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In vitro and *in vivo* synergistic interaction of substituted chalcone derivatives with norfloxacin against methicillin resistant *Staphylococcus aureus*

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Thirty chalcone derivatives were synthesized *via* a base catalyzed Claisen Schmidt condensation and evaluated for their anti-methicillin-resistant *Staphylococcus aureus* (MRSA) activity alone and in combination with norfloxacin. Among these, 5 derivatives namely *trans*-3-(1*H*-indol-3-yl)-1-(4′-benzyloxyphenyl)-2-propen-1-one (**2**), 1-(4″-biphenyl)-3-(3′4′-dihydroxyphenyl)-2-propen-1-one (**11**), 1-(4″-hydroxy-3″-methylphenyl)3-(4′-hydroxyphenyl)-2-propen-1-one (**14**), 3-(4′-chlorophenyl)-1-(4″-hydroxyphenyl)2-propen-1-one (**17**), and LTG-oxime (**27**) showed significant antibacterial activity with MIC 12.5–50 μg mL^{-1} respectively. In combination studies, derivatives **2** and **14** significantly reduced the MIC of norfloxacin by up to 16 fold (FICI < 0.5), while derivatives **11**, **17** and **27** reduced it by up to eight fold (FICI ≤ 0.5). Flow cytometry analysis results clearly indicated that derivatives **2** and **14** significantly promote the accumulation and inhibition of the Et-Br efflux, which was further validated through spectrofluorimeter using clinical isolate MRSA-ST2071. In systemically infected Swiss albino mice model, both the compounds significantly ($P < 0.001$, $P < 0.01$) lowered the systemic bacterial load in blood, liver, kidney, lung and spleen tissues. This study supports the promising use of chalcones in the development of economical antibacterial combinations.

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) are increasingly encountered and difficult to treat with available antibiotics.[1] Most multidrug-resistant *S. aureus* strains are nosocomially acquired (HA-MRSA) and cause an array of site specific infections in hospitalized patients, including bloodstream infections, pneumonia, surgical site infections, and urinary tract infections.[2] However, in the past few years, they also increase in community as community-acquired MRSA (CA-MRSA) which are more virulent than HA-MRSA.[3] Most of the MRSA strains are now becoming resistant to most of the clinically used antibiotics like penicillins and other β-lactam antimicrobial drugs, macrolide and quinolones. Due to the emergence of glycopeptides resistance strains like vancomycin hetero-intermediate (hVISA), vancomycin-intermediate (VISA), and vancomycin-resistant (VRSA) *S. aureus*; it is difficult for clinicians to manage these infections.[4,5] Therefore, efforts are being directed towards the design, synthesis and evaluation of the substituted chemical therapeutics as an alternative to the existing antimicrobial therapy.

Chalcone is an open-chain flavonoid with α,β-unsaturated carbonyl group and is one of the important compound groups of flavonoid derived from nature which are considered as precursors for the preparation of various flavonoids and exhibit interesting pharmacological activities.[6] Flavonoids represent an outstanding class of naturally occurring compounds with a 1,3-diarylpropane skeleton, which may assume different cyclic or alicyclic arrangements, according to varying levels of oxidation. Thousands of chalcone derivatives have been synthesized chemically, till date. Chalcones have received a great deal of attention due to their relatively simple structures and wide variety of pharmacological activities. Both natural and synthetic chalcones exhibit a wide variety of activities like antimicrobial,[6,7] antimalarial[8] anti-inflammatory,[9] anti-cancer[10] antioxidant[11] and many more. These activities are largely being attributed due to the unsaturated ketone moiety.[12–14] Recently, many reports have confirmed the anti-MRSA activity of such flavonoid derivatives alone and in combination with some antibiotics by increasing the antibacterial efficacy of antibiotics used together or to restore the effect of separate invalid antibiotics.[15–17] In the present study 30 chalcone analogues with different substituent were synthesized and evaluated for their

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antibacterial and synergistic interaction with norfloxacin against clinical isolates of *S. aureus* (MRSA) through *in vitro* and *in vivo* assays.

Materials and methods

Chemistry

General methods. Melting points were determined on a Toshniwal melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1719 FT-IR spectrophotometer. NMR spectra were obtained in acetone-d_6, DMSO-d_6 and pyridine-d_5 on a Bruker Avance, 300 MHz and 400 MHz instrument using TMS as internal standard. The chemical shift values are reported in ppm and coupling constants in Hz. ESI-MS spectra were recorded on a Perkin-Elmer Turbo Mass/Shimadzu LC-MS. TLC analyses were carried out on precoated silica gel 60 F_{254} plates (Merck) using solvent system, $CHCl_3$: MeOH (9 : 1). The compounds were visualized by either exposure of TLC plates to I_2 vapors or by spraying with vanillin–sulfuric acid reagent, followed by heating at 110 °C for 15 minutes. Silica-gel, 60–120 mesh (spectrochem) was used in the column chromatography for the purification of metabolites. The compounds were identified by their spectral IR, ID (^{1}H, ^{13}C, DEPT) and 2D (COSY, HSQC, HMBC) NMR and ESIMS analysis. HPLC analysis was performed using a Shimadzu LC-10AD Liquid Chromatography equipped with two LC-10A pumps controlled by a CBM-10 interface module, SPD-M10A VP diode array detector, and a SIL-10ADVP auto injector. Data were collected and analyzed using a class LC-10 Work Station. The samples were analyzed by using reverse phase chromatography on waters Spherisorb ODS2 (250 × 4.6 mm i.d.,10 mm) column using binary gradient elution with acetonitrile and water containing 0.1% TFA mobile phase (30 : 70) at a flow rate of 0.6 mL min^{-1}, a column temperature of 25 °C and UV detection at λ 254 nm.

General synthesis of chalcone derivatives

*Synthesis of indolyl chalcones, **1–4**.* To a solution of indol-3-carboxaldehyde **A** (1 mmol) and appropriate acetophenone **B** (1 mmol) in anhydrous methanol (20 mL), sodium hydroxide was added; 10% for compounds **1**, **2**, 30% for **3** and 40% for **4** and stirred the reaction mixture at RT for 2–8 h. The contents of reaction mixture were poured into ice-cold water and neutralized with dilute hydrochloric acid. The solid so obtained was filtered, column chromatographed and recrystallized from ethanol to afford pure **1–4**.

*trans-3-(1H-Indol-3-yl)-1-(4′-flouro-3′-methylphenyl)-2-propen-1-one (**1**).* Orange powder; 20% yield; mp 59–60 °C; IR ν^{max} (KBr): 3422, 1548, 1154, 737 (NH), 1653 (chalcone C=O), 1520, 1491, 1440 (aromatics) cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6): δ 2.35 (3H, s, CH_3), 7.19 (1H, d, J = 9.3 Hz, H-5″), 7.25–7.29 (2H, m, H-5′, H-6′), 7.54 (1H, dd, J = 8.1, 1.2 Hz, H-4′), 7.70 (1H, d, J = 15.6 Hz, H-2), 7.98 (1H, d, J = 2.7 Hz, H-2″), 8.04 (1H, dd, J = 8.7, 2.7 Hz, H-6″), 8.08 (1H, brs, H-2′), 8.11 (1H, d, J = 15.6 Hz, H-3), 8.10 (1H, d, J = 8.1, 1.2 Hz, H-7′), 10.90 (1H, brs, NH); ^{13}C NMR (75 MHz, acetone-d_6): δ 14.3 (CH_3), 113.0 (C-4′), 114.3 (C-1′), 115.4[a] (C-5″), 115.7[a] (C-2), 121.1 (C-7′), 121.9 (C-6′), 123.6 (C-5′), 126.3 (C-3′), 128.9 (C-6″), 132.6[b] (C-2″), 132.7[b] (C-2′), 136.1 (C-1″), 138.6 (C-8′), 139.3 (C-3), 162.7[c] (C-3″), 166.0[c] (C-4″), 188.3 (C-1) (a,b,c = interchangeable); ESI-MS, MeOH (Positive): *m*/*z* 280 $[M + H]^+$, 302 $[M + Na]^+$, (Negative): 278 $[M - H]^-$, HRMS (ESI) calcd for $C_{18}H_{14}FNO$ $[M + H]^+$ 280.1132, found 280.1129.

*trans-3-(1H-Indol-3-yl)-1-(4′-benzyloxyphenyl)-2-propen-1-one (**2**).* Yellow solid; 60%, yield; mp 74–75 °C; IR ν^{max} (KBr): 3448, 1562, 1120, 735 (NH), 1654 (chalcone C=O), 1523, 1495, 1437 (aromatics) cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6): δ 5.29 (2H, s, H_2-7″), 7.21–7.24 (2H, m, H-5′, H-6′), 7.26–7.29 (1H, m, H-4″), 7.33–7.36 (3H, m, H-10″, H-11″, H-12″), 7.46–7.50 (2H, m, H-4′, H-5″), 7.51–7.55 (2H, m, H-9″, H-13″), 7.68 (1H, d, J = 15.6 Hz, H-2), 7.70 (1H, s, H-2″), 7.72 (1H, d, J = 7.8, 2.1 Hz, H-7′), 8.01 (1H, d, J = 2.7 Hz, H-2′), 8.06–8.10 (1H, m, H-6″), 8.10 (1H, d, J = 15.6 Hz, H-3), 10.92 (1H, brs, NH); ^{13}C NMR (75 MHz, acetone-d_6): δ 70.1 (C-7″), 112.7 (C-4′), 114.0 (C-1′), 114.2 (C-2″), 116.8 (C-2), 119.3 (C-4″), 120.8 (C-6″), 121.1 (C-7′), 121.6 (C-6′), 123.2 (C-5′), 126.0 (C-3′), 128.0 (C-9″, C-13″), 128.2 (C-5″), 128.8 (C-10″, C-12″), 130.0 (C-11″), 132.3 (C-2′), 137.7 (C-8′), 138.3 (C-1″), 139.6 (C-3), 141.1 (C-8″), 159.3 (C-3″), 189.0 (C-1); ESI-MS, MeOH (Positive): *m*/*z* 354 $[M + H]^+$, (Negative): 352 $[M - H]^-$, HRMS (ESI) calcd for $C_{24}H_{20}NO_2$ $[M + H]^+$ 354.1488, found 354.1488.

*trans-3-(1H-Indol-3-yl)-1-(benzofuran)-2-propen-1-one (**3**).* Dark brown solid; 40%, yield; mp 59–60 °C; IR ν^{max} (KBr): 3395, 1151, 1156, 736 (NH), 1654 (chalcone C=O), 1509, 1483, 1427 (aromatics) cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6): δ 7.09–7.13 (2H, m, H-5′, H-6′), 7.35 (1H, d, J = 15.6 Hz, H-2), 7.47–7.51 (3H, m, H-4′, H-5″, H-6″), 7.53 (1H, d, J = 15.6 Hz, H-3), 7.61 (1H, d, J = 2.7 Hz, H-2′), 7.92 (1H, dd, J = 8.4, 2.1 Hz, H-7′), 8.01 (1H, dd, J = 6.9, 2.7 Hz, H-4″), 8.12 (1H, dd, J = 7.2, 2.4 Hz, H-8″), 8.62 (1H, s, H-2″), 13.05 (1H, brs, NH); ^{13}C NMR (75 MHz, acetone-d_6): δ 112.9 (C-4′), 113.3 (C-1′), 120.6[a] (C-2), 121.8[a] (C-7′), 123.4 (C-6′), 124.7 (C-5′), 125.7[b] (C-3′, C-2″), 125.9[b] (C-7″), 126.6 (C-4″), 127.9 (C-5″), 128.7 (C-3″), 129.0 (C-6″), 131.7 (C-8″) 133.1 (C-2′), 136.6[c] (C-8′), 138.4[c] (C-1″), 142.7 (C-3), 198.9 (C-1), (a,b,c = interchangeable); ESI-MS, MeOH (Positive): *m*/*z* 288 $[M + H]^+$, (Negative): 286 $[M - H]^-$, HRMS (ESI) calcd for $C_{19}H_{14}NO_2$ $[M + H]^+$ 288.1019, found 288.1019.

*trans-3-(1H-Indol-3-yl)-1-(2′-chlorophenyl)-2-propen-1-one (**4**).* Yellow shiny crystals, 85% yield, mp 208–210 °C ESI-MS, MeOH (Positive): *m*/*z* 282 $[M + H]^+$, (Negative): 281 $[M - H]^+$, molecular formula $C_{17}H_{12}NO$.[18a]

*trans-3-(1H-Indol-3-yl)-1-(4′-hydroxyphenyl)-2-propen-1-one (**5**).* To a solution of **A** (indol-3-carboxaldehyde) (1 mmol) and *p*-hydroxyacetophenone **B** (1 mmol) piperidine (10 mL) was added and refluxed the reaction mixture for 2 h. The contents of reaction mixture were poured into ice-cold water. The solid so obtained was column chromatographed in ($CHCl_3$: MeOH), filtered, dried and recrystallized from ethanol to afford pure **5**. Yellow crystals; 45% yield; mp 181–182 °C; ESI-MS, MeOH (Positive): *m*/*z* 264 $[M + H]^+$, (Negative): 262 $[M - H]^+$, molecular formula $C_{17}H_{13}NO_2$.[18b]

*Synthesis of chalcones (**6–24**).* To a solution of respective acetophenone **B** (3-acetylindole for **6–11**) (1 mmol) and appropriate aldehyde **A** (1 mmol) in methanol (20 mL) was added $SOCl_2$ (2 mL) and stirred the reaction mixture for 2 h. The contents of reaction mixture were poured into ice-cold water. The solid so obtained was column chromatographed in ($CHCl_3$: MeOH), filtered, dried and recrystallized from ethanol to afford pure **6–24**.

*trans-1-Indolyl-3-(2′,3′,4′-trimethoxyphenyl)-2-propen-1-one (**6**).* Creamish powder; 70% yield; mp 163–164 °C; IR ν^{max} (KBr): 3431 1586, 1201, 754 (NH), 1640 (chalcone C═O), 1525, 1493, 1414 (aromatics) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.67 (3H, s, OCH_3), 3.76 (6H, s, 2 × OCH_3), 6.71 (1H, d, J = 9.0 Hz, H-5′), 7.10–7.14 (2H, m, H-5″, H-6″), 7.40 (1H, d, J = 6.6 Hz, H-4″), 7.60 (1H, d, J = 15.6 Hz, H-2), 7.66 (1H, d, J = 9.0 Hz, H-6′), 7.74 (1H, d, J = 15.56 Hz, H-3), 8.25 (1H, d, J = 6.6 Hz, H-7″), 8.57 (1H, brs, H-2″), 11.79 (1H, brs, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 56.8, 61.3, 62.3 (3 × OCH_3), 109.2 (C-5′), 113.0 (C-4″), 118.6 (C-1″), 122.6 (C-6″, C-7″), 122.4 (C-1′), 123.4 (C-6′), 123.9 (C-5″), 124.0 (C-2), 126.8 (C-3″), 134.6 (C-3), 135.1 (C-2″), 137.7 (C-8″), 142.7 (C-3′), 153.5 (C-2′), 155.8 (C-4′), 184.7 (C-1); ESI-MS, MeOH (Positive): m/z 338 $[M + H]^+$, (Negative): 336 $[M - H]^+$, HRMS (ESI) calcd for $C_{20}H_{20}NO_4$ $[M + H]^+$ 338.1386, found 338.1386.

*trans-3-(3′-Ethoxy-4′-acetylphenyl)-1-indolyl-2-propen-1-one (**7**).* Light yellow crystals; 70% yield; mp 154–155 °C; IR ν^{max} (KBr): 1741 (ester CO), 1660 (chalcone C═O), 1603, 1478, 1356, 1260, 1061, 967, 875, 706 (NH) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.31 (3H, t, J = 6.9 Hz, OCH_2CH_3), 2.25 (3H, s, $COOCH_3$), 4.15 (2H, q, J = 6.9 Hz, OCH_2–), 7.16 (1H, d, J = 7.5 Hz, H-5′), 7.40 (1H, d, J = 6.6 Hz, H-6′), 7.21–7.24 (2H, m, H-5″, H-6″), 7.58 (1H, s, H-2′), 7.50 (1H, dd, J = 8.4, 1.5 Hz, H-4″), 8.72 (1H, d, J = 2.7 Hz, H-2″), 7.60 (1H, d, J = 15.0 Hz, H-2), 7.79 (1H, d, J = 15.6 Hz, H-3), 8.34 (1H, dd, J = 8.1, 1.8 Hz, H-7″), 12.11 (1H, brs, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 15.4 (OCH_2CH_3), 21.2 ($COOCH_3$), 65.0 (OCH_2–), 113.0 (C-4″), 113.9 (C-2′), 122.3[a] (C-5′), 118.6 (C-1″), 139.9 (C-2), 122.6[a] (C-6″), 123.9 (C-7″), 124.0 (C-5″), 126.7 (C-3″), 135.0 (C-1′, C-4′), 135.6 (C-2″), 137.7 (C-8″), 141.8 (C-3), 151.8 (C-3′), 169.3 ($COOCH_3$), 184.4 (C-1); ESI-MS, MeOH (Positive): m/z 350 $[M + H]^+$, HRMS (ESI) calcd for $C_{21}H_{20}NO_4$ $[M + H]^+$ 350.1386, found 350.1386.

*trans-1-Indolyl-3-(3′,5′-dimethoxy-4′-hydroxyphenyl)-2-propen-1-one (**8**).* Creamy crystals; 70% yield; mp 210–211 °C; IR ν^{max} (KBr): 3445, 1580, 1191, 740 (NH), 3445 (OH), 1640 (chalcone C═O), 1522, 1491, 1404 (aromatics) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.51 (6H, brs, 2 × OCH_3), 6.49 (2H, br s, H-2′), 6.60–6.63 (2H, m, H-5″, H-6″), 6.90 (1H, dd, J = 6.9, 1.5 Hz, H-4″), 6.99 (1H, d, J = 15.3Hz, H-2), 7.04 (1H, d, J = 15.3 Hz, H-3), 7.70 (1H, dd, J = 8.1, 1.8 Hz, H-7″), 8.06 (1H, d, J = 2.7 Hz, H-2″), 11.43 (1H, brs, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 57.0 (2 × OCH_3), 107.0 (C-2′, C-6′), 113.0 (C-4″), 118.5 (C-1″), 122.4 (C-2), 122.6 (C-7″), 122.8 (C-6″), 124.0 (C-5″), 126.4 (C-1′), 126.6 (C-3″), 135.2 (C-2″),137.6 (C-8″), 138.6 (C-4′), 141.8 (C-3), 148.9 (C-3′, C-5′), 185.1 (C-1); ESI-MS (Positive): m/z 324 $[M + H]^+$, (Negative): 322 $[M - H]^-$, HRMS (ESI) calcd for $C_{19}H_{18}NO_4$ $[M + H]^+$ 324.1230, found 324.1229.

*trans-1-Indolyl-3-(2′-methylphenyl)-2-propen-1-one (**9**).* Obtained as brown solid; 80% yield; mp 140–142 °C; IR ν^{max} (KBr): 3422, 1562, 1156, 748 (NH), 1639 (chalcone C═O), 1520, 1442, 1492 (aromatics) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.34 (3H, s, CH_3), 7.13–7.18 (5H, m, H-3′, H-4′, H-5′, H-5″, H-6″), 7.40 (1H, dd, J = 8.1 Hz, 2.1 Hz, H-4″), 7.63 (1H, d, J = 15.6 Hz, H-2), 7.82 (1H, d, J = 15.6 Hz, H-3), 7.87 (1H, dd, J = 7.5, 2.4 Hz, H-6′), 8.25 (1H, dd, J = 6.6, 2.1Hz, H-7″), 8.63 (1H, d, J = 3.0 Hz, 1H, H-2″), 12.05 (1H, brs, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 20.2 (CH_3), 113.0 (C-4″), 118.5 (C-1″), 122.6 (C-7″), 122.7 (C-6″), 123.9 (C-5″), 126.3 (C-2), 126.8 (C-3″), 127.1 (C-5′), 127.3 (C-6′), 130.4 (C-4′), 131.5 (C-3′), 134.6 (C-1′), 135.6 (C-2″), 137.5 (C-3), 137.7 (C-8″), 138.2 (C-2′), 184.5 (C-1); ESI-MS, MeOH (Positive): m/z 262 [M + H]+, 284 $[M + Na]^+$, (Negative): 260 $[M - H]^-$, HRMS (ESI) calcd for $C_{18}H_{16}NO$ $[M + H]^+$ 262.1226, found 262.1224.

*trans-1-Indolyl-3-(thiophenyl)-2-propen-1-one (**10**).* Creamish white powder; 70% yield; mp 181–182 °C; IR ν^{max} (KBr): 3448 1578, 1199, 754 (NH), 1632 (chalcone C═O), 1523, 1493, 1438, 1315 (aromatics) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.03 (1H, d, J = 4.2 Hz, H-2′), 6.08–6.12 (2H, m, H-5″, H-6″), 6.33–6.35 (1H, m, H-4″), 6.36 (1H, d, J = 15.3 Hz, H-2), 6.45 (1H, brs, H-4′), 6.55 (1H, d, J = 4.2 Hz, H-3′), 6.66 (1H, d, J = 15.3 Hz, H-3), 7.50 (1H, d, J = 7.5 Hz, H-7″), 7.52 (1H, brs, H-2″), 10.95 (1H, brs, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 113.0 (C-4″), 118.3 (C-1″), 122.6 (C-7″), 122.7 (C-6″), 124.0 (C-5″), 124.0 (C-2), 126.7 (C-3″), 129.3 (C-2′), 129.7 (C-3′), 132.0 (C-4′), 133.3 (C-3), 135.4 (C-2″), 137.7 (C-8″), 141.0 (C-1′), 184.0 (C-1); ESI-MS, MeOH (Positive): m/z 254 $[M + H]^+$, 276 $[M + Na]^+$, (Negative): 252 $[M - H]^-$, HRMS (ESI) calcd for $C_{15}H_{12}NOS$ $[M + H]^+$ 254.0634, found 254.0635.

*1-4″-(Biphenyl)-3-3′4′-(dihydroxyphenyl)-2-propen-1-one (**11**).* 35% yield; mp 198–200 °C; IR ν^{max} (KBr): 3448 (OH), 1654 (chalcone CO), 1561, 1459, 1401, 1035 (aromatics) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6):δ 6.70 (1H, d, J = 8.1Hz, H-5′), 7.06 (1H, d, J = 8.1 Hz, H-6′), 7.16 (1H, s, H-2′), 7.26-7.29 (3H, m, H-9″, H-10″, H-11″), 7.56–7.58 (2H, m, H-2, H-3), 7.58 (2H, d, J = 7.8Hz, H-8″, H-12″), 7.67 (2H, d, J = 8.1 Hz, H-3″, H-5″), 8.03 (2H, d, J = 8.1, H-2″, H-6″); ^{13}C NMR, DEPT (75 MHz, DMSO-d_6): δ 116.3 (C-2′), 116.6 (C-5′), 119.2 (C-2), 123.2 (C-6′), 127.1 (C-1′), 127.7 (C-8″, C-12″), 127.8 (C-3″, C-5″), 129.1 (C-10″), 129.4 (C-9″, C-11″), 129.9 (C-2″, C-6″), 137.6 (C-1″), 139.8 (C-7″), 145.0 (C-4″), 145.8 (C-3), 146.5 (C-3′), 149.7 (C-4′), 189.3 (C-1); ESI-MS, MeOH (Positive): m/z 317 $[M + H]^+$, HRMS (ESI) calcd for $C_{21}H_{17}O_3$ $[M + H]^+$ 317.1172, found 317.1172.

*trans-1-(4″-Biphenyl)3-(3′-ethoxy-4′-hydroxyphenyl)-2-propen-1-one (**12**).* 55% yield; mp 116–118 °C; IR ν^{max} (KBr): 3444 (OH), 1652 (chalcone CO), 1563, 1459, 1401, 1034 (aromatics) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.33 (3H, t, J = 6.6 Hz, OCH_2CH_3), 4.10 (2H, q, J = 6.0 Hz, OCH_2–), 6.83 (1H, d, J = 8.1 Hz, H-5′), 7.25 (1H, d, J = 7.8 Hz, H-6′), 7.44–7.47 (3H, m, H-2′, H-9″, H-11″), 7.73 (1H, d, J = 16.0 Hz, H-2), 7.69 (1H, d, J = 16.0 Hz, H-3), 7.69–7.72 (2H, m, H-8″, H-12″), 7.80 (2H, d, J = 6.9 Hz, H-3″, H-5″), 8.19 (2H, d, J = 7.2 Hz, H-2″, H-6″); ^{13}C NMR, DEPT (75 MHz, DMSO-d_6): δ 15.5 (OCH_2CH_3), 64.9 (OCH_2–), 113.8 (C-2′), 116.5 (C-5′), 119.4 (C-2), 125.0 (C-6′), 127.1 (C-1′), 127.7 (C-3″, C-5″), 127.8 (C-8″, C-12″), 129.2 (C-10″), 129.9 (C-9″, C-11″), 130.0 (C-2″, C-6″), 137.6 (C-1″), 139.8 (C-4′), 145.0 (C-7″), 145.8 (C-3), 148.0 (C-4″), 150.8 (C-3′), 189.3 (C-1); ESI-MS, MeOH (Positive): m/z 345 $[M + H]^+$, HRMS (ESI) calcd for $C_{23}H_{21}O_3$ $[M + H]^+$ 345.1485, found 345.1485.

*1-(3″-Chloro-4″-methoxyphenyl)-4′-hydroxyphenyl-2-propenen-1one (**13**).* Orange powder; 55% yield; mp 135–138 °C; IR ν^{max} (KBr): 3407 (OH), 1658 (chalcone CO), 1560, 1458, 1400, 1035 (aromatics) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6):δ 3.93 (3H, s, OCH_3), 6.84 (2H, d, J = 8.14 Hz, H-3′, H-5′), 7.24 (1H, d, J = 8.40 Hz, H-5″), 7.68 (1H, d, J = 17.36 Hz, H-3), 7.71 (1H, d, J = 17.92 Hz H-2), 7.71–7.74 (2H, m, H-2′, H-6′), 8.11 (1H, d, J = 8.56 Hz,

H-6″), 8.16 (1H, s, H-2″); ^{13}C NMR, DEPT (100 MHz, DMSO-d_6): δ 56.9 (O*C*H$_3$), 112.8 (C-5″), 116.3 (C-3′, C-5′), 118.3 (C-2), 122.0 (C-3″), 126.2 (C-1″), 129.8 (C-6″), 130.4 (C-2″), 131.5 (C-2′, C-6′), 131.7 (C-1′), 144.9 (C-3), 158.5 (C-4′), 160.5 (C-4″), 186.9 (C-1); ESI-MS, MeOH (Positive): *m*/*z* 289 [M + H]$^+$, HRMS (ESI) calcd for $C_{16}H_{14}ClO_3$ [M + H]$^+$ 289.0625, found 289.0625.

*1-(4″-Hydroxy-3″-methylphenyl)3-(4′-hydroxyphenyl)-2-propen-1-one (**14**).* Yellow shiny crystals, 88% yield, mp 198–200 °C ESI-MS, MeOH (Positive): *m*/*z* 255 [M + H]$^+$, (Negative): 253 [M − H]$^+$, molecular formula $C_{16}H_{14}O_3$.[18c]

*trans-3-(Benzofuranyl)-1-(4′-hydroxyphenyl)-2-propen-1-one (**15**).* Purple shiny crystals, 80% yield, mp 185–187 °C; IR ν^{max} (neat): 1659 (chalcone), 1592, 1510, 1452, 1031, 984, (aromatics), 750 (furan moiety) cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6): δ 6.98 (2H, dd, *J* = 8.7, 1.2 Hz, H-3′, H-5′), 7.29 (1H, dd, *J* = 7.8, 0.9 Hz, H-5″), 7.46 (1H, dd, *J* = 8.1, 1.2 Hz, H-6″), 7.49 (1H, d, *J* = 8.1 Hz, H-7″), 7.61 (1H, d, *J* = 15.8 Hz, H-2), 7.73 (3H, dd, *J* = 8.7, 2.1 Hz, H-2′, H-6′, H-4 ″), 7.70 (1H, d, *J* = 15.6 Hz, H-3), 7.87 (1H, brs, H-2″); ^{13}C, DEPT-NMR (75 MHz, acetone-d_6): δ 112.5 (C-2″), 113.4 (C-7″), 115.5 (C-3′, C-5′), 119.6 (C-4″), 123.7 (C-5″), 124.3 (C-6″), 127.9 (C-1′), 127.9 (C-3″), 128.5 (C-2), 131.0 (C-2′, C-6′), 143.9 (C-3), 154.5 (C-8″), 156.0 (C-1″), 161.8 (C-4′), 179.0 (C-1); ESI-MS (Positive): *m*/*z* 265 [M + H]$^+$, (Negative): 263 [M − H]$^-$, HRMS (ESI) calcd for $C_{17}H_{13}O_3$ [M + H]$^+$ 265.0864, found 265.0859.

*3-(2′-Chlorophenyl)-1-(4″-hydroxyphenyl)-2-propen-1-one (**16**).* Orange powder, 65% yield, mp 192–193 °C; ESI-MS, MeOH (Positive): *m*/*z* 259 [M + H]$^+$, (Negative): 257 [M − H]$^+$, molecular formula $C_{15}H_{11}O_2Cl$.[18d]

*3-(4′-Chlorophenyl)-1-(4″-hydroxyphenyl)-2-propen-1-one (**17**).* Orange crystals, 72% yield, mp 196–198 °C; ESI-MS, MeOH (Positive): *m*/*z* 259 [M + H]$^+$, (Negative): 257 [M − H]$^+$, molecular formula $C_{15}H_{11}O_2Cl$.[19]

*1-(5″-Chlorothiophen-2-yl)-3-(4′-hydroxyphenyl)-2-propen-1-one (**18**).* Yellow powder; yield: 71%; mp 210–212 °C; ESI-MS, MeOH (Positive): *m*/*z* 265 [M + H]$^+$, (Negative): 263 [M − H]$^+$, molecular formula $C_{13}H_9ClO_2S$.[20a]

*1-(Furanyl)-3-(4′-hydroxyphenyl)-2-propen-1-one (**19**).* Light yellow solid, yield: 75% mp 160–162 °C IR ν^{max} (neat): 1657 (chalcone), 1594, 1511, 1463, 1029, 985 (aromatics), 793 (furan moiety) cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6): δ 6.69 (1H, d, *J* = 3.6, H-4″), 7.02 (2H, d, *J* = 8.7 Hz, H-3′, H-5′), 7.52 (1H, d, *J* = 3.0, H-3″),7.51 (1H, d, *J* = 15.6, H-2), 7.72 (2H, d, *J* = 3.6 Hz, H-2″), 7.74 (2H, d, *J* = 8.7, H-2′, H-6′), 7.76 (1H, d, *J* = 15.6 Hz, H-3); ^{13}C, DEPT-NMR (75 MHz, acetone-d_6): δ 112.7 (C-3″), 115.5 (C-3′, C-5′), 117.7 (C-2″), 119.7 (C-2), 128.0 (C-1′), 130.8 (C-2′, C-6′), 143.2 (C-3), 147.3 (C-4″), 154.3 (C-1″), 161.6 (C-4′), 177.3 (C-1); ESI-MS (Positive): *m*/*z* 215 [M + H]$^+$, 213 [M − H]$^-$, HRMS (ESI) calcd for $C_{13}H_{11}O_3$ [M + H]$^+$ 215.0702, found 215.0702.

*3-(2′,3′,4′-Trimethoxyphenyl)-1-(4″-hydroxyphenyl)-2-propen-1-one (**20**).* Yellow powder; yield: 81%; mp 192–194 °C; ESI-MS, MeOH (Positive): *m*/*z* 315 [M + H]$^+$, (Negative): 313 [M − H]$^+$, molecular formula $C_{18}H_{18}O_5$.[20b]

*3-(2′,4′-Dimethoxyphenyl)-1-(4″-hydroxyphenyl)-2-propen-1-one (**21**).* Dark pink; yield: 85%; mp 215–217 °C; ESI-MS, MeOH (Positive): *m*/*z* 285 [M + H]$^+$, (Negative): 283 [M − H]$^+$, molecular formula $C_{17}H_{16}O_4$.[21]

*3-(2′,4′-Dimethoxyphenyl-3′-hydroxy)-1-(4″-hydroxyphenyl)-2-propen-1-one (**22**).* Orange crystals; yield: 85%; mp 240–242 °C; ESI-MS, MeOH (Positive): *m*/*z* 285 [M + H]$^+$, (Negative): 283 [M − H]$^+$, ESI-MS, MeOH (Positive): *m*/*z* 301 [M + H]$^+$, molecular formula $C_{17}H_{16}O_5$.[22]

*3-(3′,4′-Dimethoxy-4′-hydroxyphenyl)-1-(3″-hydroxyphenyl)-2-propen-1-one (**23**).* Orange crystals, 90% yield; mp 160–162 °C; IR ν^{max} (KBr): 3418 (OH), 1659 (chalcone CO), 1551, 1462, 1388, 1030 (aromatics) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.81 (2 × O*C*H$_3$), 6.88–6.92 (2H, m, H-2′, H-6′), 6.94 (1H, d, *J* = 8.4 Hz, H-4″), 7.28 (1H, d, *J* = 8.1 Hz, H-5″), 7.73 (1H, d, *J* = 15.6 Hz H-2), 7.74 (2H, s, H-2″), 7.61 (1H, d, *J* = 15.3 Hz, H-3), 8.04 (1H, d, *J* = 8.1 Hz, H-6″); ^{13}C NMR, DEPT (75 MHz (DMSO-d_6): δ 56.3, 56.5 (2 × O*C*H$_3$), 111.4 (C-2′, C-6′), 112.4 (C-2″), 116.2 (C-4″), 120.5 (C-2), 124.3 (C, C-3″, C-6″), 128.5 (C-4′), 130.2 (C-1″), 131.9 (C-5″), 144.1 (C-3), 149.8 (C-3′, C-5′), 188.2 (C-1); ESI-MS, MeOH (Positive): *m*/*z* 301 [M + H]$^+$, 323 [M + Na]$^+$, HRMS (ESI) calcd for $C_{17}H_{17}O_5$ [M + H]$^+$ 301.1070, found 301.1070.

*3-(3′-Ethyloxy-4′-hydroxyphenyl)-1-(4″-hydroxyphenyl)-2-propen-1-one (**24**).* Orangish yellow; yield: 91%; mp 250–252 °C; ESI-MS, MeOH (Positive): *m*/*z* 285 [M + H]$^+$, (Negative): 283 [M − H]$^+$, molecular formula $C_{17}H_{16}O_4$.[23]

*Isoliquiritigenin (**25**).* To a mixture of 4-hydroxybenzaldehyde (1.12 g, 9.1 mmol), 2,5-dihydroxyacetophenone (1.36 g, 8.94 mmol), and ethanol (1.12 mL) was added potassium hydroxide solution (8 mL, 60% w/w). The mixture was heated at 100 °C for 2 h. The reaction mixture poured over ice cold water (30 mL). The mixture was acidified to pH 5 with conc. HCl (13 mL). The solid was filtered, washed with water (50 mL) and dried at 80 °C. The crude product was column chromatographed over silica gel using ethyl acetate–hexane (40 : 60) as eluent to yield the compound **25** (1.1 g, 48%) as a yellow solid.[34]

*Liquiritigenin (**26**).* A mixture of **25** (2.5 g, 9.76 mmol), methanol (125 mL) and conc. HCl (10 mL) was refluxed for 48 h. The reaction mixture was concentrated under vacuum and diluted with water (100 mL). The product was filtered, washed with water and dried. The compound was obtained as a pale yellow solid (2.33 g, 92%). Mp: 235–237 °C.[34]

*LTG-oxime (**27**).* It was prepared by treating (0.256 g, 0.001 mol) **26** with hydroxylamine hydrochloride (0.069 g, 0.01 mol) in ethanol and sodium acetate trihydrate (0.136 g, 0.001 mol) in water. The solution was heated on a water bath for 4 h with constant stirring. On cooling and water addition, the cream compound precipitated out. It was filtered, washed with water, and dried. The product was crystallized from ethanol to yield 0.235 g (91.7%), mp 125–126 °C; IR ν^{max} (KBr): 3398 (phenolic OH) 3600–2700, 1615, 1467, 1375, 931, 824 (CN-OH), 1517, 1243, (aromatics) cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD): δ 2.62 (1H, dd, *J* = 17.1, 12.0 Hz, H-3a), 3.35 (1H, dd, *J* = 17.1, 3.0 Hz, H-3b), 4.91 (1H, dd, *J* = 12.0, 2.7 Hz, H-2), 6.31 (1H, d, *J* = 2.4 Hz, H-8), 6.41 (1H, dd, *J* = 8.4, 2.1 Hz, H-6), 6.79 (2H, d, *J* = 8.4 Hz, H-3′, H-5′), 7.26 (1H, d, *J* = 8.4 Hz, H-2′, H-6′), 7.66 (1H, d, *J* = 8.7 Hz, H-5); ^{13}C and DEPT NMR (75 MHz, MeOD): δ 31.1 (CH_2, C-3), 78.4 (CH, C-2), 104.3 (CH, C-8), 110.9 (CH, C-6), 111.7 (C, C-4a), 116.2 (CH, C-3′, C-5′), 126.0 (CH, C-5), 128.7 (CH, C-2′, C-6′), 132.4 (C, C-1′), 150.8 (C═O, C-4), 158.4 (C, C-4′), 159.2 (C, C-8a) 161.1 (C,

C-7); ESI-MS, MeOH (Positive): *m*/*z* 272 $[M + H]^+$, 294 $[M + Na]^+$, (Negative): *m*/*z* 270 $[M - H]^-$, HRMS (ESI) calcd for $C_{15}H_{14}O_4N$ $[M + H]^+$ 272.0921, found 272.0922.[38]

*LTG-oxime-tribenzoate (**28**).* LTG-oxime **27** (0.198 g, 0.70 mmol) and benzoyl chloride (0.5 mL) were stirred in pyridine (2 mL) at room temperature. After completion, the reaction mixture was work-up as usual which afforded a residue. The residue upon crystallization in ethyl acetate–hexane furnished dark orange crystals, 0.372 g (96%); mp 90–91 °C. IR ν^{max} (KBr): 3393 (phenolic OH), 1738 (ester CO), 1518, 1234, 1023 (aromatics) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.03 (1H, dd, J = 13.5, 13.5 Hz, Ha-3), 3.74 (1H, d, J = 15.9 Hz, Hb-3), 5.25 (1H, d, J = 12.6 Hz, H-2), 6.92–6.95 (2H, m, H-6, H-8), 7.31 (2H, d, J = 8.1 Hz, H-3′, H-5′), 7.50–7.54 (9H, m, H-4a, H-4b, H-4c, H-5a, H-5b, H-5c, H-6a, H-6b, H-6c), 8.18–8.23 (6H, m, H-3a, H-3b, H-3c, H-7a, H-7b, H-7c), 7.63 (1H, d, J = 8.1 Hz, H-2′, H-6′), 8.31 (1H, d, J = 9.0 Hz, H-5); ^{13}C and DEPT NMR (75 MHz, $CDCl_3$): δ 32.9 (C-3), 77.4 (C-2), 111.8 (C-8), 116.4 (C-6), 114.6 (C-4a), 122.6 (C-3′, C-5′), 127.3 (C-5), 128.0 (C-2′, C-6′), 129.0 (C-4a/C-4b/C-4c, C-6a/C-6b/C-6c), 129.6 (C-1′), 130.1 (C-3a/C-3b/C-3c, C-7a/C-7b/C-7c), 134.2, 133.9 (C-5a/C-5b/C-5c), 136.9 (C-2a/C-2b/C-2c), 151.6 (C-4), 155.0 (C-4′), 156.7 (C-7), 158.9 (C-8a), 164.0/165.0/165.6 (C-1a/C-1b/C-1c); ESI-MS, MeOH (Positive): *m*/*z* 704 $[M + H]^+$, 726 $[M + Na]^+$, HRMS (ESI) calcd for $C_{46}H_{26}O_7N$ $[M + H]^+$ 704.1709, found 704.1673.

*Synthesis of **29–30**.* 2,4-Dihydroxyflavanone (1 eq.) and allyl bromide (3 eq.) was refluxed in acetone in the presence of K_2CO_3 for 2 h. Two spots developed on TLC which were separated by column chromatography in $CHCl_3$: MeOH (Scheme 2).

*4′,2″,4″-Triallyloxyisoliquiritigenin (**29**).* 45% yield; yellow, viscous compound; IR ν^{max} (KBr): 1654 (chalcone CO), 1551, 1450, 1411, 1022 (aromatics) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6):δ 4.61 (2H, d, J = 5.08 Hz, H_2-1b), 4.65 (2H, d, J = 5.16 Hz, H_2-1c), 4.69 (2H, d, J = 4.64 Hz, H_2-1a), 5.24–5.27 (3H, m, Ha-3a, Ha-3b, Ha-3c), 5.39–5.42 (3H, m, Hb-3a, Hb-3b, Hb-3c), 6.03–6.07 (3H, m, H-2a, H-2b, H-2c), 6.65 (1H, d, J = 8.64 Hz, H-5″), 6.99 (2H, d, J = 8.48 Hz, H-3′, H-5′), 6.70 (1H, s, H-3″), 7.52 (1H, d, J = 15.60 Hz H-2), 7.45 (1H, d, J = 15.60 Hz, H-3), 7.58 (2H, d, J = 8.60 Hz, H-2′, H-6′), 7.63 (1H, d, J = 8.56 Hz, H-6″); ^{13}C NMR, DEPT (75 MHz, DMSO-d_6): δ 69.0 (C-1c), 68.7 (C-1b), 69.3 (C-1a), 100.7 (C-3″), 115.6 (C-3′, C-5′), 117.9 (C-3b), 118.1 (C-3a), 118.3 (C-3c), 122.4 (C-1″), 125.3 (C-2), 127.9 (C-1′), 130.5 (C-2′, C-6′), 132.4 (C-6″), 133.5 (C-2b), 133.6 (C-2c), 133.7 (C-2a), 141.5 (C-3), 159.3 (C-2″), 160.4 (C-4′), 163.0 (C-4″), 107.3 (C-5″), 189.8 (C-1); ESI-MS, MeOH (Positive): *m*/*z* 377 $[M + H]^+$, HRMS (ESI) calcd for $C_{24}H_{25}O_4$ $[M + H]^+$ 377.1743, found 377.1743.

*4′,4″-Diallyloxy,2″-hydroxyisoliquiritigenin (**30**).* 35% yield; yellow, viscous compound; IR ν^{max} (KBr): 3448 (OH), 1657 (chalcone CO), 1546, 1454, 1408, 1015 (aromatics) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6):δ 4.64–4.67 (4H, m, H_2-1a, H_2-1b), 5.29 (2H, d, J = 9.9 Hz, Ha-3a, Ha-3b), 5.41 (2H, d, J = 17.1 Hz, Hb-3a, Hb-3b), 6.08–6.11 (2H, m, H-2a, H-2b), 6.58 (1H, d, J = 9.0 Hz, H-5″), 7.04 (2H, d, J = 8.4 Hz, H-3′, H-5′), 6.51 (1H, d, J = 1.8 Hz, H-3″), 7.83–7.87 (4H, m, H-2, H-3, H-2′, H-6′), 8.26 (1H, d, J = 9.0 Hz, H-6″); ^{13}C NMR, DEPT (75 MHz, DMSO-d_6): δ 69.2 (C-1a), 69.5 (C-1b), 102.6 (C-3″), 108.5 (C-5″), 114.8 (C-1″), 116.0 (C-3′, C-5′), 118.6 (C-3a), 118.8 (C-3b), 119.4 (C-2), 128.2 (C-1′), 131.9 (C-2′, C-6′), 133.4 (C-6″), 133.8 (C-2a), 134.1 (C-2b), 145.0 (C-3), 161.3 (C-4′), 165.6 (C-4″), 166.4 (C-2″), 192.7 (C-1); ESI-MS, MeOH (Positive): *m*/*z* 337 $[M + H]^+$, HRMS (ESI) calcd for $C_{21}H_{21}O_4$ $[M + H]^+$ 337.1435, found 337.1435.

Bacterial strains and antimicrobial agents

The reference strain of *S. aureus* MTCC-96 (SA-96) was procured from Microbial Type Culture Collection, CSIR-Institute of Microbial Technology, Chandigarh, India. Clinical isolates of *S. aureus* (MRSA) were obtained from the Clinical Microbiology Laboratory of Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India, which are being maintained in their repository. The numbers mentioned alongside the strains represent the repository accession number. Clinical isolates were characterized and maintained as reported earlier[24] Norfloxacin (Sigma-Aldrich, St. Louis, MO, USA) was used as positive control.

Anti-staphylococcal activity

Determination of minimum inhibitory concentration (MIC). The antibacterial activity of substituted chalcone derivatives was determined by the broth microdilution assay using 96 'U'-bottom micro-titre plates as per CLSI guidelines (Wayne, 2006). Derivatives were serially diluted twofold (in the range 100–0.195 μg mL^{-1}) in Mueller Hinton Broth (MHB). The broth was inoculated with 10.0 μL of diluted 24 h-grown culture of the test organism with a titre equivalent to 0.5 McFarland standards. The inoculated plates were then incubated at 37 °C for 16–24 h and the growth was recorded spectrophotometrically at 600 nm using a SpectraMax 190 microplate reader (Molecular Devices, Sunnyvale, CA, USA). The minimum inhibitory concentration (MIC) value was determined from the turbidimetric data as the lowest concentration of derivatives showing growth inhibition equal to or greater than 80% as compared to the control. Experimental observations were performed in triplicate to rule out any error during the procedure. An antibiotic norfloxacin were used as the positive control.

Interaction study of substituted chalcones derivatives with norfloxacin

The interactions of substituted chalcones derivatives with norfloxacin against clinical isolates (MRSA) were assessed through checkerboard method.[25] The synergy between substituted chalcones derivatives and norfloxacin was evaluated as a fractional inhibitory concentration index (FICI). The FIC was calculated as the MIC of an antibiotic or chalcone derivatives in combination, divided by the MIC of the antibiotic or chalcone derivatives alone. The FIC was then summed to derive the FIC index, which indicated the interaction when the index values were the following: FICI ≤ 0.5 = synergy, FICI > 4.0 = antagonism, and FICI > 0.5–4 = no interaction.[26]

$$\mathrm{FIC}\ (\text{drug 1}) = \frac{\text{MIC of drug 1 in combination}}{\text{MIC of drug 1 on its own}}$$

$$\text{FIC (drug 2)} = \frac{\text{MIC of drug 2 in combination}}{\text{MIC of drug 2 on its own}}$$

$$\text{FIC Index (FICI)} = \text{FIC (drug 1)} + \text{FIC (drug 2)}$$

Bacterial-killing assay

The *in vitro* bactericidal activity of selective chalcones derivatives in combination with norfloxacin against clinical isolates of *S. aureus* (MRSA-ST 2071) was studied at different MIC combination in accordance to the method described by McKay *et al.* 2009.[27] Each analysis was done in triplicate with a control without test sample. Time kill curves were derived by plotting $\log_{10}$ CFU mL^{-1} against time (h).

Ethidium bromide accumulation assay (flow cytometric analysis)

Ethidium bromide accumulation assay was done as the methods describe previously.[28] Bacterial strain (MRSA-ST2071) was cultured in 10 mL of MHB medium at 37 °C and 200 rpm until an OD600 of 0.6. Aliquots of 1.0 mL were centrifuged at 16 000*g* for 5 minutes, the supernatant discarded and the pellet washed twice with PBS. The absorbance of bacterial suspension was adjusted to 0.3 using Phosphate Buffer Saline (PBS) without glucose. Et-Br was added at a final concentration of 1 μg mL^{-1} and reserpine was added to a final concentration of 20 μg mL^{-1}. Following incubation at 25 °C for 60 minutes, aliquots of 0.5 mL were taken for fluorescence measurement as Et-Br was excited at 488 nm and the fluorescence detected through 585 nm filter (FL-2 channel) using the flow cytometer FACS Calibur™ (BD Biosciences, San Jose, CA, USA). Data was collected for at least 10 000 events per sample.

Ethidium bromide efflux assay

The spectrofluorometric determination of ethidium bromide (Et-Br) efflux was performed as described previously.[28] Bacterial (MRSA-ST2071) culture was grown to reach optical density (OD) of 0.6 at 600 nm. The cells were collected by centrifugation and washed with PBS. The suspension (0.3 OD) was exposed to 5 μg mL^{-1} Et-Br for 60 min at 25 °C in the presence of chalcone derivatives at 10 μmg mL^{-1}. The cells were harvested by centrifugation and re-suspended in fresh buffer. Loss of fluorescence was recorded for 30 min at 1 min intervals at an excitation and emission wavelength of 530 nm and 585 nm respectively using spectrofluorimeter (FLUO star omega; BMG Labtech, Ortenberg, Germany).

In vivo efficacy of substituted chalcone derivatives in systemically infected Swiss mice model

The therapeutic efficacies of identified chalcone derivatives were evaluated through intraperitoneal (i.p.) route. Five groups, each with six Swiss mice (5–6 weeks old weighing 18 to 22 g), were infected by intravenous injection of 0.2 mL (10^6 CFU) of *S. aureus* (SA-MTCC96). Chalcone derivatives at graded doses of 100, 50, 25 and 12.5 mg kg^{-1} body weight comprised the treatment groups. The vehicle control group was administered with an equivalent volume of 0.1% cremophor (Fluka, USA). The treatment commenced 2 h after the infusion of infection and continued till day 7 post-infection once daily. Blood was collected from the retro-orbital plexus 24 h after the last dose for bacterial load which was estimated through plate counting on brain heart infusion agar. All the animals were then sacrificed for the collection of lung, liver, kidney and spleen tissues. The tissue homogenates were prepared in 2 mL of chilled, normal saline solution with a glass tissue homogenizer under aseptic conditions. Homogenates were suitably diluted and plated on agar plates to enumerate the bacterial load per gram of tissue.[24,29] Bacterial elimination was assessed by comparing the reduction of bacterial load of each organ in the infected groups and vehicle control.

Statistical analysis

One-way analysis of variance was used to analyse the mean values obtained for the treatment and vehicle groups. Tukey's test was used to compare the treatment and vehicle groups and statistical significance was set at $P \leq 0.001$, $P < 0.01$.

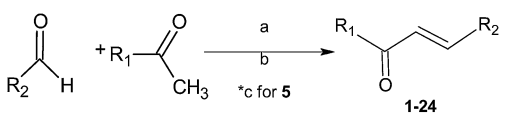
Ethical clearance

The study was approved by the Institutional Bio-safety Committee and Institutional Animal Ethics Committee under the Committee for the Supervision and Experimentation on Animals, Ministry of Environment, Government of India.

Results

Synthesis of substituted chalcones

Analogues based on the α,β-unsaturated ketone core can be prepared by Claisen–Schmidt condensations between corresponding aldehydes and ketones. Indolyl chalcones **1–4** were prepared by the reaction of indol-3-carboxaldehyde with appropriate acetophenone in the presence of NaOH at RT (Scheme 1).[30–32] Further, the reaction of 3-acetylindole with appropriate aldehyde in the presence of $SOCl_2$ resulted in the formation of indolyl chalcones **6–10** (ref. 33). Claisen–Schmidt condensation reactions were carried out between indole-3-carboxaldehyde and *p*-hydroxyacetophenone catalyzed by piperidine, which efficiently provided indole-chalcone **5**. As piperidine acts as a catalyst, we initially carried out the aldol condensations in the presence of catalytic amounts of piperidine. However, we generally observed higher yields when



Scheme 1 General routes used for the chalcone synthesis. Reagents and conditions: (a) NaOH, MeOH, RT; (b) $SOCl_2$, MeOH, RT; (c) piperidine, reflux for **5**.

Table 1 Antibacterial activity of substituted chalcone derivatives against clinical isolates of *S. aureus* (MRSA)

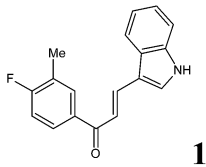
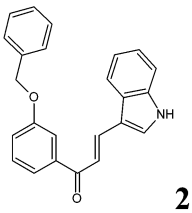
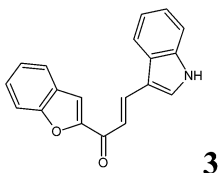
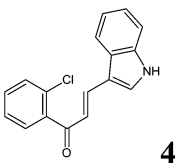
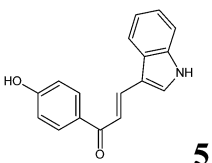
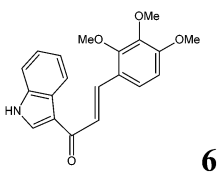
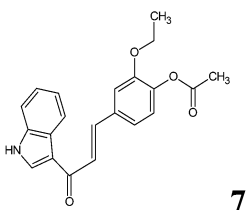
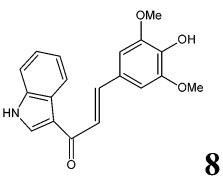
	Minimum inhibitory concentration (μg mL^{-1})										
Structure of test compound	SA-96	MRSA ST-1745	MRSA ST-2071	MRSA ST-2438	MRSA B-10732	MRSA P-8029	MRSA ST-5457	MRSA ST-10342	MRSA B-10760	MRSA ST-3151	MRSA P-6642
 **1**	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
 **2**	**12.5**	**50**	**50**	**12.5**	**50**	**12.5**	**50**	**50**	**25**	**25**	**50**
 **3**	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
 **4**	100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
 **5**	**50**	**100**	**100**	**50**	**50**	**50**	**50**	**100**	**50**	**50**	**50**
 **6**	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
 **7**	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
 **8**	100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100

Table 1 (*Contd.*)

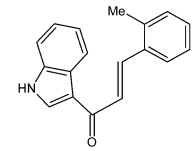
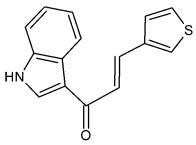
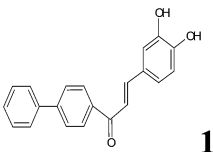
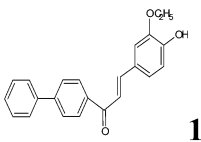
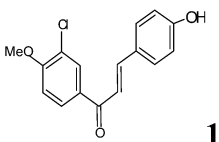
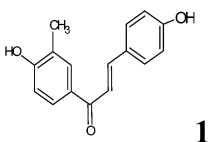
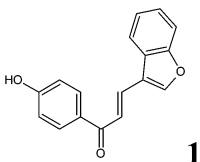
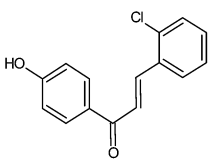
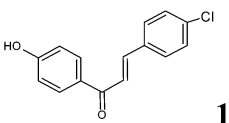
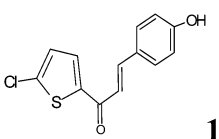
Structure of test compound	Minimum inhibitory concentration (μg mL^{-1})										
	SA-96	MRSA ST-1745	MRSA ST-2071	MRSA ST-2438	MRSA B-10732	MRSA P-8029	MRSA ST-5457	MRSA ST-10342	MRSA B-10760	MRSA ST-3151	MRSA P-6642
 **9**	100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
 **10**	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
 **11**	**50**	**50**	**50**	**25**	**25**	**25**	**50**	**25**	**25**	**50**	**25**
 **12**	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
 **13**	100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
 **14**	**12.5**	**50**	**50**	**12.5**	**50**	**12.5**	**12.5**	**12.5**	**25**	**25**	**12.5**
 **15**	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
 **16**	100	>100	>100	100	>100	100	100	>100	>100	>100	>100
 **17**	**50**	**50**	**50**	**25**	**50**	**25**	**50**	**50**	**50**	**50**	**50**
 **18**	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100

Table 1 (*Contd.*)

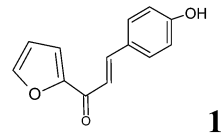
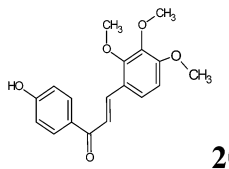
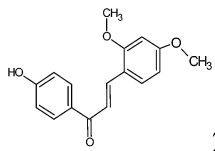
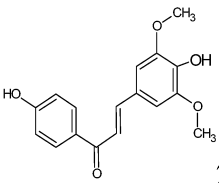
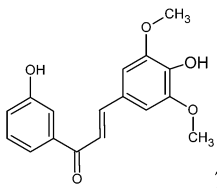
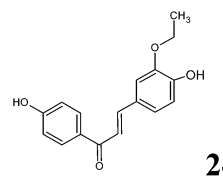
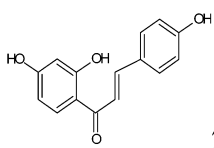
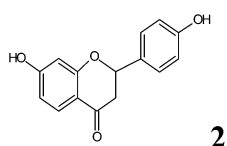
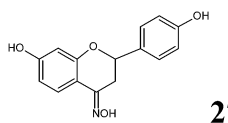
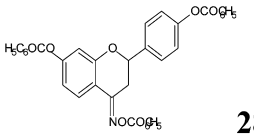
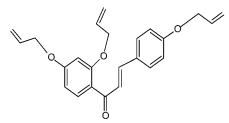
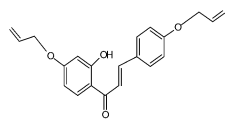
Structure of test compound	Minimum inhibitory concentration (μg mL^{-1})										
	SA-96	MRSA ST-1745	MRSA ST-2071	MRSA ST-2438	MRSA B-10732	MRSA P-8029	MRSA ST-5457	MRSA ST-10342	MRSA B-10760	MRSA ST-3151	MRSA P-6642
 **19**	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
 **20**	100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
 **21**	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
 **22**	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
 **23**	100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
 **24**	**50**	**100**	**100**	**25**	**50**	**25**	**50**	**50**	**50**	**50**	**50**
 **25**	**50**	**100**	**100**	**100**	**50**	**50**	**100**	**100**	**100**	**50**	**100**
 **26**	**50**	**100**	**100**	**50**	**50**	**50**	**50**	**100**	**100**	**100**	**100**
 **27**	**25**	**50**	**50**	**25**	**50**	**25**	**50**	**50**	**50**	**50**	**25**

Table 1 (*Contd.*)

Structure of test compound	Minimum inhibitory concentration (μg mL^{-1})										
	SA-96	MRSA ST-1745	MRSA ST-2071	MRSA ST-2438	MRSA B-10732	MRSA P-8029	MRSA ST-5457	MRSA ST-10342	MRSA B-10760	MRSA ST-3151	MRSA P-6642
 **28**	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
 **29**	100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
 **30**	100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100

piperidine was used in excess. In almost all instances, the product was precipitated from the solution and was thus easily purified from excess catalyst (or starting material) by simple rinsing. Compounds **14–24** were synthesized by reported procedure and their structures were elucidated on the basis of spectral data reported in the literature.[18,20,34] Natural flavanone liquiritigenin was synthesized by reported procedure by cyclization of trihydroxy chalcone isoliquritigenin[34] and its antibacterial activity reported by us is comparable with that reported in literature.[35–37] Oxime derivative of flavanone was synthesized by reported procedure earlier described by us.[38] Oxime derivative of flavanone **26** was further benzoylated in the presence of benzoyl chloride in pyridine. Allyl ether derivatives of isoliquiritigenin resulted from ring opening of natural flavanone liquiritigenin in the presence of allyl bromide and K_2CO_3 in acetone in reflux condition.

All new structures of compounds were satisfactorily confirmed by IR, ^{1}H NMR and EI-MS data. IR spectra showed absorptions at 1650–1720 cm^{-1} (C=O), and sharp bands at 3200 cm^{-1} (NH). In the ^{1}H NMR spectra chalcones showed peaks in the region of δ 2.54–2.78 (CH_3), 2.88–2.98, 7.14–7.26 (CO–CH) and 7.53–7.58 (Ar–CH). The C_α–C_β double bond in the enone moiety of chalcones can potentially adopt either a *Z* or an *E* configuration. The ^{1}H NMR spectrum of each compounds exhibited CH=CH protons around 7.14–7.63 ppm, with $J > 15$, would suggest that the compounds were produced with an (*E*) configuration. A library of compounds (chalcones, flavanones and their derivatives) were synthesized in our lab but the most active ones are being reported here.

Anti-staphylococcal activity

Minimum Inhibitory Concentration (MIC). The results of MIC assay showed that, among 30 substituted chalcone derivatives only five derivatives namely *trans*-3-(1*H*-indol-3-yl)-1-(4′-benzyloxyphenyl)-2-propen-1-one (**2**), 1-(4″-biphenyl)-3-(3′4′-dihydroxyphenyl)-2-propen-1-one (**11**), 1-(4″-hydroxy-3″-methylphenyl)-3-(4′-hydroxyphenyl)-2-propen-1-one (**14**), 3-(4′-chlorophenyl)-1-(4″-hydroxyphenyl)-2-propen-1-one (**17**), LTG-oxime (**27**) exhibits significant antibacterial activity against all clinical isolates of *S. aureus* (MRSA) as well as reference strain SA-MTCC96 with MIC value ranging from 12.5–50 μg mL^{-1} (Table 1). Norfloxacin as positive control, exhibit MIC in the range 125–500 μg mL^{-1} against all the tested clinical isolates of MRSA.

In vitro combination study

The combination of most active substituted chalcone derivatives (**2**, **11**, **14**, **17** and **27**) and norfloxacin were evaluated through checker board assay. The result showed synergistic interaction of chalcone derivatives with 2–16 fold reduction in MIC of norfloxacin (FICI = 0.312 to 0.50) against all the clinical isolates of methicillin resistance *S. aureus* (Table 2).

The combination of norfloxacin with compound **2** showed synergistic interaction (2–16 fold reduction in MIC of norfloxacin) against five clinical isolates (MRSA-ST1745, ST2071, P8029, ST5457 and ST10760) with FICI ranging from 0.312 to 0.50.

Combination of norfloxacin with compound **11** showed synergistic interaction (2–8 fold reduction in MIC of norfloxacin) against four clinical isolates (MRSA-ST1745, ST2071, P8029, and ST10760) with FICI ranging from 0.375 to 0.50.

Combination of norfloxacin with compound **14** exhibits strongest synergistic interaction (4–16 fold reduction in MIC of norfloxacin) against seven clinical isolates (MRSA-ST1745, ST2071, ST2438, P8029, ST5457, ST10342 and ST3151) with FICI ranging from 0.312 to 0.50.

Combination of norfloxacin with compound **17** showed synergistic interaction (2–8 fold reduction in MIC of

Table 2 *In vitro* combination study of most active substituted chalcone derivatives and norfloxacin against clinical isolated of methicillin resistant *S. aureus* (MRSA)

Strains	MIC of NOR alone ($\mu g\ mL^{-1}$)	MIC of NOR in presence of **2** ($\mu g\ mL^{-1}$)			MIC of NOR in presence of **11** ($\mu g\ mL^{-1}$)			MIC of NOR in presence of **14** ($\mu g\ mL^{-1}$)			MIC of NOR in presence of **17** ($\mu g\ mL^{-1}$)			MIC of NOR in presence of **27** ($\mu g\ mL^{-1}$)		
		MIC of NOR	FICI	FR*	MIC of NOR	FICI	FR*	MIC of NOR	FICI	FR*	MIC of NOR	FICI	FR*	MIC of NOR	FICI	FR*
MRSA-ST1745	500	62.5	**0.375**	8	62.5	**0.375**	8	31.25	**0.312**	16	62.5	**0.375**	8	125	**0.5**	4
MRSA-ST2071	500	31.25	**0.312**	16	62.5	**0.375**	8	31.25	**0.312**	16	62.5	**0.375**	8	125	**0.5**	4
MRSA-ST2438	250	31.25	0.625	8	62.5	0.75	4	31.25	**0.375**	8	62.5	0.75	4	31.25	**0.375**	8
MRSA-B10732	250	31.25	0.625	8	62.5	0.75	4	31.25	0.625	8	62.5	0.75	4	62.5	0.75	4
MRSA-P8029	125	31.25	**0.50**	4	31.25	**0.50**	4	31.25	**0.50**	4	62.5	1.0	2	62.5	1.0	2
MRSA-ST5457	125	31.25	**0.50**	4	62.5	1.0	2	31.25	**0.50**	4	62.5	1.0	2	62.5	1.0	2
MRSA-ST10342	250	62.5	0.75	4	62.5	0.75	4	31.25	**0.375**	8	62.5	0.75	4	62.5	0.75	4
MRSA-B10760	500	62.5	**0.375**	8	62.5	**0.375**	8	62.5	0.75	8	62.5	**0.375**	8	125	**0.50**	4
MRSA-ST3151	250	62.5	0.750	4	62.5	0.750	4	31.25	**0.375**	8	62.5	0.750	4	62.5	0.750	4
MRSA-P6642	125	62.5	1.0	2	62.5	1.0	2	31.25	0.75	4	62.5	1.0	2	62.5	1.0	2

norfloxacin) against three clinical isolates (MRSA-ST1745, ST2071, and ST10760) with FICI = 0.375.

Similarly combination of compound **27** with norfloxacin, reveal synergistic interaction (2–8 fold reduction in MIC of norfloxacin) against four clinical isolates (MRSA-ST1745, ST2071, ST2438, and ST10760) with FICI ranging from 0.375 to 0.50 (Table 2). Since clinical isolate MRSA-ST2071 also exhibited higher level of resistance towards the maximum number of antibiotics (data not shown).

Combination effect of chalcone derivatives with norfloxacin on bacterial killing kinetics

Times kill kinetics study of substituted chalcone derivatives (**2**, **11**, **14**, **17** and **27**) with norfloxacin alone as well as in combination were evaluated at two different MIC concentrations. At 1/2MIC and MIC concentration derivatives **2** and **14** diminish the viability of bacterial cells up to 2 and 3 folds, while derivatives **11**, **17** and **27** reduce up to 1.5 folds respectively after 24 h of incubation.

Combination of norfloxacin with derivative **2** (1/2MIC Comp **2** + 1/2MIC Nor, MIC Comp **2** + MIC Nor), diminish in viability of bacterial cells up to 5 and 10 folds at 1/2MIC and MIC respectively after 24 h incubation. The combination of compound **14** with norfloxacin at 1/2MIC concentration (1/2MIC Comp **14** + ½MIC Nor) diminish the viability of bacterial cells up to 6 folds after 24 h of incubation, while at MIC concentrations (MIC Comp **14** + MIC Nor) not a single colony was observed after 24 h of incubation indicating bactericidal effect of combination.

Combination of norfloxacin with derivatives **11**, **17**, and **27** at different MIC concentrations, diminish the viability of bacterial cells only up to 3 folds (Fig. 1). Since maximum synergy (up to 16 folds) of derivatives **2** and **14** was observed against clinical isolate MRSA-ST2071, it was selected for further experiments.

Ethidium bromide accumulation and efflux assay

Taking advantage of Et-Br being a substrate for many MDR efflux pump, the ability of both compound **2** and **14** to accumulate and inhibit efflux of Et-Br in bacterial cells as Et-Br bound to intracellular nucleic acid were measured *via* flow cytometer and spectrofluorimeter. Et-Br accumulation assay using flow cytometer clearly indicate that both substituted chalcone derivative **2** and **14** blocks the MDR efflux pump as shown by the increase in Et-Br accumulation as increase in Et-Br fluorescence in the bacterial cells. A significant shift (1.739 for compound **2** and 2.374 fold for compound **14**) in the intensity of red fluorescence was observed inside the bacterial cells as compare to untreated control (Fig. 2). Similarly, spectrofluorimeter analysis revealed that rapid decrease in Et-Br efflux over the time period of 30 min. As shown in Fig. 1 only control cells without compounds (**2** and **14**) extruded Et-Br, resulting significant decrease in fluorescence over the time period 30 min. In presence of both compound **2** and **14**, loss of florescence was significantly reduced, reflecting strong interference with Et-Br efflux by both compounds (Fig. 3).

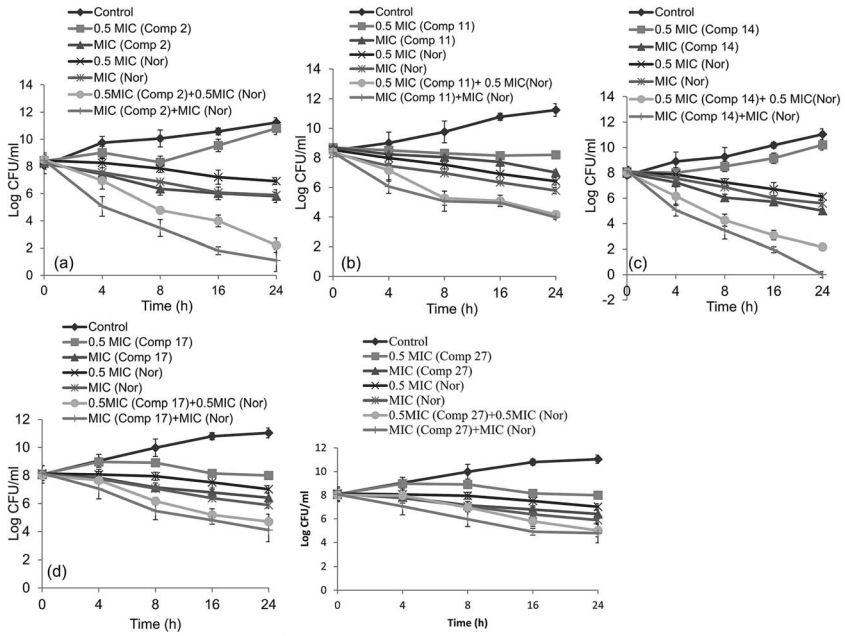


Fig. 1 Time kill kinetics study of chalcone derivatives **2** (a) **11** (b), **14** (c), **17** (d) and **27** (e) alone as well as in combination with Norfloxacin at different MIC concentrations against clinical isolates MRSA ST-2071. The data are expressed as mean ± SEs.

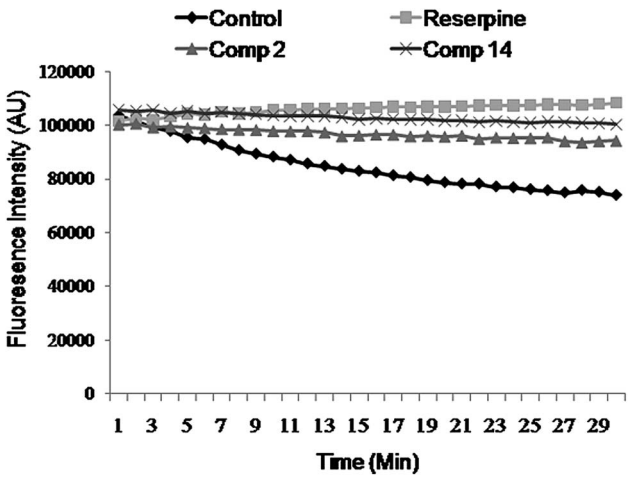


Fig. 2 Ethidium bromide (Et-Br) efflux inhibition assay in presence of chalcone derivatives **2** and **14** in clinical isolates MRSA-ST2071. Reserpine used as positive control.

In vivo anti-staphylococcal activity of identified substituted chalcone derivatives

The staphylococcal loads on various tissues (liver, spleen, kidney and lung) and blood upon treatment with both substituted chalcone derivatives (**2** and **14**) at various doses ranging from 12.5 to 100 mg kg^{-1} body weight are shown in Fig. 4 and 5 respectively. In animals treated with compound **2**, a significant reduction of staphylococcal load was observed ($P < 0.01$, $P < 0.001$) in blood and different tissues such as liver, spleen, kidney and lung. Similarly, significant reduction of staphylococcal load was also observed ($P < 0.001$) in blood and different tissue when animal treated with compound **14** in a dose-dependent manner as compared to untreated control.

Discussion

Currently, most of the pathogenic bacteria have been emerged as multidrug resistant (MDR) strains as they were resistant towards numerous classes of antibiotics through involvement of efflux pump.[39] Efflux is a process by which antimicrobial compounds are removed from cell by membrane based proteins, resulting in sub-lethal antimicrobial concentration at the active site.[35–37] *S. aureus* have an array of antiporter type efflux proteins, energized by proton motive force or ATP hydrolysis that excludes a wide range of antimicrobial compounds,[40] which result in resistance to broad spectrum of compounds including fluoroquinolones, biocides (acriflavine and cetrimide) and dye such as ethidium bromide.[41,42] There are many natural compounds as reserpine,[43] flavonolignan and flavone compounds,[44,45] *N*-caffeoylphenalkylamide derivatives,[46] piperine and piperine analogues,[47] have been reported as drug resistance reversal agents. Some synthetic inhibitors such as indoles,[48] phenothiazines and thioxanthenes,[49,50] quinolines and quinolones[51] and tricyclic compounds[52,53] have also been reported as drug resistance reversal agents. Many more chalcones have been reported for its antibacterial and resistance modifying activity,[15,54,55] which encourage us to design and synthesizes some substituted chalcone derivatives. Keeping the above, thirty chalcone derivatives were synthesized and investigated for their antibacterial and drug resistance reversal potential against clinical isolates of methicillin resistance *S. aureus* (MRSA).

On the basis of MIC data, among thirty derivatives, only five derivatives namely *trans*-3-(1*H*-indol-3-yl)-1-(4′-benzyloxyphenyl)-2-propen-1-one (**2**), 1-(4′-biphenyl)-3-(3′4′-dihydroxyphenyl)-2-propen-1-one (**11**), 1-(4″-hydroxy-3″-methylphenyl)-3-(4′-hydroxy-

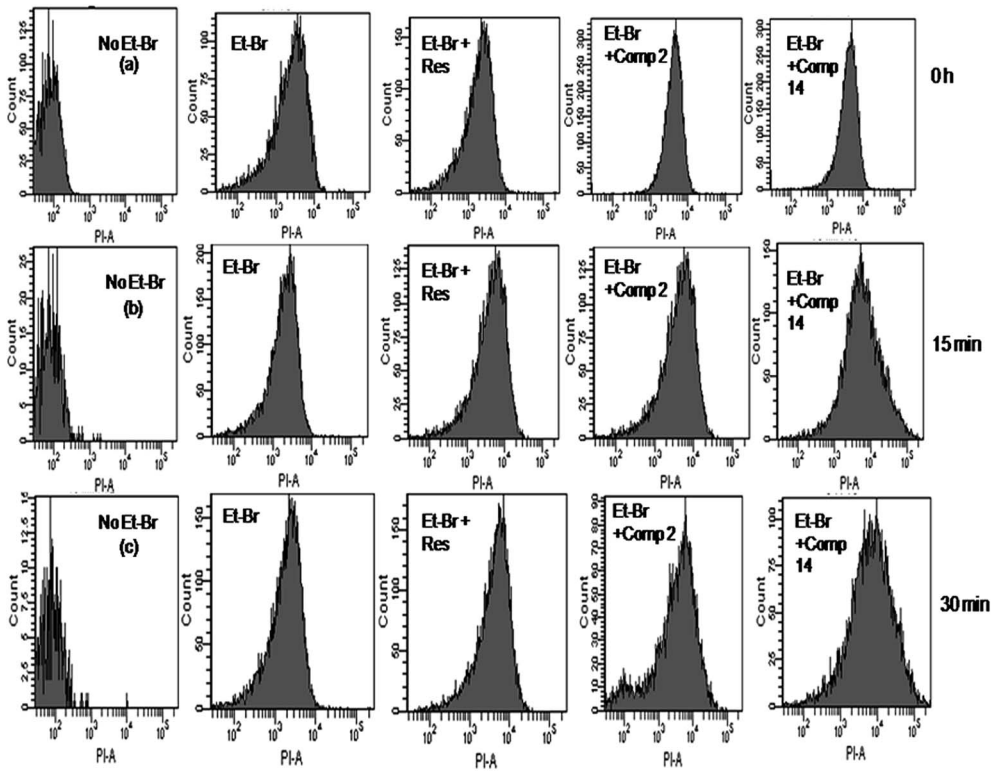


Fig. 3 Ethidium bromide (Et-Br) accumulation by chalcone derivatives **2** and **14** treated cells of MRSA-ST2071. Cells treated with chalcone derivatives **2** and **14** were examine immediately (a), after 15 min (b), and after 30 min (c) exposure to Et-Br. Reserpine used as positive control.

phenyl)-2-propen-1-one (**14**), 3-(4′-chlorophenyl)-1-(4″-hydroxyphenyl)-2-propen-1-one (**17**), LTG-oxime (**27**) showed significant antibacterial activity against all clinical isolates of MRSA. The results of quantitatively determining antibacterial coordination ability proved that combinations between norfloxacin and chalcone derivatives (**2**, **11**, **14**, **17** and **27**) were predominantly synergistic as 2–16 fold reduction in MICs of norfloxacin was observed. The combination of norfloxacin with derivatives **2** and **14** had most synergistic effect against MRSA in which the rates in increasing susceptibility of bacteria with norfloxacin were up to sixteen fold, respectively.

Times kill kinetics study revealed that combination of compound **2** with norfloxacin diminish the viability of bacterial cells up to 5 and 10 folds at 1/2MIC and MIC respectively after 24 h incubation. The combination of **14** with norfloxacin at 1/2MIC diminish the viability of bacterial cells up to 6 folds,

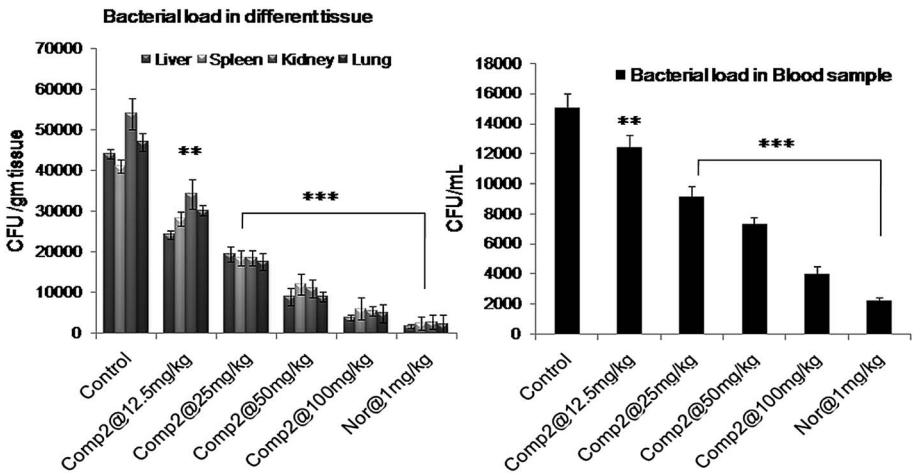


Fig. 4 Efficacy of chalcone derivative (**2**) at various doses in terms of reduction of bacterial burden (*S. aureus* MTCC-96) in multiple organs and blood. The infection was induced through the intravenous injection of *S. aureus* cells (0.5Mc Farland) in a volume of 0.2 mL. The data are expressed as mean ± SEs.

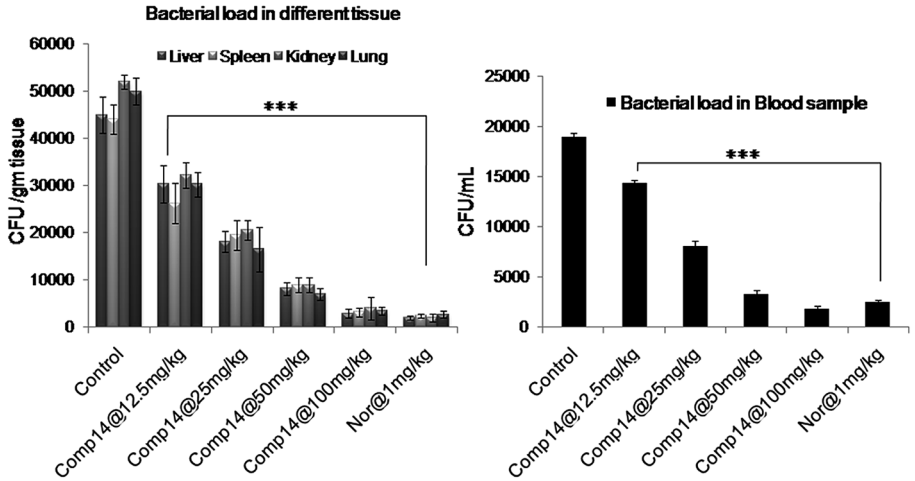
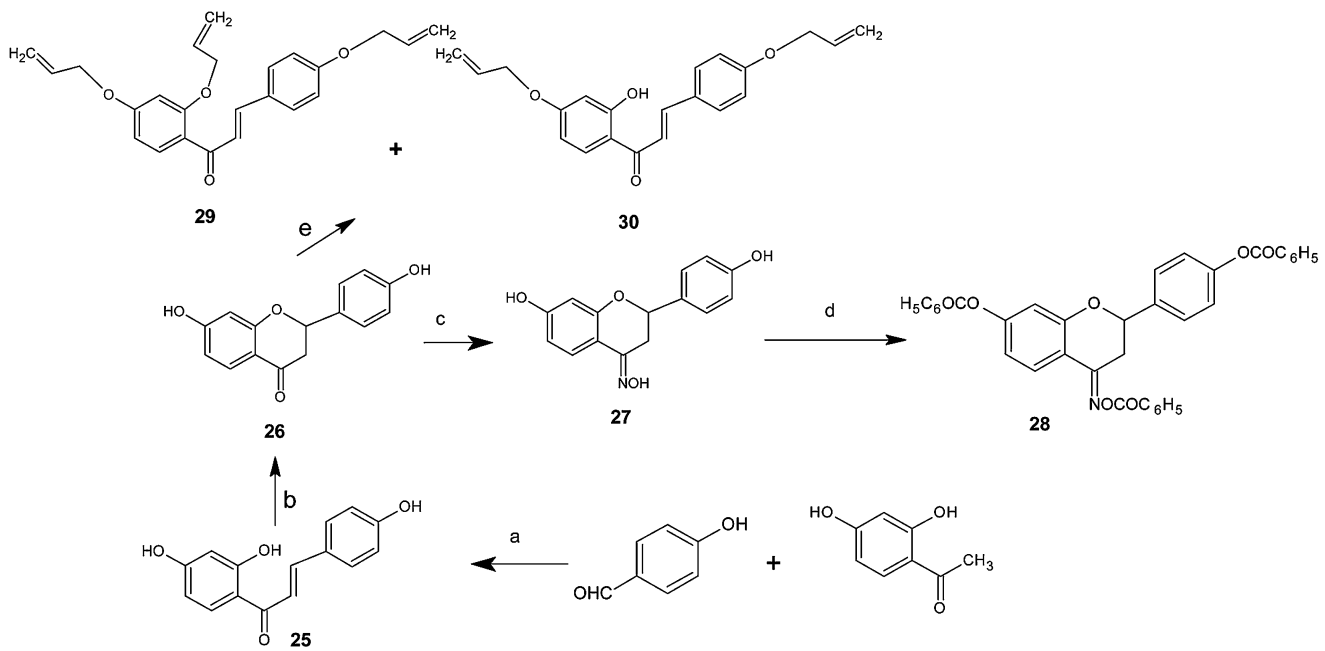


Fig. 5 Efficacy of chalcone derivative **14** at various doses in terms of reduction of bacterial burden (*S. aureus* MTCC-96) in multiple organs and blood. The infection was induced through the intravenous injection of *S. aureus* cells (0.5Mc Farland) in a volume of 0.2 mL. The data are expressed as mean ± SEs.

while after 24 h, at MIC concentrations not a single colony was observed indicating bactericidal combinations. Bactericidal activity defined as decrease in 3 log 10 CFU mL^{-1} corresponding to 99.9% killing.[46]

From the results of bioactivities, no clear cut structure–activity relationship can be drawn due to diversity of chalcone derivatives but presence of a free hydroxyl group in the aromatic ring appears to be very important for anti-MRSA activity alone or in combination with antibiotics; methylation to hydroxyl group might also be responsible for the abolishment in the anti MRSA activity. It was found that ring opened chalcone derivatives showed better activity than its ring cyclized derivatives (flavanones, **26–28**).

Since both chalcone derivatives **2** and **14** were found to act synergistically with norfloxacin, as they were evaluated for their efflux pump modulation potentials. Results of an Et-Br accumulation assay using flow cytometer clearly indicate that both chalcone derivative **2** and **14** modulate the bacterial efflux pump as shown by the increase in Et-Br fluorescence in the cells which might be due to accumulation of Et-Br inside the cells. Similar observations were reported by Kalle and Rizvi, 2011,[28,54] wherein celecoxib was found to inhibit bacterial MDR efflux pump.



Scheme 2 Reagents and conditions: (a) 60%, aq. KOH, 100 °C, 2 h, 48%, (b) concd HCl, MeOH, reflux, 48 h, 92%, (c) $NH_2OH \cdot HCl$, CH_3COONa, EtOH, reflux; (d) pyridine, benzoyl chloride acetone, (e) allyl bromide, K_2CO_3, reflux.

Similarly, spectrofluorimeter assay revealed the decrease in Et-Br efflux over the time period of 30 min as compared to untreated bacterial cells indicates towards a strong interference of substituted chalcone derivatives in modulation of efflux pump. Although there are many reports on *in vitro* antibacterial activity of substituted chalcone derivatives, but no any reports on *in vivo* efficacy using *S. aureus* infected Swiss albino mice model. During *in vivo* study through the infectious model, no any mortality or morbidity was recorded in any group of mice. Substituted chalcone derivatives were able to repress the staphylococcal burden of blood, lung, liver, kidney and spleen tissues in a dose-dependent manner ($P > 0.001$; $P > 0.01$), indicating the possible use of chalcones in the treatment of MRSA infections.[53,56]

Conclusion

In conclusion, the present investigation has clearly shown that, certain phenolic hydroxyl groups and/or phenyl side chain substituted chalcone derivatives namely *trans*-3-(1*H*-indol-3-yl)-1-(4′-benzyloxyphenyl)-2-propen-1-one (**2**), 1-(4″-biphenyl)-3-(3′4′-dihydroxyphenyl)-2-propen-1-one (**11**), 1-(4″-hydroxy-3″-methylphenyl)-3-(4′-hydroxyphenyl)-2-propen-1-one (**14**), 3-(4′-chlorophenyl)-1-(4″-hydroxyphenyl)-2-propen-1-one (**17**), LTG-oxime (**27**) diminish the escalation of bacterial cells and exhibits positive synergistic interaction with norfloxacin under both *in vitro* and *in vivo* conditions. Related to the antibacterial mechanisms, our synthesized chalcones could effect on clinical isolates of *S. aureus* (MRSA) by modulating the bacterial efflux pump. These results may be of immense helpful in development of anti-MRSA drug combinations from economical and non toxic natural product.

Abbreviations

MIC	Minimum inhibitory concentrations
DEPT	Distortionless enhancement by polarization transfer
ESI-MS	Electrospray ionization mass spectrometry
COSY	Correlation spectroscopy
HSQC	Heteronuclear single quantum correlation
HMBC	Heteronuclear multiple-bond correlation spectroscopy
CFU	Colony-forming unit
CLSI	Clinical and Laboratory Standards Institute
FICI	Fractional Inhibitory Concentration Index
MIC	Minimum Inhibitory Concentration
MTCC	Microbial Type Culture Collection
MFS	Major facilitator superfamily
MRSA	Methicillin-resistant *Staphylococcus aureus*
NOR	Norfloxacin

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