Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 12 (2004) 3047–3054
Bioorganic & Medicinal Chemistry

# Antibacterial chalcones—bioisosteric replacement of the 4′-hydroxy group

Simon Feldbæk Nielsen,[†] Thomas Boesen,[‡] Mogens Larsen,[§] Kristian Schønning and Hasse Kromann*,[‡]

*Lica Pharmaceuticals A/S, Fruebjergvej 3, DK-2100 Copenhagen, Denmark*

Received 16 December 2003; accepted 5 March 2004

**Abstract**—Hydroxy chalcones, for example, Licochalcone A, has for several years been known to be antibacterial. The low aqueous solubility and the medium antibacterial potency have limited the usefulness of the compounds. We describe the bioisosteric replacement of the essential 4′-hydroxy group in the hydroxy chalcones with bioisosters of varied degrees of acidity resulting in both more potent and more soluble compounds. The more acidic 4′-hydroxy analogues (e.g., 3′-fluoro- or 3′,5′-difluoro-) gave almost inactive compounds whereas exchanging the hydroxy group with a carboxy group resulted in a potent compound with a high aqueous solubility. Further optimisation and SAR-analysis resulted in soluble and potent carboxy chalcones [e.g., 3,5-dibromo- and 3,5-di(trifluoromethyl)–].

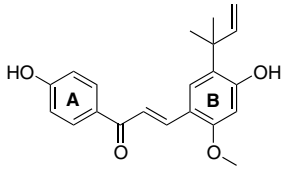

## 1. Introduction

The rapid development of resistance in clinically important Gram-positive bacteria represents a serious public health threat. Every country in the world has antibiotic-resistant bacteria and more than 70% of the bacteria giving rise to hospital acquired infections in the United States are resistant to at least one of the main antimicrobial agents that are commonly used to fight these infections.[1] The proportion of *Staphylococcus aureus* resistant to traditional antibiotics such as methicillin, oxacilin or nafcillin continues to rise and is now more than 50% in intensive care units in the United States.[2] Vancomycin was the last resort for the treatment of multiple drug-resistant *S. aureus*, but recently the first case of fully vancomycin-resistant *S. aureus* has been reported.[3]

Ironically, no new classes of antibiotics have been introduced to the market in the 37 years between the introduction of nalidixic acid in 1962 and linezolid in 2000. All of the antibacterials introduced in this period were modifications of existing molecules. Bacteria have exploited this window of opportunity by developing resistance to all commonly used antibiotics, making the need for new antibiotics more pressing.[4]

Compounds with a chalcone-based structure have shown an array of pharmacological activities, such as antiprotozoal,[5–7] antiinflammatory,[8] immunomodulatory,[9] nitric oxide inhibition,[10] inhibition of the production of interleukin-1[11] and anticancer[12] activities. Licochalcone A (Chart 1), an oxygenated chalcone isolated from the root of Chinese liquorice[13] was shown to posses activity against Gram-positive strains of bacteria.[14,15]

Recently we submitted for publication a study that identified the groups responsible for the antibacterial effect of Licochalcone A against *Staphylococcus aureus*.[16] It was shown that the 4′-hydroxy group in the A-ring was essential for the activity preferably in




**Licochalcone A**

**Chart 1.**

* Corresponding author. Tel.: +45-39178301; e-mail: hk@medchem.dk
[†] Present address: LEO Pharma., Ballerup DK-2750, Denmark.
[‡] Present address: MedChem Aps., Copenhagen DK-2100, Denmark.
[§] Present address: H. Lundbeck A/S., Valby DK-2500, Denmark.

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.03.071

combination with lipophilic substituents in the B-ring. The generally low solubility of chalcones has been one of the major obstacles in the development of this type of compounds to usable drugs: The compounds do not dissolve in the intestine and are not available for absorption, making the bioavailability extremely low and the dose level unrealistic high.

In this study the possibility of changing the proteolytic properties of the 4′-hydroxy group making it more acidic and exchanging the hydroxy group with carboxylic acids or a bioisoster of this group was investigated. We have prepared a number of acidic chalcone analogues in order to find the optimal bioisoster with regard to antibacterial potency. In addition, having an acidic group in the molecule markedly increases aqueous solubility of the compounds compared to the parent phenol compound. A detailed structure–activity relationship analysis of the most promising group of compounds was performed.

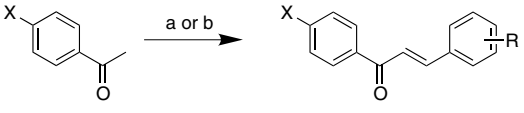
In order to simplify the synthetic work the following investigation is based on the simple chalcone analogue **1**[5] (Table 1), designed on the basis of the SAR described above, as the lead compound. The compound has a 4′-hydroxy group and lipophilic substituents in the B-ring being equipotent to Licochalcone A against *S. aureus*.

## 2. Results and discussion

### 2.1. Chemistry

The Claisen–Schmidt condensation of hydroxy acetophenones or carboxy acetophenones is well described in the literature, using either basic[17,18] or acidic[19–21] catalysis. We found that the best results were obtained using basic catalysis. All the chalcone products described were prepared using 1.5 eq. of sodium hydroxide in ethanol (Scheme 1, Tables 1 and 2).

The 4-(1H-tetrazol-5-yl)-acetophenone **I** was prepared in 85% yield from the corresponding nitrile using sodium azide and triethylammonium chloride in toluene by the method described by Koguro et al.[22] (Scheme 2). Demethylation of 3-fluoro-4-methoxyacetophenone using pyridine hydrochloride at elevated temperature afforded the 3-fluoro-4-hydroxy-acetophenone **II**.[23] THP protection of the hydroxy group gave compound **III**, which was used in the subsequent condensation. The

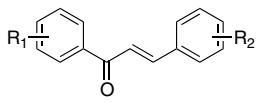



**1-30**

X=OH, OTHP, COOH, Tetrazole.

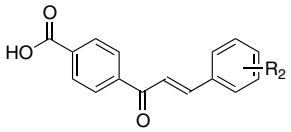
**Scheme 1.** Claisen–Schmidt condensation. Reagents and conditions: (a) 1.5 eq. NaOH, benzaldehyde, EtOH, Reflux; (b) (i) 1.5 eq. NaOH, benzaldehyde, EtOH, Reflux; (ii) aqueous HCl.

**Table 1.** In vitro antibacterial activity against *S. aureus* ATCC 29213




| Compound | $R_1$ | $R_2$ | MIC (μM) |
|---|---|---|---|
| LicA | 4-OH | 2-$OCH_3$-4-OH-5-(1,1-Dimethylallyl) | 20 |
| **1**[5] | 4-OH | 2,4-Di-Cl | 20 |
| **2** | 2-F-4-OH | 2,4-Di-Cl | 20 |
| **3** | 3-F-4-OH | 2,4-Di-Cl | 300 |
| **4** | 3,5-Di-F-4-OH | 2,4-Di-Cl | 150 |
| **5** | 4-COOH | 2,4-Di-Cl | 40 |
| **6** | 4-Tetrazolyl | 2,4-Di-Cl | 300 |
| Ciprofloxacin[31] | | | 2 |
| Linezolid[32,33] | | | 2 |

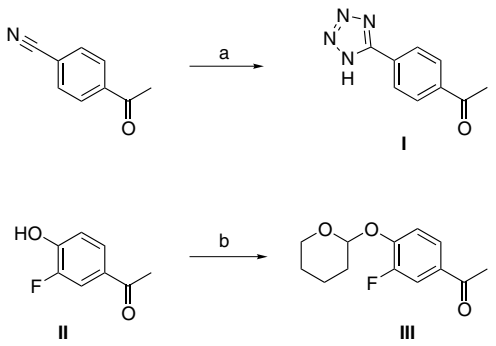
**Table 2.** In vitro antibacterial activity of carboxy chalcones against *S. aureus* ATCC 29213




| Compound | $R_2$ | log *P*[a] | MIC (μM) |
|---|---|---|---|
| **7**[28] | H | 0 | >300 |
| **8** | 4-$CF_3$ | 0.88 | 150 |
| **9** | 4-Cl | 0.71 | 150 |
| **10** | 4-$CH_3$ | 0.56 | >300 |
| **11**[29] | 4-$OCH_3$ | −0.02 | >300 |
| **12** | 4-OH | −0.67 | >300 |
| **13** | 4-OPh | 2.1 | NT[b] |
| **14** | 3-$CF_3$ | 0.88 | 40 |
| **15** | 3-Br | 0.86 | 75 |
| **16**[29] | 3-Cl | 0.71 | 75 |
| **17** | 3-$NO_2$ | −0.28 | 300 |
| **18** | 3-OH | −0.67 | >300 |
| **19** | 3-OPh | 2.1 | NT[b] |
| **20** | 2-$CF_3$ | 0.88 | 300 |
| **21** | 2-Br | 0.86 | 150 |
| **22** | 2-Cl | 0.71 | 150 |
| **23** | 2-OH | −0.67 | >300 |
| **24** | 2-OBu | 1.6 | NT[b] |
| **25** | 3,5-Di-$CF_3$ | 1.76 | 2 |
| **26** | 3,5-Di-Br | 1.72 | 2 |
| **27** | 3,5-Di-Cl | 1.42 | 40 |
| **28** | 3,5-Di-$CH_3$ | 1.12 | 75 |
| **29** | 3,5-Di-F | 0.28 | 150 |
| **30** | 3,5-Di-$OCH_3$ | −0.04 | >300 |

[a] Log *P* is calculated as Σπ of substituents on the B-ring.
[b] The compounds show antibacterial activity but due to precipitation of the compounds in the assay the data are unreliable.

3,5-difluoro-4-hydroxyacetophenone was prepared in a two-step synthesis from the 2,6-difluorophenol as previously described.[24] Acetylation of 2,6-difluoro-phenol and subsequent $AlCl_3$ promoted rearrangement gave the desired product.




**Scheme 2.** Syntheses of starting materials. Reagents and conditions: (a) $NaN_3$, $Et_3N \times HCl$, toluene, 95 °C; (b) 3,4-dihydro-2*H*-pyran, pyridinium *p*-toulenesulfonate, $CH_2Cl_2$.

## 2.2. In vitro activity

Tables 1 and 2 summarise the antibacterial activity of the synthesised chalcones, expressed in terms of antibacterial Minimal Inhibitory Concentrations (MIC) against the Gram-positive bacterium *Staphylococcus aureus*.

Two types of bioisosteric replacements have been investigated: (A) Changing the acidity of the phenol group by fluor-substitution of the A-ring; (B) exchanging the phenol with a carboxylic acid or the tetrazole bioisoster of the carboxylic acid (Table 1).

As a consequence of having a fluor-atom in the *ortho*-position to a phenol group, the acidity of the phenol group increases. A phenol group having two neighbouring fluorine atoms has been described to be almost as acidic as a carboxylic acid.[25,26] In order to investigate the antibacterial effect of lowering the p$K_a$-value of the phenol group the 3′,5′-difluoro-4′-hydroxy analogue **4** and the 3′-fluoro-4′-hydroxy analogue **3** were prepared. The data clearly demonstrate that an increase in acidity of the phenol group causes a dramatic loss of activity (Table 1). The 2-fluoro-analogue **2** believed to have the same p$K_a$ value as the parent compound **1** is not surprisingly equipotent to **1**. Even though the compounds are expected to be more soluble at physiological pH this approach failed to give potent antibacterial compounds.

The second approach by exchanging the hydroxy group with a carboxylic group was much more successful. The 4′-carboxy analogue (**5**) of the parent compound and the 4′-tetrazole bioisoster (**6**) were prepared and it was shown that the carboxylic acid analogue **5** was almost equipotent to the phenol analogue (Table 1). In addition, **5** was much more soluble at physiological pH (0.6 mg/mL) than **1** (<0.01 mg/mL). On the other hand **6** was shown to be almost inactive (MIC: 300 μM) proving that the 4′-tetrazol group is in this case not a true bioisoster of the carboxylic acid (Table 1).

The observation that the hydroxy group in the 4′-position can be exchanged by a carboxy group without loss of activity and at the same time increasing the solubility prompted us to make a thorough structure–activity relationship evaluation of the 4′-carboxy chalcones.

The substituents in the B-ring were systematically changed in order to get the most diverse set of analogues (Table 2). The substituents are selected on the basis of size, lipophilicity and electronic properties using the previously published principal component analyses of selected aromatic substituents.[5,27]

Substituents in the B-ring were initially changed once at a time in all positions of the ring. As the B-ring was monosubstituted only the 2-, 3- or 4-positions were relevant due to the symmetry of the ring. The three positions in the B-ring have different influence on the antibacterial activity of the compounds prepared. Introduction of substituents in the 4-position (**8**–**13**) resulted in compounds that were either inactive or marginally more potent than the nonsubstituted analogue **7**.[28] The lipophilicity of the compounds, expressed as $\log P$[30] of the substituents in the B-ring, appears to be main predictor for the activity. Compounds having the lipophilic bromo-, chloro- or trifluoromethyl groups showed antibacterial activity whereas compounds with the more polar hydroxy and methoxy groups were inactive.

Compounds with substitution in the 3-position of the B-ring (**14**–**19**) seem to be more potent. As seen for the 4-position it appears that lipophilicity of the substituent in the 3-position is modulating the activity. The most potent compound (**14**) has the lipophilic and electron withdrawing trifluoromethyl-group in the 3-position. In order to determine if the electronic properties is essential for the activity, compound **17**, having the polar and electron withdrawing nitro-substituent in the 3-position, was prepared. The compound was almost inactive indicating that lipophilic properties rather than electronic properties were responsible for the high activity of **14**. The fact that compound **18**, having the polar hydroxy group in the 3-position, was inactive supports this observation.

The 2-position appears to be of marginal importance for the activity as all the 2-substituted compounds (**20**–**24**) are either inactive or low potent. The antibacterial activity of the compounds follows the same trend as seen for the 4- and 3-substituted compounds, that is the potency correlates with the lipophilicity of the substituents.

To further investigate the effect of having substituents in the B-ring a number of 3,5-disubstituted analogues were prepared (Table 2).

As seen for the mono-substituted analogues the activity of the compounds correlated with the lipophilicity of the substituents in the B-ring. The lipophilic compounds (**25**, **26**) were very potent and as the substituents become more polar the activity gradually decreased (**27**–**30**). Having two substituents in the B-ring gave a synergistic increase in activity resulting in very potent carboxy chalcones (**25**, **26**: MIC 2 μM). The in vitro activity being equipotent to Ciprofloxacin[31] and the recently launched antibiotic Linezolid.[32,33]

**2.2.1. Antibacterial profile of the compounds.** In order to evaluate the antibacterial profile of the compounds tested, the kinetics of the bacterial killing was investigated. Surprisingly, the antimicrobial profile of the compounds changed when the hydroxy group was exchanged with a carboxy group.

Figure 1a shows the antibacterial profile of **1**. The profile is clearly bacteriocidal with a relative fast bacterial killing at concentration equivalent to the MIC value and above. Figure 1b illustrates the killing kinetics of the carboxy analogue **26** and shows that the compound has an inhibitory effect on bacterial growth but does not cause bacterial killing even at concentration 16 times the MIC, thereby proving a bacteriostatic profile. Additional time–kill studies on a selection of carboxy-chalcones, including a number of compounds listed in Table 2, confirmed this observation.

## 3. Conclusion

Bioisosteric replacement of the 4-hydroxy group in the antibacterial compound **1** with a 4-carboxy group resulted in a number of very interesting antibacterial compounds. In addition to being more potent than the lead compound the carboxy chalcones turned out to have markedly improved solubility. Furthermore the mechanism of action of the carboxy chalcones was different as they were bacteriostatic, while the hydroxy chalcones were bacteriocidal. This might very well be an advantage, as it is believed that bactereocidal compounds are far more toxic to mammalian cells than bacterostatic compound. In fact no cytotoxicity (measured as MTT reduction[34]) was observed for the carboxy chalcones, even at concentrations of 100 μM.

The best compounds (Compounds **25**, **26**) showed high in vitro activity (MIC 2 μM) and no cytotoxicity and are currently under further evaluation.

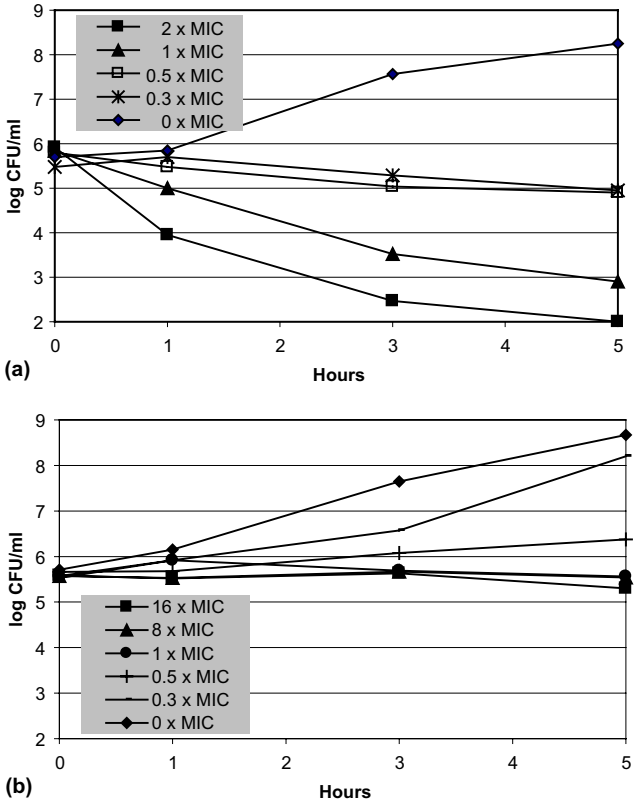



**Figure 1.** Time–kill curve of **1** (a) and **26** (b) against *S. aureus* (E2371) at different concentrations. CFU = colony forming units; MIC = minimum inhibitory concentration.

## 4. Experimental section

### 4.1. Chemistry

Thin-layer chromatography (TLC) was performed on silica gel $F_{254}$ plates (Merck). All compounds were detected using UV light. $^1$H spectra were recorded on a 400 MHz Varian Gemini spectrometer or a Bruker AC-300 F spectrometer, using $CDCl_3$ or DMSO as the solvent. Chemical shifts are given in ppm ($\delta$) using TMS as the internal standard, and coupling constants ($J$) are given in Hertz. Mass spectra (LC–MS) were recorded using a Waters Alliance HPLC-system coupled to a Quatro Microtriple quadropol mass spectrometer (Micromass) operating in positive (ESI) mode. Separation was performed on a XTerra MS $C_{18}$ column (150*2.1 mm ID, 3.5 μm particle size). Purity of the final products (>95%) was determined using a Waters Alliance 2690 separation module and Waters 996 PDA-detector. Separation was performed on a XTerra MS $C_{18}$ column (150*2.1 mm ID, 3.5 μm particle size) using 40% mobile phase A (acetonitrile) and 60% mobile phase B (10 mM ammonium acetate pH 9.5). During the first 20 min, the mobile phase was changed via a linear gradient to 90% A and 10% B.

Column chromatography (CC) was performed on Merck silica gel 60 (0.063–0.200 mm). All solvents and reagents were obtained from Fluka or Aldrich and used without further purification.

**4.1.1. 1-[4-(1H-Tetrazol-5-yl)-phenyl]-ethanone (I).** A solution of 4-cyanobenzonitrile (7.26 g, 50 mmol), sodium azide (4.2 g, 65 mmol) and triethylammonium chloride (8.9 g, 65 mmol) in anhydrous toluene was stirred at 95 °C for 18 h. The mixture was extracted with aqueous NaOH (2 M, 40 mL) and the aqueous phase was poured into aq HCl (6 M, 100 mL). The precipitate was recovered by filtration and recrystallised from methanol to give the product **I** (8 g, 85%) as white crystals (mp 191.7–192.3 °C). $^1$H NMR ($CDCl_3$): $\delta$ 8.09 (2H, d $J$ = 8.7 Hz), 8.01 (2H, d $J$ = 8.7 Hz), 2.64 (3H, s).

**4.1.2. 1-[3-Fluoro-4-(tetrahydro-pyran-2-yloxy)-phenyl]-ethanone (III).** A solution of 3′-fluoro-4′-hydroxy acetophenone (20 g, 130 mmol), 3,4-dihydro-2*H*-pyran

(23.7 mL, 260 mmol) and pyridinium *p*-toluenesulfonate (1.3 g cat) in methylene chloride was stirred for 18 h at rt. The mixture was washed with aqueous NaOH (1 M, 50 mL) and the solvent was removed under reduced pressure. Distillation of the resulting oil (132–140 °C at 0.05 mbar) gave the product **III** as a pure colourless oil (23 g, 74%). $^1$H NMR ($CDCl_3$): $\delta$ 7.86 (1H, t $J = 8.8$ Hz), 6.87 (1H, dd $J = 8.8$ Hz, 2.5 Hz), 6.81 (1H, dd $J = 13.2$ Hz, 2.3 Hz), 5.49 (1H, t $J = 2.8$ Hz), 3.82 (1H, m), 3.64 (1H, m), 2.60 (3H, d $J = 5.3$ Hz), 2.02–1.56 (6H, m).

### 4.2. General procedure A—the Claisen–Schmidt condensation

A solution of acetophenone (3 mmol) and benzaldehyde (3.6 mmol) in ethanol was added NaOH (1.5 equiv). The mixture was refluxed for 18 h and the solvent was removed under reduced pressure. The residue was added an aqueous solution of HCl (2 M, 10 mL) and extracted with ethyl acetate. The organic phase was removed under reduced pressure. The residue was purified by recrystallisation (ethanol), CC or precipitation as the ammonium salt (the resulting residue was dissolved in diethyl ether and $NH_3$ (g) was passed through the solution. The precipitate was collected by filtration).

**4.2.1. 4′-Hydroxy-2,4-dichloro-chalcone (1).**[5] General procedure A followed by recrystallisation gave the desired product as yellow crystals (0.11 g, 13%). LC–MS: 291.0 (M−1. $^1$H NMR (DMSO-$d_6$): $\delta$ 10.48 (1H, s), 8.23 (1H, d $J = 8.5$ Hz), 8.09 (2H, d $J = 8.8$ Hz), 7.99 (1H, d $J = 15.4$ Hz), 7.91 (1H, d $J = 15.4$ Hz), 7.76 (1H, d $J = 2.0$ Hz), 7.53 (1H, dd $J = 7.5$ Hz, 2.0 Hz), 6.90 (2H, d $J = 8.5$ Hz).

**4.2.2. 2′-Fluoro-4′-hydroxy-2,4-dichloro-chalcone (2).** General procedure A using equivalent amount of acetophenone and benzaldehyde (2 mmol) at rt gave the desired product as yellow crystals (0.30 g, 44%) after recrystallisation. LC–MS: 309.0 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 7.90 (2H, d $J = 8.5$ Hz), 7.73–7.67 (3H, m), 7.60–7.45 (4H, m).

**4.2.3. 3′-Fluoro-4′-hydroxy-2,4-dichloro-chalcone (3).** A slightly modified General procedure A (2 mmol scale) followed by CC and recrystallisation gave the desired product as colourless crystals (0.39 g, 63%). In order to hydrolyse the THP group the mixture was stirred with aqueous hydrochloric acid (excess) for 30 min prior to the evaporation of ethanol. LC–MS: 309.0 (M−1). $^1$H NMR ($CDCl_3$/DMSO-$d_6$): $\delta$ 7.89 (1H, d $J = 15.6$ Hz), 7.62–7.55 (3H, m), 7.33 (1H, d $J = 15.6$ Hz), 7.30 (1H, d $J = 2.0$ Hz), 7.15 (1H, dd $J = 8.4$ Hz, 2.1 Hz), 6.89 (1H, t $J = 8.4$ Hz).

**4.2.4. 3′,5′-Difluoro-4′-hydroxy-2,4-dichloro-chalcone (4).** General procedure A (1.6 mmol scale, rt) followed by CC and recrystallisation gave the desired product as yellow crystals (0.16 g, 26%). LC–MS: 327.0 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.30 (1H, d $J = 8.6$ Hz), 8.01 (1H, d $J = 15.3$ Hz), 7.91 (1H, d $J = 15.3$ Hz), 7.87–7.83 (2H, m), 7.75 (1H, d $J = 2.0$), 7.56 (1H, dd $J = 8.6$ Hz, 2.0 Hz).

**4.2.5. 4′-Carboxy-2,4-dichloro-chalcone (5).** General procedure A (2.8 mmol scale) followed by recrystallisation gave the desired product as yellow crystals (0.41 g, 46%). LC–MS: 321.0 (M+1). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.12 (2H, d $J = 8.5$ Hz), 8.12–8.04 (3H, m), 7.82 (1H, d $J = 8.0$ Hz), 7.58 (1H, d $J = 16.0$ Hz), 7.48 (1H, d $J = 2.0$ Hz), 7.35 (1H, dd $J = 8.1$ Hz, 2.0 Hz).

**4.2.6. 4′-Tetrazoyl-2,4-dichloro-chalcone (6).** General procedure A (2 mmol scale) followed by recrystallisation gave the desired product as yellow crystals (0.25 g, 36%). LC–MS: 345.0 (M+1). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.43 (2H, d $J = 8.7$ Hz), 8.34 (1H, d $J = 8.5$ Hz), 8.28 (2H, d $J = 8.7$ Hz), 8.14 (1H, d $J = 15.5$ Hz), 8.04 (1H, d $J = 15.5$ Hz), 7.82 (1H, d $J = 2.1$ Hz), 7.62 (1H, dd $J = 8.5$ Hz, 2.1 Hz).

**4.2.7. 4-(3-Phenyl-acryloyl)-benzoic acid (7)[27] ammonium salt.** General procedure A followed by CC and ammonium salt formation gave the desired product as yellow crystals (0.41 g, 53%). LC–MS 251.1 (M+1). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.18 (2H, d $J = 8.5$ Hz), 8.05 (2H, d $J = 8.5$ Hz), 7.78 (1H, d $J = 15.8$ Hz), 7.7–7.65 (2H, m), 7.52 (1H, d $J = 15.8$ Hz), 7.46–7.40 (3H, m).

**4.2.8. 4′-Carboxy-4-trifluoromethyl-chalcone (8).** General procedure A followed by recrystallisation gave the desired product as off-white crystals (0.51 g, 53%). LC–MS: 319.1 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.26 (2H, d $J = 8.2$ Hz), 8.12 (4H, m), 8.09 (1H, d $J = 15.6$ Hz), 7.83 (1H, d $J = 15.6$ Hz), 7.82 (2H, d $J = 8.2$ Hz).

**4.2.9. 4′-Carboxy-4-chloro-chalcone (9).** General procedure A followed by recrystallisation gave the desired product as white crystals (0.43 g, 50%). LC–MS: 285.0 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.22 (2H, d $J = 8.3$), 8.09 (2H, d $J = 8.3$), 7.97 (1H, d $J = 15.6$ Hz), 7.97 (2H, d $J = 8.5$), 7.76 (1H, d $J = 15.6$), 7.53 (2H, d $J = 8.5$).

**4.2.10. 4′-Carboxy-4-methyl-chalcone (10).** General procedure A followed by recrystallisation gave the desired product as yellow crystals (0.27 g, 33%). LC–MS: 265.1 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 13.33 (s, 1H), 8.23 (2H, d $J = 8.4$ Hz), 8.09 (2H, d $J = 8.4$ Hz), 7.90 (1H, d $J = 15.4$ Hz), 7.80 (2H, d $J = 8.1$ Hz), 7.75 (1H, d $J = 15.4$ Hz), 7.29 (2H, d $J = 8.1$ Hz), 2.36 (3H, s).

**4.2.11. 4′-Carboxy-4-methoxy-chalcone (11).**[28] General procedure A followed by recrystallisation gave the

desired product as yellow crystals (0.18 g, 21%). LC–MS: 281.1 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.21 (2H, d $J = 8.4$ Hz), 8.09 (2H, d $J = 8.4$ Hz), 7.86 (2H, d $J = 8.8$ Hz), 7.80 (1H, d $J = 15.6$ Hz), 7.74 (1H, d $J = 15.6$ Hz), 7.03 (2H, d $J = 8.8$ Hz), 3.83 (3H, s).

**4.2.12. 4′-Carboxy-4-hydroxy-chalcone (12).** General procedure A followed by CC and recrystallisation gave the desired product as yellow crystals (0.02 g, 2%). LC–MS: 267.1 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.14 (2H, d $J = 8.2$ Hz), 8.04 (2H, d $J = 8.4$ Hz), 7.8–7.7 (4H, m), 6.84 (2H, d $J = 8.4$ Hz).

**4.2.13. 4′-Carboxy-4-phenoxy-chalcone (13).** General procedure A followed by recrystallisation gave the desired product as yellow crystals (0.12 g, 12%). LC–MS: 343.1 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 13.34 (s, 1H), 8.23 (2H, d $J = 8.5$ Hz), 8.10 (2H, d $J = 8.5$ Hz), 7.94 (2H, d $J = 8.8$ Hz), 7.87 (1H, d $J = 15.6$ Hz), 7.77 (1H, d $J = 15.6$ Hz), 7.45 (2H, t $J = 7.5$ Hz), 7.22 (1H, b t, $J = 7.5$ Hz), 7.13–7.04 (m, 4H).

**4.2.14. 4′-Carboxy-3-trifluoromethyl-chalcone (14).** General procedure A followed by recrystallisation gave the desired product as yellow crystals (0.54 g, 56%). LC–MS: 319.1 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.35 (1H, b s), 8.27 (2H, d $J = 8.8$ Hz), 8.18 (1H, b d $J = 7.8$ Hz), 8.13 (1H, d $J = 15.9$ Hz), 8.11 (2H, d $J = 8.8$ Hz), 7.85 (1H, d $J$ 15.9 Hz), 7.82 (1H, b d $J = 7.8$ Hz), 7.70 (1H, t $J = 7.8$ Hz).

**4.2.15. 4′-Carboxy-3-bromo-chalcone (15).** General procedure A followed by recrystallisation gave the desired product as yellow crystals (0.78 g, 78%). LC–MS: 329.0 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.22 (1H, b s); 8.11–7.98 (m, 5H); 7.87 (1H, b d $J = 7.8$ Hz); 7.69 (1H, d $J = 15.7$ Hz); 7.63 (1H, b d $J \approx$ Hz); 7.41 (1H, t $J = 7.8$ Hz).

**4.2.16. 4′-Carboxy-3-chloro-chalcone (16).[28]** General procedure A followed by recrystallisation gave the desired product as yellow crystals (0.41 g, 48%). LC–MS: 285.0 (M−1). $^1$H NMR (DMSO-$d_6$) $\delta$ 8.24 (2H, d $J = 8.1$), 8.06 (3H, m), 8.01 (1H, d $J = 15.8$ Hz), 7.81 (1H, d $J = 6.6$ Hz), 7.74 (1H, d $J = 15.8$ Hz), 7.5 (2H, m).

**4.2.17. 4′-Carboxy-3-nitro-chalcone (17).** General procedure A followed by recrystallisation gave the desired product as yellow crystals (0.01 g, 10%). LC–MS: 296.1 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 13.37 (s, 1H), 8.81 (1H, t $J = 1.9$ Hz), 8.36 (1H, b d $J = 7.9$ Hz), 8.32–8.27 (3H, m), 8.19 (1H, d $J = 15.9$ Hz), 8.11 (2H, d $J = 8.6$ Hz), 7.90 (1H, d $J = 15.9$ Hz), 7.77 (1H, t $J = 7.9$ Hz).

**4.2.18. 4′-Carboxy-3-hydroxy-chalcone (18).** General procedure A followed by recrystallisation gave the desired product as yellow crystals (0.33 g, 41%). LC–MS: 267.1 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 13.29 (1H, s), 9.64 (1H, s), 8.22 (2H, d $J = 8.5$ Hz), 8.09 (2H, d $J = 8.5$ Hz), 7.83 (1H, $J = 15.6$ Hz), 7.68 (1H, d $J = 15.6$ Hz), 7.34–7.24 (3H, m), 6.89 (1H, b d $J \approx 8.6$ Hz).

**4.2.19. 4′-Carboxy-3-phenoxy-chalcone (19).** General procedure A followed by recrystallisation gave the desired product as yellow crystals (0.49 g, 47%). LC–MS: 343.1 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.07 (2H, d $J = 8.3$ Hz), 8.02–7.92 (3H, m); 7.74–7.67 (3H, m); 7.50–7.38 (3H, m); 7.15 (1H, b t $J = 7.4$ Hz); 7.07–7.03 (m, 3H).

**4.2.20. 4′-Carboxy-2-trifluoromethyl-chalcone (20).** General procedure A followed by recrystallisation gave the desired product as yellow crystals (0.30 g, 31%). LC–MS: 319.1 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.34 (1H, b d $J = 7.8$ Hz), 8.27 (2H, d $J = 8.5$ Hz), 8.11 (2H, d $J = 8.8$ Hz), 8.05 (1H, d $J = 15.1$ Hz), 7.98 (1H, d $J = 15.1$ Hz), 7.85 (1H, d $J = 7.8$ Hz), 7.81 (1H, t $J = 7.8$ Hz), 7.69 (1H, t $J = 7.8$ Hz).

**4.2.21. 4′-Carboxy-2-bromo-chalcone (21).** General procedure A followed by recrystallisation gave the desired product as yellow crystals (0.46 g, 46%). LC–MS: 329.0 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 13.37 (1H, s); 8.26 (2H, d $J = 8.5$ Hz); 8.22 (1H, dd $J = 7.7$ Hz, 1.7 Hz); 8.11 (2H, d $J = 8.5$ Hz); 8.04 (1H, d $J = 15.7$ Hz); 7.96 (1H, d $J = 15.7$ Hz); 7.76 (1H, dd $J = 7.9$ Hz, 1.3 Hz); 7.51 (1H, td $J = 7.5$ Hz, 1.3 Hz); 7.41 (1H, td $J = 7.9$ Hz, 1.9 Hz).

**4.2.22. 4′-Carboxy-2-chloro-chalcone (22).** General procedure A followed by recrystallisation gave the desired product as yellow crystals (0.43 g, 51%). LC–MS: 285.0 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.24 (2H, d $J = 8.3$ Hz), 8.21 (1H, dd $J = 7.3$ Hz, 3.0 Hz), 8.10 (2H, d $J = 8.3$ Hz), 8.06 (1H, d $J = 15.9$ Hz), 7.98 (1H, d $J = 15.9$ Hz), 7.57 (1H, dd $J = 7.3$ Hz, 3.0 Hz), 7.48 (2H, m).

**4.2.23. 4′-Carboxy-2-hydroxy-chalcone (23).** General procedure A followed by CC and recrystallisation gave the desired product as green crystals (0.04 g, 5%). LC–MS: 267.1 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 13.32 (1H, s), 10.33 (1H, s), 8.18 (2H, d $J = 8.5$ Hz), 8.09 (2H, d $J = 8.5$ Hz), 8.06 (1H, d $J = 15.8$ Hz), 7.87 (1H, b d $J = 8.1$ Hz), 7.85 (1H, d $J = 15.8$ Hz), 7.29 (1H, td $J = 8.1$ Hz, 1.5 Hz), 6.95 (1H, dd $J = 8.1$ Hx, 1.0 Hz), 6.89 (1H, b t $J = 8.1$ Hz).

**4.2.24. 4′-Carboxy-2-butoxy-chalcone (24).** General procedure A followed by recrystallisation gave the desired product as white crystals (0.56 g, 57%). LC–MS: 323.1 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.03–7.88 (7H, m), 7.42 (1H, td $J = 7.5$ Hz, 1.6 Hz), 7.11 (1H, d

$J = 7.5$ Hz), 7.02 (1H, b t $J = 7.5$ Hz), 4.11 (2H, t $J = 6.2$ Hz), 1.85–1.76 (2H, m), 1.58–1.47 (2H, m), 0.98 (3H, t $J = 7.5$ Hz).

**4.2.25. 4′-Carboxy-3,5-bis-trifluoromethyl-chalcone (25).** General procedure A (6 mmol scale) followed by recrystallisation gave the desired product as yellow crystals (0.38 g, 16%). LC–MS: 387.1 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 13.38 (1H, s), 8.68 (2H, s), 8.33–8.28 (3H, m), 8.15 (1H, b), 8.12 (2H, d $J = 8.3$ Hz), 7.93 (1H, d $J = 15.9$ Hz).

**4.2.26. 4′-Carboxy-3,5-dibromo-chalcone (26).** General procedure A followed by recrystallisation gave the desired product as yellow crystals (0.1 g, 7%). LC–MS: 406.9 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 13.37 (1H, s), 8.29 (2H, d $J = 8.4$ Hz), 8.23 (2H, d $J = 1.7$ Hz), 8.14–8.09 (3H, m), 7.91 (1H, t $J = 1.7$ Hz), 7.71(1H, d $J = 15.6$ Hz).

**4.2.27. 4′-Carboxy-3,5-dichloro-chalcone (27) ammonium salt.** General procedure A followed by recrystallisation and ammonium salt formation gave the desired product as white crystals (0.1 g, 8%). LC–MS: 319.0 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.17–8.07 (5H, m), 7.99 (2H, d $J = 8.1$ Hz), 7.72–7.67 (2H, m).

**4.2.28. 4′-Carboxy-3,5-dimethyl-chalcone (28).** General procedure A followed by recrystallisation gave the desired product as yellow crystals (0.26 g, 31%). LC–MS: 179.1 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.23 (2H, d $J = 8.3$ Hz), 8.09 (2H, d $J = 8.3$ Hz), 7.88 (1H, d $J = 15.6$ Hz), 7.68 (1H, d $J = 15.6$ Hz), 7.50 (2H, s), 7.09 (1H, s), 2.31 (6H, s).

**4.2.29. 4′-Carboxy-3,5-difluoro-chalcone (29).** General procedure A followed by recrystallisation gave the desired product as yellow crystals (0.27 g, 31%). LC–MS: 287.1 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 13.36 (s, 1H); 8.28 (2H, d $J = 8.5$ Hz); 8.12–8.06 (3H, m); 7.77–7.72 (3H, m); 7.35 (1H, tt $J = 8.9$ Hz, 2.3 Hz).

**4.2.30. 4′-Carboxy-3,5-dimethoxy-chalcone (30).** General procedure A followed by recrystallisation gave the desired product as yellow crystals (0.35 g, 37%). LC–MS: 311.1 (M−1). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.25 (2H, d $J = 8.5$ Hz), 8.07 (2H, d $J = 8.5$ Hz), 7.74 (1H, d $J = 15.8$ Hz), 7.45 (1H, d $J = 15.8$ Hz), 6.79 (2H, d $J = 2.2$ Hz), 6.55 (1H, t $J = 2.2$ Hz), 3.50 (6H, s).

### 4.3. Determination of MIC

MIC was determined in triplicate in a microdilution assay using Mueller–Hinton agar as described by NCLLS (National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Fifth Edition. M7-A5 NCCLS 2000) modified to include uninoculated dilution series of test compounds to facilitate MIC determination if the test compound should precipitate. MIC was determined as the lowest concentration of test compound able to inhibit visible growth of bacteria.

### 4.4. Kinetics of bacterial killing

For the determination of the killing curve of a test compound a dilution series of test compound was made using 2 mL MH-broth cultures in microtitre plates and an inoculum of approximately 5×10E5 CFU/mL as described by Amsterdam (Amsterdam, D. Susceptibility testing of antimicrobials in liquid media. In Lorian, V., Ed.; Antibiotics in Laboratory Medicine, 4th edition; Williams & Wilkins 1996). At the time points indicated 100 μL samples was withdrawn from the test tubes, serially diluted and spotted in duplicate on unselective agar plates to determine CFU. Test compounds with bactericidal activity were capable of decreasing surviving colony counts (CFU/mL) when incubated with bacteria.

### 4.5. Determination of solubility

Solubility of the compounds was determined by preparing a saturated solution of compound in 0.3 M phosphate buffer (pH 7.4 ± 0.3) in a brown glass tube. The suspensions were rotated slowly for 24 h. Aliquots were centrifuged for 10 min at 14,000 rpm and supernatants were diluted in 40% (v/v) acetonitrile in water prior to HPLC analysis. Concentrations of analytes were quantified against a standard curve and used as term of solubility. The HPLC–UV method used for the assessment of solubility was the same as used in the determination of purity of the compounds.

## References and notes


1. Coates, A.; Hu, Y.; Page, C. *Nature Reviews—Drug Discovery* **2002**, *1*, 895.
2. National Nosocomial Infections Surveillance (NNIS) System Report, Data Summary from January 1990–May 1999, Issued June 1999 (*Am. J. Infect. Control* **1999**, *27*, 520).
3. Sievert, D. M.; Boulton, M. L.; Stoltman, G.; Johnson, D.; Stobierski, M. G.; Downes, F. P.; Somsel, P. A.; Rudrik, J. T.; Brown, W.; Hafeez, W.; Lundstrom, T.; Flanagan, E.; Johnson, R.; Mitchell, J.; Chang, S. *MMWR* **2002**, *51*, 565.
4. Bax, R. P.; Mullan, N. *Balliere Clin. Infect. Dis.* **1999**, *5*, 289.
5. Nielsen, S. F.; Christensen, S. B.; Cruciani, G.; Kharazmi, A.; Liljefors, T. *J. Med. Chem.* **1998**, *41*, 4819.
6. Li, R.; Kenyon, G. L.; Cohen, F. E.; Chen, X.; Gong, B.; Dominguez, J. N.; Davidson, E.; Kurzban, G.; Miller, R. E.; Nazum, E. O.; Rosenthal, P. J.; McKerrow, J. H. *J. Med. Chem.* **1995**, *38*, 5031.

7. Liu, M.; Wilairat, P.; Go, M.-L. *J. Med. Chem.* **2001**, *44*, 4443.
8. Hsieh, H. K.; Tsao, L. T.; Wang, J. P.; Lin, C. N. *J. Pharm. Pharmacol.* **2000**, *52*, 163.
9. Barfod, L.; Kemp, K.; Hansen, M.; Kharazmi, A. *Int. Immunopharmacol.* **2002**, *2*, 545.
10. Rojas, J.; Dominguez, J. N.; Charris, J. E.; Lobo, G.; Paya, M.; Ferrandiz, M. L. *Eur. J. Med. Chem.* **2002**, *37*, 699.
11. Batt, D. G.; Goodman, R.; Jones, D. G.; Kerr, J. S.; Mantegna, L. R.; McAllister, C.; Newton, R. C.; Nurnberg, S.; Welch, P. K.; Covington, M. B. *J. Med. Chem.* **1993**, *36*, 1434.
12. Ducki, S.; Forrest, R.; Hadfield, J. A.; Kendall, A.; Lawrence, N. J.; McGown, A. T.; Rennison, D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1051.
13. Saitoh, T.; Shibata, S. *Tetrahedron Lett.* **1975**, *16*, 4461.
14. Okada, K.; Tamura, Y.; Yamamoto, M.; Inoue, Y.; Takagaki, R.; Takahashi, K.; Demizu, S.; Kajiyama, K.; Hiraga, Y.; Kinoshita, T. *Chem. Pharm. Bull.* **1989**, *37*, 2528.
15. Bowden, K.; Dal Pozzo, A.; Duah, C. K. *J. Chem. Res. (S)* **1990**, 377.
16. Kromann, H.; Larsen, M.; Boesen, T.; Schønning, K.; Nielsen, S. F. Syntheses of Prenylated Benzaldehydes and Their Use in the Synthesis of Analogues of Licochalcone A. *Bioorg. Med. Chem.* **2004**, *12*, Submitted for publication.
17. Dhar, D. N.; Lal, J. B. *J. Org. Chem.* **1958**, *23*, 1159.
18. Wattanasin, S.; Murphy, W. S. *Synthesis* **1980**, *8*, 647.
19. Sipos, G. *Nature* **1964**, *202*, 489.
20. Davey, W.; Tivey, D. J. *J. Chem. Soc.* **1958**, 1230.
21. Lyle, R. E.; Paradis, L. P. *J. Am. Chem. Soc.* **1955**, *77*, 6667.
22. Koguro, K.; Oga, T.; Mitsui, S.; Orita, R. *Synthesis* **1998**, *6*, 910.
23. Buu-Hoi, N. P.; Xuong, N. D.; Lavit, D. *J. Org. Chem.* **1953**, *18*, 910.
24. Xu, L.; Giese, R. W. *J. Fluorine Chem.* **1994**, *67*, 47.
25. Qiu, J.; Stevenson, S. H.; O'Beirne, M. J.; Silverman, R. B. *J. Med. Chem.* **1999**, *42*, 329.
26. Stefanidis, D.; Cho, S.; Dhe-Paganon, S.; Jencks, W. P. *J. Am. Chem. Soc.* **1993**, *115*, 1650.
27. Skageberg, B.; Bonelli, D.; Clementi, S.; Cruciani, G.; Ebert, C. *Quant. Struct.-Act. Relat.* **1989**, *8*, 32.
28. Rupe, H.; Steinbach, A. *Chem. Ber.* **1910**, *43*, 3468.
29. Pfeiffer, A.; Kollbach, T.; Haack, E. *Justus Liebigs Ann. Chem.* **1928**, *460*, 148.
30. Kubinyi, H. In *QSAR Hansch Analysis and Related Approaches*; Mannhold, R.; Krogsgaard-Larsen, P.; Timmerman, H., Eds.; VCH: Weinheim, 1993; pp 21–55.
31. Watt, B.; Brown, F. V. *J. Antimicrob. Chemother.* **1986**, *17*, 605.
32. Zurenko, G. E.; Yagi, B. H.; Schaadt, R. D.; Allison, J. W.; Kilburn, J. O.; Glickman, S. E.; Hutchinson, D. K.; Barbachyn, M. R.; Brickner, S. J. *Antimicrob. Agents Chemother.* **1996**, *40*, 839.
33. Clemett, D.; Markham, A. *Drugs* **2000**, *59*, 815.
34. Plumb, J. A. *Methods in Molecular Medicine* **2004**, *88*, 165.