM-M and 20 μL Neutral Red (3.3 g L^{-1}; Sigma–Aldrich) was added to each well. After 2 h incubation at 37 °C with shaking, cells were washed twice with PBS then 200 μL of 1 % acetic acid in 50 % EtOH was added to each well to solubilise the stain. After 15 min incubation at 37 °C with shaking the optical densities of the wells at 690 nm were determined and subtracted from the OD540. Values were then blanked against wells to which no cells had been added, and converted to a ratio of the OD of the negative control. Ratios of $\leq$ 0.5 indicated a cytostatic effect. Testing was performed on two separate occasions.

Ames test: The Ames test for mutagenicity was based on the method published by Zeiger and Mortelmans,[32] in the absence of a metabolic activation system. To prepare inocula, single colonies from overnight BA cultures of three commonly used Salmonella tester strains, *S. typhimurium* TA98, TA100 and TA1535, were used to inoculate 10 mL of nutrient broth. After incubation for 15–18 h at 37 °C with shaking, the concentration of the culture was appropriate for use in the test (~1–2 × 109 cfu/mL). Glucose minimal agar plates (20 mL volume) were dried thoroughly. Molten top agars (2 mL) were prepared, supplemented with biotin and trace histidine, and maintained at 43–48 °C before adding 50 μL of the bacterial culture (~1 × 108 cells) and 100 μL of test solution (see below). The molten top agar was then poured directly over the surface of the glucose minimal agar and gently swirled to ensure even distribution. Once solidified, plates were incubated at 37 °C for 48 h and then bacterial colonies were counted. Test solutions were prepared as follows and included three concentrations of compound **10** or **17**, a negative solvent control and a positive control (selected from the recommended positive control chemicals and test concentrations) for each strain. Compounds **10** and **17** were assessed by incorporating 100 μL of the stock solution (10 mg mL^{-1} in DMSO) directly into molten top agar to test the compound at 1000 μg/plate. Dilutions of the stock were prepared in sterile distilled water to also test 300 μg/plate and 100 μg/plate. DMSO (100 μL of 100 %) was incorporated into molten top agar as the negative solvent control. Positive controls were 4-nitro-o-phenlenediamine at 2.5 μg/plate (for TA98) and sodium azide at 5 μg/plate (for TA100 and TA1535). A mutagenic effect is indicated when colony counts are two to three times greater with test compound than on the negative solvent control plate and this is regarded as 'positive'. In these cases, the increase in colonies is usually dose related. A positive result in this test is highly predictive of rodent carcinogenicity. Testing was performed in duplicate on two separate occasions.

Acknowledgements

We are indebted for the NMR support provided by Prof. L. Byrne and MS support provided by Dr A. Reeder. The authors acknowledge the facilities, and the scientific and technical assistance of the Australian Microscopy & Microanalysis Research Facility at the Centre for Microscopy, Characterisation & Analysis, The University of Western Australia, a facility funded by the University, State and Commonwealth Governments. Part of this work was supported by an NH&MRC project grant (353619) to A.J.M., B.M. and T.V.R. This work was also supported by a UWA Pathfinder Grant to A.J.M., T.V.R. and S.G.S. We thank Dr. A. Sierakowski and S. Handford for development and commercialisation advice. R.A.B. acknowledges support from a University Postgraduate Award and N.Y.M. acknowledges support from an Australian Postgraduate Award.

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[41] X-ray crystal-structure analysis is documented in the Supporting Information. CCDC-931125 (**17**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
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Received: August 7, 2013
Published online: ■■ ▮▮, 0000

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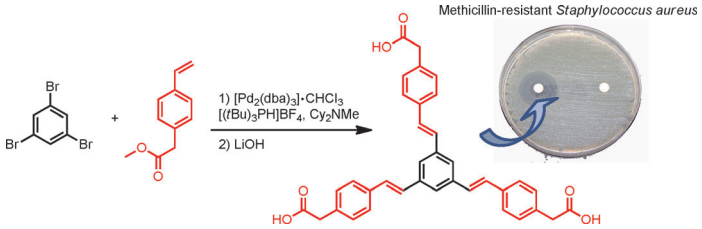

Routes to new antibiotics: The preparation of and antibacterial-based structure–activity relationship studies on a range of stilbene based compounds revealed two related compounds, each bearing carboxylic acid moieties, exhibiting pronounced activity against several bacterial strains including methicillin-resistant *Staphylococcus aureus*, MRSA (see scheme).