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ARTICLE

Glabridin-Chalcone hybrid molecules: Drug resistance reversal agent against clinical isolates of Methicillin-Resistant *Staphylococcus aureus*†

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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A novel series of glabridin-chalcone hybrid molecules (GCHMs) were synthesized, evaluated for their antibacterial and resistance reversal activity against clinical isolates of methicillin resistant strain of *Staphylococcus aureus* (MRSA) alone and in combination with norfloxacin. Glabridin showed significant anti-staphylococcal activity against various MRSA clinical isolates with MICs 12.5 µg/ml. However, all its synthesized derivatives displayed moderate to weak activity (MICs ranging from 12.5- >100 µg/ml). Among all the synthesized hybrid molecules, compounds 6f, 6h, 8f and 8h along with glabridin were chosen for combination study with norfloxacin. Among all tested compounds, 8h exhibited marked synergism up to 16 folds reduction in MICs with norfloxacin (FICI range from 0.312-0.375). In systemically infected *Swiss* albino mice model, compound 8h significantly (p<0.01, p<0.05) lowered the systemic bacterial load in blood, liver, kidney, lung and spleen tissues. The present study reports the potential use of GCHMs in the development of economical anti-infective combinations for treatment of infection caused by clinical MRSA isolates.

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CIMAP Communication no- CIMAP/PUB/2015/01

† The authors declare no competing interests.
Electronic Supplementary Information (ESI) available: [Tables containing Biochemical characterization of various clinical isolates of *S. aureus* used in the study, antibiotic resistance profile and PCR amplification of MecA gene in clinical MRSA strains]. See DOI: 10.1039/x0xx00000x

Introduction

Bacterial infections particularly caused by *Staphylococcus aureus* remains a major killer worldwide and antibiotic resistance continues to plague the effective control of this pandemic health problem.[1] Methicillin resistant *S. aureus* is one of the very common pathogens in both hospital (Hospital acquired, HA-MRSA) and community settings (Community acquired, CA-MRSA). It causes significant morbidity, mortality and are often associated with an array of life-threatening infections including surgical site infections, bacteremia, pneumonia and catheter-associated infections.[2,3] Now a day's most of the MRSA strains are resistant to many of the clinically used antibiotics such as penicillin and other β-lactam, macrolide, quinolones and even glycopeptide such as vancomycin.[4,5] This probably might be due to the presence of resistance genes encoded on mobile genetic elements (MGEs) such as plasmids and transposons. Therefore, efforts are being directed towards the design, synthesis and evaluation of a series of glabridin-chalcone hybrid molecules (GCHMs) as an alternative to the existing antimicrobial therapy.

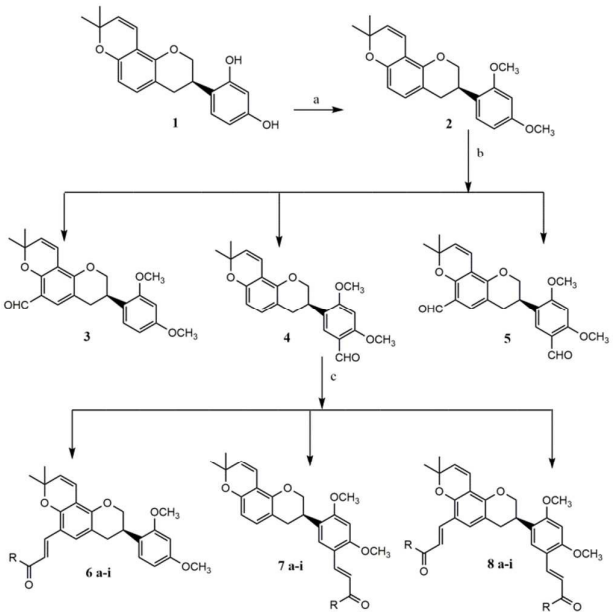
Natural products and its derivatives are always the good source of lead compounds. Glabridin **1** is a prenylated isoflavan found in the roots of *Gylyrrhiza glabra.*[6] It is a major phenolic compound isolated from hydrophobic fraction of licorice. It has a wide range of biological activities such as antimicrobial, antioxidant, anti-inflammatory, estrogenic, anticancer, anti-neuroprotective, anti-osteoporoticetc.[7-16]

Chalcones, bioprecursors of flavonoids in plants are the open-chain flavonoid in which two aromatic rings are joined by the α, β-unsaturated carbonyl system. Chalcones are the versatile molecules having diverse biological activities including antimicrobial,[17] anticancer,[18] antioxidant,[19] anti-inflammatory,[20] antimalarial,[21] hepatoprotective[22] and many more. In view of these biological activities of both the molecules, it was envisaged to synthesize the hybrid molecules where both the chalcone and glabridin skeleton are in the same molecule. Twenty seven GCHMs were synthesized and all these molecules were evaluated for their anti-MRSA activity alone as well as in combination with norfloxacin. Norfloxacin belongs to fluoroquinolones antibiotics which mainly act as substrate for efflux pump and it mainly responsible for Multi Drug Resistance (MDR) generation. The main aspect of this study may lead the discovery of novel GCHMs as efflux pump inhibitor.

Results and Discussion

The synthetic route for the preparation of GCHMs is depicted in Shceme I. Synthesis of 2',4'-dimethoxyglabridin **2** had already been reported in the literature.[23] Compounds **3**, **4**, and **5** were synthesized through Vilsmeier-Haack formylation reaction of compound **2**. The target molecules **6 a-i**, **7a-i**, and **8 a-i**, were synthesized by the Claisen Schmidt condensation reaction of compounds **3**, **4** and **5** with appropriate ketone derivatives. The structures of desired compounds were confirmed by mass, IR, ^{1}H and ^{13}C spectroscopy.

Emergence of Multi Drug Resistant (MDR) strains of *S. aureus* is now the major challenge for clinicians to manage the MRSA infections and also threatened the population worldwide. Therefore, investigators continue to search for novel effective antimicrobial compounds or their combinations.[24] Interaction of plant compounds with antibiotic as combination therapy, a new therapeutic strategy is widely accepted worldwide for combating the infections caused by such drug-resistant bacteria.[25] Many studies have reported of combination of natural product with antibiotics against infectious diseases.[26] Reuk-ngam et al. 2014

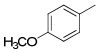
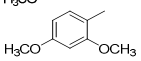
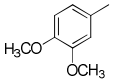
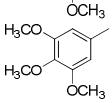
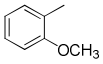
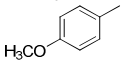
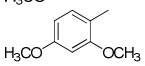
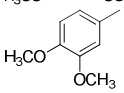
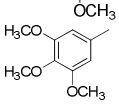
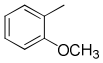
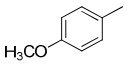
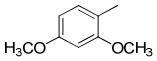
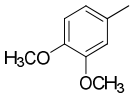
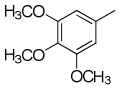


Scheme 1. Reagents and conditions: a) CH_3I, K_2CO_3, acetone, reflux 4 hrs b) DMF, $POCl_3$ in acetonitrile, rt, overnight c) $RCOCH_3$, KOH in MeOH, rt, 4-8 hrs.

reported the antimicrobial activity of coronarin D and its synergistic potential with antibiotics.[27] Similarly Mun et al. 2013 reported synergistic antibacterial effect of curcumin against MRSA.[28] These findings may encourage us to study *in vitro* antibacterial efficacy of GCHMs alone as well as in combination with fluoroquinolones antibiotics against clinical MRSA strains. All the clinical isolates of *S. aureus* (MRSA) were characterized by using various biochemical parameters including *S. aureus* identification test kit (Microxpress, India). Based on these tests, it was confirmed that all the clinical isolates were *S. aureus* (Table S1). Further PCR amplification of *mecA* gene (310 bp) results showed that all the clinical isolates were MRSA (Fig S1). Antibiotic sensitivity profiling results showed that all the clinical isolates used in this study were found to be resistant toward ampicillin, oxacillin, penicillin, nalidixic acid, cefoxitin, carbenicillin, cefazolin, rifampicin, streptomycin, kanamycin, neomycin, norfloxacin and erythromycin. Since all the clinical isolates showed resistance to multiple drugs, they were considered as multidrug resistant (MDR) Table S2. In this study we have focused mainly on anti-MRSA activity of fluoroquinolones antibiotics, norfloxacin (MIC ranges from 250-500 µg/ml) and GCHMs alone as well as in combinations. The results of MIC assay showed that free hydroxyl groups on glabridin is essential for its antibacterial activity as its 2,4-O-dimethoxyglabridin derivative was found to be inactive. But in the present study, it was found that the synthesized novel methoxy derivatives (**6h**, **6f**, **8f**, **8h**) were found to be more active than glabridin in combination with norfloxacin. Among 27 GCHMs, two derivatives **6h** and **8f** exhibited significant antibacterial activity against all clinical isolates of MRSA including the reference strain (SA-MTCC96) with MIC value ranging from 12.5-25 µg/ml and fourteen compounds(**6b**, **8e**, **7g**, **6c**, **8i**, **7i**, **7h**, **8h**, **6a**, **7a**, **6g**, **6f**, **7c** and **8a**) exhibited MIC ranging from 25-50 µg/ml while in case of glabridin alone the MIC ranges from 3.125-12.5 µg/ml. Norfloxacin as positive control, exhibited MIC in the range 125–500 µg/ml against all the tested clinical isolates of MRSA(Table1).

Table 1 Antibacterial activity of Glabridin and their derivatives against clinical isolates of *S. aureus* (MRSA).Minimum inhibitory concentration (µg ml^{-1})

Comp	R	SA	MRSA- 2071	MRSA-4629	MRSA-4423	MRSA-10760	MRSA-10342	MRSA-4650
6a		25	25	25	25	25	25	25

6b		25	50	25	50	50	50	25
6c		25	25	25	25	25	25	25
6d		>100	>100	>100	>100	>100	>100	>100
6e		>100	>100	>100	>100	>100	>100	>100
6f		25	50	50	50	50	50	50
6g		25	25	25	25	25	25	25
6h		12.5	25	12.5	12.5	25	12.5	12.5
6i		100	>100	>100	>100	>100	>100	>100
7a		25	25	25	25	25	25	25
7b		>100	>100	>100	>100	>100	>100	>100
7c		25	50	50	50	50	50	50
7d		50	>100	>100	>100	>100	>100	>100
7e		12.5	25	25	25	25	25	12.5
7f		100	>100	>100	>100	>100	>100	>100
7g		25	25	25	25	25	25	25
7h		25	25	25	25	25	25	25
7i		25	50	50	50	50	50	50
8a		25	25	25	25	25	25	25
8b		50	100	100	100	100	100	100
8c		>100	>100	>100	>100	>100	>100	>100
8d		100	>100	>100	>100	>100	>100	>100
8e		50	50	50	50	50	50	50
8f		12.5	25	12.5	25	25	12.5	12.5
8g		50	100	100	100	100	100	100
8h		25	50	25	50	50	50	25

8i		50	50	50	50	50	50	50
1 (Glabridin)		3.125	12.5	12.5	12.5	12.5	12.5	12.5
2 (2′,4′-dimethoxyglabridin)		>100	>100	>100	>100	>100	>100	>100
3 (6-formyl-2′,4′-dimethoxyglabridin)		>100	>100	>100	>100	>100	>100	>100
4 (5′-formyl-2′,4′-dimethoxyglabridin)		>100	>100	>100	>100	>100	>100	>100
5- (5′,6-diformyl-2′,4′-dimethoxyglabridin)		>100	>100	>100	>100	>100	>100	>100
Norfloxacin		1.56	500	500	250	500	250	125

Table 2 *In vitro* combination study of identified derivatives with norfloxacin against clinical isolated of methicillin resistant *S. aureus* (MRSA)

Strains	MIC of Nor alone (µg/ml)	MIC of NOR in presence of 1 (µg/ml)			MIC of NOR in presence of 6h (µg/ml)			MIC of NOR in presence of 8h (µg/ml)			MIC of NOR in presence of 6f (µg/ml)			MIC of NOR in presence of 8f (µg/ml)		
		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-2071	500	250	0.75	2	62.5	0.375	8	31.25	0.312	16	125	0.50	4	125	0.50	4
MRSA-4629	500	125	0.50	4	125	0.625	4	31.25	0.312	16	62.5	0.375	8	125	0.50	4
MRSA-4423	250	125	1	2	31.25	0.50	8	31.25	0.375	8	62.5	0.75	4	62.5	0.75	4
MRSA-10760	500	125	0.75	4	62.5	0.375	8	62.5	0.375	8	125	0.75	4	62.5	0.375	8
MRSA-10342	250	125	1	2	31.25	0.50	8	31.25	0.375	8	31.25	0.375	8	62.5	0.75	4
MRSA-4650	250	62.5	0.5	4	62.5	0.625	4	31.25	0.375	8	62.5	0.50	4	62.5	0.75	4

FICI: ≤ 0.5= synergy; 0.5–4= additivity/ indifference; > 4 antagonism
Abbreviations for interpretations: S, synergy; A/I, additivity/indifference; ANT, antagonism, FICI, fractional inhibitory concentrations index; FR, fold reduction; MIC, minimum inhibitory concentration; NOR, norfloxacin;*FR= fold reduction

Norfloxacin was further selected for combination study with most active GCHMs (**6h** and **8f**), two moderate active GCHMs (**6f**, **8h**) and glabridin with norfloxacin were evaluated through checker board assay because it is mainly act as substrate of efflux pump, which is mainly responsible for MDR generation. Further the combination study of most active GCHMs (**6h** and **8f**), two moderate active GCHMs (**6f** , **8h**) and glabridin with norfloxacin were evaluated through checker board assay. The result of combination study showed that **8h** exhibits strongest synergistic interaction with 8–16 folds reduction (FICI= 0.312 to 0.375) in the MIC of norfloxacin i.e. MIC reduced from 500-125 to 31.25-15.725 µg/ml respectively against all the tested clinical isolates of MRSA. Similarly the MIC of 8h also reduced up to four folds (Table 2). The combination of **6h** with norfloxacin showed synergistic interaction

(4-8 folds reduction in MIC of norfloxacin and 4 fold reduction in MIC of 6h). The MIC of norfloxacin reduces from 500-250 to 62.5-31.25 µg/ml against four clinical isolates (MRSA-ST2071, P4423, ST10760 and ST10342) with FICI ranging from 0.375 to 0.50

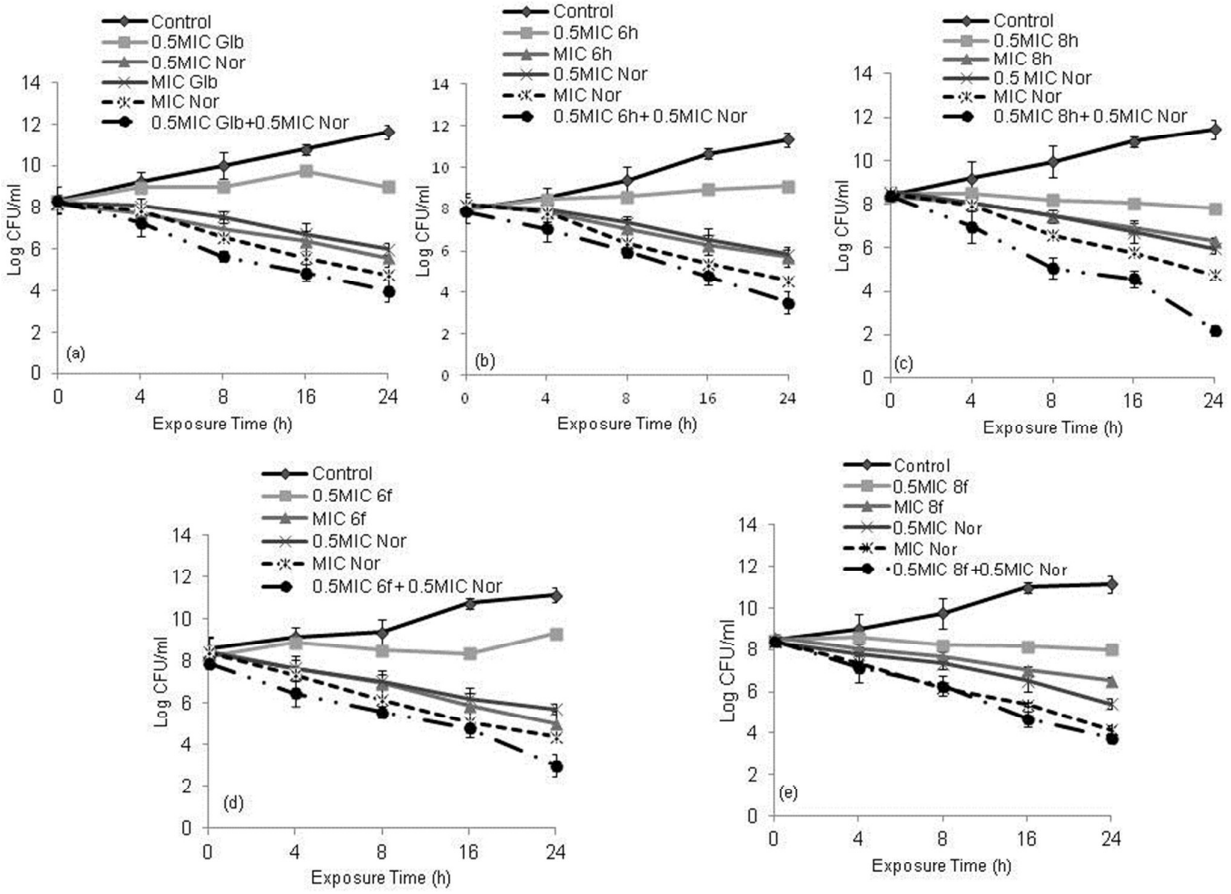


Figure 1. Time kill kinetics study of glabridin (Glb, a), and their derivatives 6h(b), 8h(c), 6f(d), and 8f(e) alone as well as in combination with norfloxicin (Nor) at different MIC concentration against clinical isolates MRSA ST-2071. Data expressed as mean ± SDs

similarly. Combination of **8f** with norfloxacin exhibited synergistic interaction (4–8 folds reduction in MIC of norfloxacin and 4 fold reduction of MIC of 8f) against three clinical isolates (MRSA-ST2071, P4423 and ST10760) with FICI ranging from 0.375 to 0.50. Combination of **6f** with norfloxacin also showed synergistic interaction (4–8 folds reduction in the MIC of norfloxacin, 500-125 to 125-31.25 µg/ml respectively) against four clinical isolates (MRSA-ST2071, P4629, ST10342 and P4650) with FICI ranging from 0.375 to 0.50. The MIC of 6f also reduced up to 4 folds. Combination of glabridin with norfloxacin exhibit synergistic interaction (2-4 folds reduction in the MIC of norfloxacin and 4 fold reduction in MIC of glabridin) against two clinical isolates (MRSA 4629 and 4650) only. Although in combination study the GCHMs reduced the MIC of norfloxacin up to 16 folds which are still quite high from the clinical point of view, but the significant anti-MRSA activity of some GCHMs alone as well as in combination with norfloxacin against all clinical strains of *S. aureus* showed the importance of plant derived molecule

glabridin to be exploited further derivatization for its novel derivatives with clinical value. Time-kill curve represents a useful method for evaluating the kinetic interactions between bacterial cells and antimicrobial agents. Time kill kinetics study of glabridin and its derivatives (6h, 8h, 6f and 8f) with norfloxacin alone as well as in combination were evaluated at two different MIC concentrations. At 1/2MIC and MIC concentration glabridin and GCHMs derivatives diminish the viability of bacterial cells up to 1.52 and 2 folds respectively after 24 h of incubation. In Combination study, the combination of norfloxacin with glabridin, 6h and 8f at ½ MIC concentration, diminish in viability of bacterial cells up to 3 folds only after 24 h incubation. The combination of 6f with norfloxacin at 1/2MIC concentration diminish the viability of bacterial cells up to 4 folds after 24 h of incubation. While the combination of derivative 8h with norfloxacin at ½ MIC concentrations, diminish the viability of bacterial cells up to 6 folds (Fig. 1). Since maximum synergy (up to 16 folds) of derivatives 8h was observed against clinical isolate MRSA-ST2071, it was selected for further experiments. With regard to the SAR, the in vitro results showed that in case of GCHMs 6a-i having electron donationg group (-OCH_3) at ortho and para position of phenyl ring of chalcone counterpart, were less active as compared to the unsubstituted derivative (6a). Introduction of –OCH_3 group at meta position had no effect on activity. Substitution of phenyl ring by the furan led to the increased in anti-MRSA activity. In case of GCHMs 7a-i, indroduction of OCH_3 group at ortho and para position reduced Its anti- MRSA activity and at meta position resulted in no change or slightly reduction in activity. In case of compounds having di-chalcone moiety, introduction of OCH_3 group at ortho, meta and meta position led to decrease in activity. In combination study, compounds having furan ring in place of phenyl ring, showed highest reduction in MIC of norfloxacin (8-16 fold) as compared to phenyl substituent.

During *in vivo* study through the infectious model, no any mortality or morbidity was recorded in any group of mice. The staphylococcal loads on various tissues (liver, spleen, kidney and lung) and blood upon treatment with GCHM **8h** at various doses ranging from 12.5 to 100 mg/kg body weight evaluated and are

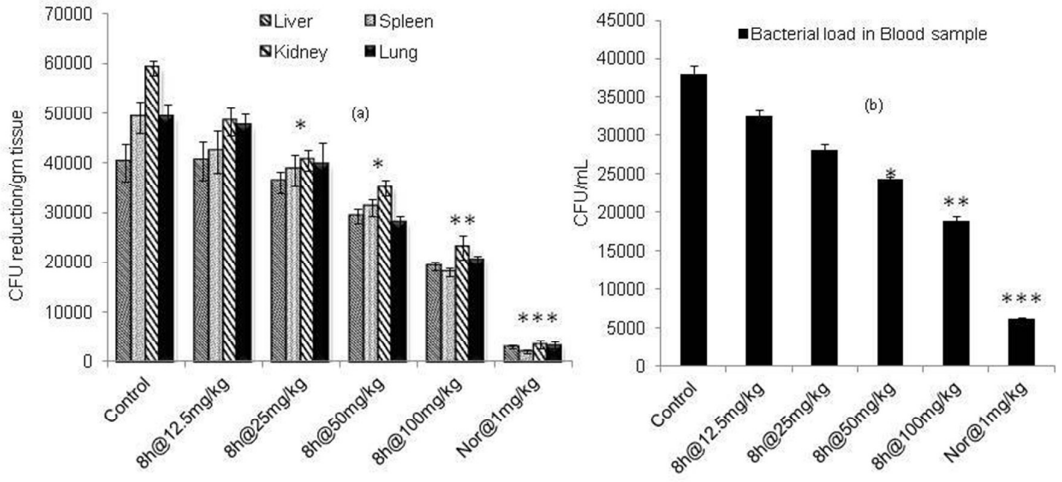


Figure 2 Efficacy of glabridin derivative (8h) at various doses in terms of reduction of bacterial burden (*S. aureus* MTCC-96) in multiple organs (a) and blood (b). The infection was induced through the intravenous injection of 0.2 mL (10^6 CFU) of *S.aureus* in a volume of 0.2 mL. Data are expressed as mean ± SEs.

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shown in fig. 2. In animals treated with GCHM **8h**, a significant reduction of staphylococcal load was observed ($p< 0.05$, $p< 0.001$) in blood and different tissues such as liver, spleen, kidney and lung in a dose-dependent manner as compared to untreated control.

Conclusions

In conclusion, we have prepared a series of novel glabridin-chalcone hybrid molecules (GCHMs) and evaluated their antibacterial activity against different clinical isolates of methicillin resistant strain of *Staphylococcus aureus* (MRSA) alone and in combination with norfloxacin. Results of *in-vitro* combination study showed that GCHMs exhibited synergistic and additive interactions (2-16 folds reduction in MICs) of norfloxicin against all tested clinical isolates of MRSA. Compound 8h exhibited marked synergism up to 16 folds reduction in MICs of norfloxacin (FICI range from 0.312-0.375). Though the MIC of norfloxacin is still high in combination for its clinical use but glabridin can be exploited to prepare better derivatives to increase the efficacy of norfloxacin against resistant bacterial strain. So we conclude from this study that the GCHMs are promising template for the development of new drugs to treat MRSA infection.

Experimental

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 Thermo Scientific Nicolet-380 FT IR spectrometer. NMR spectra were obtained in acetone-d_6, $CDCl_3$ and pyridine-d_5 on a Bruker Avance, 300 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 Perkin-Elmer turbo Mass/Shimadzu LC-MS and LC-MS-MS APC3000 (Applied Biosystem). HRMS spectra were recorded on JEOL-AccuTOF JMS-T100LC mass instrument using dry helium for ionization. TLC analyses were carried out on precoated silica gel 60 F254 plates (Merck). 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 and 100-200 mesh were used in the column chromatography for the purification of metabolites. The compounds were identified by their spectral IR, ID (^1H, ^{13}C, DEPT) and 2D (COSY, HSQC, HMBC) NMR, ESI-MS and HRMS analysis.

Plant material

Roots of *Glycyrrhiza glabra* L.(Fabaceae) were procured from the research farm of CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India in March 2011 and authenticated by taxonomist Dr S C Singh, Department of Botany and Pharmacognosy, CSIR-CIMAP, Lucknow and a voucher specimen #7401 of the material was deposited in the department.

Extraction and isolation

Fresh roots of *G. glabra* were cut into small pieces and dried in oven at temp 35°C and powdered. The dried and powdered roots of *G. glabra* (10 kg) were extracted three times with ethylacetate. The combined ethylacetate extract was concentrated on rotavapour and the residue obtained (268 gram) was column chromatoghaped

on 60-120 mesh size silica gel, eluting with hexane/EtOAc solvent system. Glabridin rich fraction (47.6 gm) was eluted in 70:30 hexane-EAA solvent system which was further purified by vaccum liquid chromatography and crystallized in benzene to afford pure white crystals of glabridin (11.6 gm).The structure of glabridin was confirmed by comparison of its spectral data (IR, mass, NMR) with those reported in literature.[29]

Formylation of Glabridin dimethyl Ether- To a solution 2',4'-dimethoxyglabridin, **2** (1mmol) in acetonitrile, DMF (4.5 mmol) and $POCl_3$ (4.5 mmol) were added and the resulting mixture was allowed to stir at room temperature for overnight. After completion of the reaction, water was added and the reaction mixture was extracted with ethylacetate (3x 100 ml). The organic layer was washed with water, dried over anhydrous sodium sulfate and concentrated in vaccum. The residue thus obtained was subjected to column chromatography on silica gel (100-200 mesh), to yield compounds **3** (20%), **4** (14.5%) and **5** (60.2%).

3- White powder, 20% yield, mp 95-98 ºC. IRv^{max} (KBr): 2849, 1674 (C=O), 1585, 1467, 1289, 1157, 1131, 1032 cm^{-1}; ^1H NMR (300Hz, acetone d_6): 1.47 (6H, s, H_3-5", H_3-6"), 2.86 (1H, m, H-4e), 3.00 (1H, dd, J=15.6, 11.4 Hz, H-4a), 3.47 (1H, m, H-3a), 3.80, 3.84 (3H each, s, 2 x OCH_3), 4.08 (1H, t, J= 10.2 Hz, H-2a), 4.38 (1H, m, H-2e), 5.76 (1H, d, J=10.2 Hz, H-3''), 6.49 (1H, dd, J= 8.4, 2.4 Hz, H-5'), 6.58 (1H, d, J= 2.1 Hz, H-3'), 6.61 (1H, d, J=10.2 Hz, H-4''), 7.08 (1H, d, J=8.4 Hz, H-6'), 7.37 (1H, s, H-5), 10.26 (1H, s, CHO); ^{13}C NMR (75 MHz, acetone d_6): 27.31, 27.46 (C-5''/C-6''), 30.36 (C-4), 31.66 (C-3), 55.11, 55.40 (2 x OCH_3), 70.99 (C-2), 77.24 (C-2''), 98.94 (C-3'), 105.08 (C-5'), 109.90 (C-8), 116.39 (C-10, C-4''), 118.49 (C-6), 120.94 (C-1'), 127.85 (C-6'), 127.97 (C-5), 129.90 (C-3''), 155.33 (C-9), 155.83 (C-7), 158.76 (C-2'), 160.55 (C-4'), 186.94 (CHO); ESI-MS, (Positive): m/ z 381 $[M + H]^+$; HRMS (ESI) calcd for $C_{23}H_{24}O_5$ $[M + H]^+$ 381.1696, found 381.1697.

4- White powder, 14.5% yield, mp 85 ºC. IRv^{max} (KBr): 2850, 1670 (C=O), 1475, 1277, 1211, 1155, 1115, 1090 cm^{-1}; ^1H NMR (300Hz, acetone d_6): 1.35, 1.36 (3H each, s, H_3-5", H_3-6"), 2.75 (1H, dd, J=4.8, 1.8 Hz, H-4e), 2.86 (1H, m, H-4a), 3.44 (1H, m, H-3a), 3.96 (1H, m, H-2a), 3.98 (4H, m, H-2e, OCH_3), 4.00 (3H, s, OCH_3), 4.31 (1H, m, H-2e), 5.59 (1H, d, J=10.2 Hz, H-3''), 6.28 (1H, d, J= 8.1 Hz, H-6), 6.59 (1H, d, J=9.9 Hz, H-4"), 6.78 (1H, s, H-3'), 6.81 (1H, d, J=8.1, H-5), 7.57 (1H, s, H-6'), 10.24 (1H, s, CHO) ; ^{13}C NMR (75 MHz, acetone d_6), 27.36, 27.53 (C-5''/C-6''), 30.48 (C-4), 31.77 (C-3), 55.99, 56.11 (2 x OCH_3), 70.02 (C-2), 75.60 (C-2''), 95.76 (C-3'), 108.97 (C-6), 110.02 (C-8), 114.55 (C-10), 117.17 (C-4''), 118.50 (C-5'), 122.39 (C-1'), 126.70 (C-6'), 129.19 (C-3''), 129.70 (C-5), 150.00 (C-9), 152.42 (C-7), 163.64 (C-2'), 164.33 (C-4'), 187.00 (CHO); ESI-MS, (Positive): m/ z 381 $[M + H]^+$; HRMS (ESI) calcd for $C_{23}H_{24}O_5$ $[M + H]^+$ 381.1696, found 381.1697.

5- White powder, 60.2 % yield, mp 75-76 ºC. IRv^{max} (KBr): 2847, 1673 (C=O), 1582, 1462, 1278, 1209, 1132, 1115, 1025 cm^{-1}; ^1H NMR (300Hz, acetone d_6): 1.47, 1.52 (3H each, s, H_3-5", H_3-6"), 2.87 (1H, m, H-4e), 3.05 (1H, dd, J=15.6, 11.1, H-4a), 3.48 (1H, m, H-3a), 3.99, 4.05 (3H each, s, 2 x OCH_3), 4.14 (1H, t, J= 10.2 Hz, H-2a), 4.42 (1H, m, H-2e), 5.76 (1H, d, J=10.2 Hz, H-3''), 6.61 (1H, d, J=9.9 Hz, H-4"), 6.81 (1H, s, H-3'), 7.38 (1H, s, H-5), 7.58 (1H, s, H-6'), 10.24, 10.26 (1H each, s, 2 x CHO); ^{13}C NMR (75 MHz, acetone d_6): 27.32, 27.43 (C-5''/C-6''), 30.11 (C-4), 31.57 (C-3), 56.01, 56.15 (2xOCH_3), 70.62 (C-2), 77.27 (C-2''), 95.88 (C-3'), 109.94 (C-8), 116.06 (C-10), 116.35 (C-4''), 118.52 (C-6), 118.57 (C-5'), 121.67 (C-1'), 126.75 (C-6'), 127.90 (C-5), 129.97 (C-3''), 155.19 (C-9), 155.87 (C-7), 163.78 (C-2'), 164.33 (C-4'), 186.95 (2 x CHO); ESI-MS,(Positive): m/ z 409

$[M + H]^+$; HRMS (ESI) calcd for $C_{24}H_{24}O_6[M + H]^+$409.1645, found 409.1646.

Synthesis of Glabridin-Chalcone hybrid molecules

General procedure- Chalcones were prepared by Claisen–Schmidt condensation reaction of mono/di formyl glabridin and different substituted acetophenone derivatives. In case of mono formyl glabridin (**3**&**4)**, 1mmol of mono formyl glabridin and 1 mmol of acetophenone derivatives were dissolved in methanol with stirring. KOH (10 mmol) was added. Resulting solution was stirred for 4-8 hours. Then the reaction mixture was poured into ice –cold water and acidified by addition of 1 N HCl. The crude product was extracted with ethylacetate (50 ml x 3) and the organic phase was washed with water, dried over anhydrous Na_2SO_4 and evaporated in vacuo. Resulting solid was chromatographed on 100-200 mesh size silica gel column. In case of diformylglabridin (**5**), 2 mmol of acetophenone derivatives were used.

6a- Yellow powder, 70 % yield, mp 55-58 ºC. IRv^{max} (KBr): 1656 (C=O), 1584, 1463, 1280, 1175, 1129, 1039 cm^{-1}; ^1H NMR (300Hz, acetone d_6): 1.48, 1.49 (3H each, s, H_3-5", H_3-6"), 2.88 (1H, m, H-4e), 3.02 (1H, dd, J=15.3, 10.8 Hz, H-4a), 3.50 (1H, m, H-3a), 3.77, 3.85 (3H each, s, 2 x OCH_3), 4.08 (1H, t, J= 10.2 Hz, H-2a), 4.37 (1H, m, H-2e), 5.74 (1H, d, J=10.2 Hz, H-3''), 6.50 (1H, dd, J= 8.4, 2.4 Hz, H-5'), 6.58 (1H, d, J= 2.4 Hz, H-3'), 6.63 (1H, d, J=9.9 Hz, H-4"), 7.10 (1H, d, J=8.4 Hz, H-6'), 7.50 (1H, s , H-5), 7.55 (2H, dd, J=7.8, 7.8 Hz, H-3''',H-5'''), 7.60 (1H, dd, J=7.2 Hz, 1.5, H-4'''), 7.79 (1H, d, J= 15.6 Hz, H-α), 8.07(2H, dd, J=7.8, 2.1 Hz, H-2''', H-6'''), 8.05 (1H, d, J=15.6 Hz, H-β) ; ^{13}C NMR (75 MHz, acetone d_6), 27.49, 27.66 (C-5''/C-6''), 30.56 (C-4), 31.76 (C-3), 55.10, 55.44 (2 x OCH_3), 70.75 (C-2), 76.98 (C-2''), 98.93 (C-3'), 105.07 (C-5'), 110.12 (C-8), 116.09 C-10), 116.23 (C-6), 116.87 (C-4''), 119.75 (C-α), 121.31 (C-1'), 127.95 (C-6'), 128.54 (C-3'''), 128.96 (C-5, C-3'',C-5'''), 129.54 (C-2'''), 129.67 (C-6'''), 132.73 (C-4'''), 139.31 (C-1'''), 139.53 (C-β), 152.28 (C-9), 152.60 (C-7), 158.75 (C-2'), 160.49 (C-4'), 189.52 (C=O); ESI-MS, (Positive): m/ z 483 $[M + H]^+$; HRMS (ESI) calcd for $C_{31}H_{30}O_5$ $[M + H]^+$ 483.2166, found 483.2167.

6b- Yellow powder, 62 % yield, mp 58-60 ºC. IRv^{max} (KBr): 1649, 1599, 1462, 1286, 1158, 1130, 1034 cm^{-1}. ^1H NMR (300Hz, acetone d_6): 1.44, 1.45 (3H each, s, H_3-5", H_3-6"), 2.84 (1H, m, H-4e), 3.01 (1H, dd, J= 15.3, 10.8, H-4a), 3.48 (1H, m, H-3a), 3.77, 3.86, 3.90 (3H each, s, 3 x OCH_3), 4.09 (1H, t, J= 10.2 Hz, H-2a), 4.34 (1H, m, H-2e), 5.71 (1H, d, J=10.2 Hz, H-3''), 6.50 (1H, dd, J= 8.4, 2.4 Hz, H-5'), 6.58 (1H, d, J= 2.4 Hz, H-3'), 6.62 (1H, d, J=9.9 Hz, H-4''), 7.02 (1H, d, J=7.2 Hz, H-3'''), 7.06 (1H, d, J= 7.5, H-5'''), 7.10 (1H, d, J=8.4 Hz, H-6'), 7.31 (1H, s, H-5), 7.40 (1H, d, J= 15.9 Hz, H-α), 7.50 (2H, dd, J= 7.2, 1.8, H-4''', H-6'''), 7.79 (1H, d, J=16.2 Hz, H-β); ^{13}C NMR (75 MHz, acetone d_6): 27.45, 27.62 (C-5''/C-6''), 30.56 (C-4), 31.77 (C-3), 55.11, 55.40, 55.74 (3 x OCH_3), 70.74 (C-2), 76.84 (C-2''), 98.92 (C-3'), 105.08 (C-5'), 110.12 (C-8), 112.26 (C-3'''), 116.03 (C-10) ,116.25 (C-6), 116.91 (C-4''), 120.88 (C-α), 121.31 (C-1'), 125.37 (C-5'''), 127.99 (C-6'), 129.48 (C-3''), 129.56 (C-5), 130.18 (C-6'''), 130.71 (C-1'''), 132.68 (C-4'''), 138.09 (C-β), 152.06 (C-9), 152.34 (C-7), 158.41 (C-2'''), 158.74 (C-2'), 160.48 (C-4'), 192.57 (C=O); ESI-MS, (Positive): m/ z 513 $[M + H]^+$; HRMS (ESI) calcd for $C_{32}H_{32}O_6$ $[M+ H]^+$ 513.2271, found 513.2273.

6c- Yellow powder, 61 % yield, mp 50-52 ºC. IRv^{max} (KBr): 1656, 1576, 1464, 1210, 1161,1127, 1033 cm^{-1}; ^1H NMR (300Hz, acetone d_6): 1.49, 1.51 (3H each, s, H_3-5", H_3-6"), 2.87 (1H, dd, J= 15.3, 4.8 Hz, H-4e), 3.02 (1H, dd, ,J= 15.3, 10.5 Hz, H-4a), 3.50 (1H, m, H-3a), 3.78, 3.86, 3.87 (3H each, s, 3 x OCH_3), 4.09 (1H, t, J=10.2 Hz, H-2a), 4.36 (1H, dd, J=10.2, 1.8 Hz, H-2e), 5.74 (1H, d, J=9.9 Hz, H-3''), 6.50 (1H, dd, J= 8.4, 2.4 Hz, H-5'), 6.59 (1H, d, J= 2.4 Hz, H-3'), 6.64 (1H, d, J=9.9 Hz, H-4''), 6.66 (1H, d, J=6.9, Hz, H-6'''), 7.11 (1H, d,

J=8.4 Hz, H-6'), 7.16 (1H, dd, J= 7.5, 1.8, H-4'''), 7.44 (1H, m, H-5'''), 7.49 (1H, s , H-5), 7.57 (1H, d, J= 1.8 H-2'''), 7.77 (1H, d, J= 15.6 Hz, H-α), 8.04 (1H, d, J=15.6 Hz, H-β); ^{13}C NMR (75 MHz, acetone d-$_6$): 27.49, 27.66 (C-5''/C-6''), 30.56 (C-4), 31.78 (C-3), 55.09, 55.25, 55.40, (3x OCH_3), 70.75 (C-2), 76.98 (C-2''), 98.94 (C-3'), 105.10 (C-5'), 110.48 (C-8), 113.27 (C-2'''), 116.08 (C-10), 116.23 (C-6), 116.87 (C-4'''), 118.72 (C-4''), 119.90 (C-α), 120.93 (C-6'''), 121.38 (C-1'), 127.95 (C-6'), 129.49 (C-3''), 129.79 (C-5), 130.01 (C-5'''), 139.60 (C-β), 140.79 (C-1'''), 152.29 (C-7, C-9), 158.76 (C-2'), 160.50 (C-3''', C-4'), 189.28 (C=O); ESI-MS, (Positive): m/ z 513 $[M + H]^+$; HRMS (ESI) calcd for $C_{32}H_{32}O_6$ $[M + H]^+$ 513.2271, found 513.2274.

6d- Yellow powder, 64 % yield, mp 88-90 ºC. IRν^{max} (KBr): 1649, 1561, 1463, 1279, 1166, 1130, 1024 cm^{-1}; ^{1}H NMR (300Hz, acetone d$_6$): 1.49, 1.50 (3H each, s, H_3-5", H_3-6"), 2.85 (1H, m, H-4e), 2.99 (1H, m, H-4a), 3.50 (1H, m, H-3a), 3.78, 3.86, 3.89 (3H each, s, 3 x OCH_3), 4.08 (1H, t, J= 10.2 Hz, H-2a), 4.35 (1H, m, H-2e), 5.74 (1H, d, J=10.2 Hz, H-3''), 6.50 (1H, dd, J= 8.4, 2.4 Hz, H-5'), 6.59 (1H, d, J= 2.1 Hz, H-3'), 6.64 (1H, d, J=9.9 Hz, H-4''), 7.05 (2H, dd, J=9.0, 2.7 Hz, H-3''', H-5'''), 7.10 (1H, d, J=8.4 Hz, H-6'), 7.48 (1H, s, H-5), 7.80 (1H, d, J= 15.6 Hz, H-α), 8.03 (1H, d, J=15.6 Hz, H-β), 8.09 (2H, dd, J=8.9, 1.8 Hz, H-2''', H-6''') ; ^{13}C NMR (75 MHz, acetone d-$_6$): 27.50, 27.68 (C-5''/C-6''), 30.59 (C-4), 31.79 (C-3), 55.10, 55.41, (3x OCH_3), 70.73 (C-2), 76.90 (C-2''), 98.96 (C-3'), 105.10 (C-5'), 110.11 (C-8), 114.15 (C-3''', C-5'''), 115.99 (C-10), 116.41 (C-6), 116.93 (C-4''), 119.77 (C-α), 121.37 (C-1'), 127.94 (C-6'), 129.48 (C-3''), 129.60 (C-5), 130.77 (C-2''', C-6'''), 132.13 (C-1'''), 138.68 (C-β), 152.13 (C-9), 152.38 (C-7), 158.76 (C-2'), 160.49 (C-4'), 163.68 (C-4'''), 187.87 (C=O); ESI-MS, (Positive): m/ z 513 $[M + H]^+$; HRMS (ESI) calcd for $C_{32}H_{32}O_6$ $[M + H]^+$ 513.2271, found 513.2272.

6e- Yellow powder, 67 % yield, mp 63-65 ºC. IRν^{max} (KBr): 1645, 1578, 1462, 1279, 1159, 1128, 1028 cm^{-1}; ^{1}H NMR (300Hz, acetone d$_6$): 1.46, 1.48 (3H each, s, H_3-5", H_3-6"), 2.89 (1H, m, H-4e), 3.00 (1H, m , H-4a), 3.49 (1H, m, H-3a), 3.79, 3.86, 3.88, 3.94 (3H each, s, 4 x OCH_3), 4.08 (1H, t, J= 10.2 Hz, H-2a), 4.34 (1H, m, H-2e), 5.73 (1H, d, J=9.9 Hz, H-3''), 6.50 (1H, dd, J= 8.1, 2.4 Hz, H-5'), 6.53 (2H, d, J= 2.4 Hz, H-3', H-3'''), 6.61 (1H, dd, J=8.7, 2.4, Hz, H-5'''), 6.63 (1H, d, J=9.9 Hz, H-4''), 7.12 (1H, d, J=8.4 Hz, H-6'), 7.57 (1H, d, J= 15.6 Hz, H-α), 7.63 (1H, d, J=8.4 Hz, H-6'''), 7.85 (1H, d, J=15.9 Hz, CH β); ^{13}C NMR (75 MHz, acetone d-$_6$), 27.45, 27.62 (C-5''/C-6''), 30.60 (C-4), 31.81 (C-3), 55.10, 55.40, 55.43, 55.81 (4 x OCH_3), 70.71 (C-2), 76.76 (C-2''), 98.75 (C-3'''), 98.92 (C-3'), 105.09 (C-5'), 106.01 (C-5'''), 110.11 (C-8), 115.91 (C-10), 116.59 (C-6), 116.95 (C-4''), 121.37 (C-1'), 123.26 (C-1'''), 125.57 (C-α), 128.01 (C-6'), 129.47 (C-5, C-3''), 132.57 (C-6'''), 136.80 (C-β), 151.95 (C-9), 152.09 (C-7), 158.75 (C-2'), 160.48 (C-4'), 160.70 (C-2'''), 164.40 (C-4'''), 189.99 (C=O); ESI-MS, (Positive): m/ z 543 $[M + H]^+$.

6f- Yellow powder, 68 % yield, mp 64-65 ºC. IRν^{max} (KBr): 1649, 1569, 1462, 1264, 1207, 1161, 1124, 1024 cm^{-1}; ^{1}H NMR (300Hz, acetone d$_6$): 1.49, 1.51 (3H each, s, H_3-5", H_3-6"), 2.85 (1H, dd, J= 15.6, 4.8 Hz, H-4e), 3.01 (1H, dd, J= 15.6, 11.1 Hz, H-4a), 3.51 (1H, m, H-3a), 3.78, 3.86, 3.88, 3.89 (3H each, s, 4 x OCH_3), 4.07 (1H, t, J= 10.2 Hz, H-2a), 4.35 (1H, m, H-2e), 5.73 (1H, d, J=9.9 Hz, H-3''), 6.50 (1H, dd, J= 8.4, 2.4 Hz, H-5'), 6.58 (1H, d, J= 2.4 Hz, H-3'), 6.64 (1H, d, J=9.9 Hz, H-4''), 7.05 (1H, d, J=8.7 Hz, H-5'''), 7.10 (1H, d, J=8.7 Hz, H-6'), 7.63 (1H, d, J=1.8 Hz, H-2'''), 7.76 (1 H, dd, J=8.4, 1.8 Hz, H-6'''), 7.81 (1H, d, J= 15.6 Hz, H-α) , 8.00 (1H, d, J=15.6 Hz, H-β); ^{13}C NMR (75 MHz, acetone d-$_6$); 27.52, 27.70 (C-5''/C-6''), 30.58 (C-4), 31.79 (C-3), 55.10, 55.40, 55.64, 55.72 (4 x OCH_3), 70.73 (C-2), 76.90 (C-2''), 98.95 (C-3'), 105.09 (C-5'), 110.12 (C-8), 111.11 (C-5'''), 111.46 (C-2'''), 115.97 (C-10), 116.43 (C-6), 116.94 (C-4''), 119.84 (C-α), 121.36 (C-1'), 127.94 (C-6'), 129.45 (C-3''), 129.81 (C-5), 122.91 (C-6'''), 132.25 (C-1'''), 138.68 (C-β), 149.83 (C-3'''), 152.13 (

C-9), 152.34 (C-7), 153.81 (C-4'''), 158.75 (C-2'), 160.48 (C-4'), 187.87 (C=O); ESI-MS, (Positive): m/ z 543 $[M + H]^+$; HRMS (ESI) calcd for $C_{33}H_{34}O_7$ $[M + H]^+$ 543.2377, found 543.2372.

6g- Yellow powder, 73 % yield, mp 64-65 ºC. IRv^{max} (KBr): 1649, 1576, 1464, 1210, 1161, 1127, 1033 cm^{-1}; ^1H NMR (300Hz, acetone d_6): 1.49, 1.51 (3H each, s, H_3-5", H_3-6"), 2.83 (1H, m, H-4e), 3.01 (1H, dd, J= 15.6, 11.1, H-4a), 3.51 (1H, m, H-3a), 3.80, 3.82, 3.90, 3.95, 3.96 (3H each , s, 5 x OCH_3), 4.09 (1H, t, J= 10.2 Hz, H-2a), 4.36 (1H, dd, J= 10.5, 2.1 Hz, H-2e), 5.74 (1H, d, J=9.9 Hz, H-3''), 6.50 (1H, dd, J= 8.4, 2.4 Hz, H-5'), 6.59 (1H,d, J=2.1, H-3'), 6.64 (1H, d, J=9.9 Hz, H-4''), 7.11 (1H, d, J= 8.4, H-6'), 7.36 (1H, d, J=2.1, H-2'''), 7.37 (1 H, d, J=2.1, H-6'''), 7.79 (1H, d, J= 15.6 Hz, H-α), 7.99 (1H, d, J=15.6 Hz, H-β); ^{13}C NMR (75 MHz, acetone d_6): 26.06, 27.54 (C-5''/C-6''), 30.57 (C-4), 31.77 (C-3), 55.10, 55.41, 56.08, 56.13, 60.20 (5x OCH_3), 70.75 (C-2), 76.99 (C-2''), 98.93 (C-3'), 105.08 (C-5'), 106.34 (C-2''', C-6'''), 110.12 (C-8), 116.00 (C-10), 116.31 (C-6), 116.91 (C-4''), 119.88 (C-α), 121.30 (C-1'), 127.94 (C-6'), 129.47 (C-3''), 130.09 (C-5), 134.56 (C-1'''), 139.46 (C-β), 152.27 (C-9), 153.71 (C-7), 153.80 (C-3''', C-4''', C-5'''), 158.74 (C-2'), 160.49 (C-4'), 188.40 (C=O); ESI-MS, (Positive): m/ z 573 $[M + H]^+$; HRMS (ESI) calcd for $C_{34}H_{36}O_8$ $[M + H]^+$ 573.2482, found 573.2481.

6h - Yellow powder, 72 % yield, mp 90-92 ºC . IRv^{max} (KBr): 1649, 1576, 1465, 1284, 1159, 1129, 1040 cm^{-1}; ^1H NMR (300Hz, acetone d_6): 1.48, 1.50 (3H each, s, H_3-5", H_3-6"), 2.91 (1H, dd, J= 16.2, 4.5 Hz H-4e), 3.00 (1H, m, H-4a), 3.50 (1H, m, H-3a), 3.77, 3.85 (3H each, s, 2 x OCH_3), 4.07 (1H, t, J= 10.2 Hz, H-2a), 4.36 (1H, m, H-2e), 5.73 (1H, d, J=9.9 Hz, H-3''), 6.50 (1H, dd, J= 8.4, 2.4 Hz, H-5'), 6.58 (1H, d, J= 2.4 Hz, H-3'), 6.63 (1H, d, J=9.9 Hz, H-4''), 6.67 (1H, dd, J=3.6, 1.5 Hz, H-4'''), 7.09 (1H, d, J=8.4 Hz, H-6'), 7.40 (1H, d, J=3.6 Hz, H-3'''), 7.61 (1H, d, J= 15.9 Hz, H-α), 7.83 (1 H, d, J=1.5 Hz, H-5'''), 8.03 (1H, d, J=15.9 Hz, H-β); ^{13}C NMR (75 MHz, acetone d_6): 27.49, 27.66 (C-5''/C-6''), 30.55 (C-4), 31.78 (C-3), 55.10, 55.41 (2x OCH_3), 70.77 (C-2), 77.01 (C-2''), 98.96 (C-3'), 105.11 (C-5'), 110.13 (C-8), 112.66 (C-4'''), 116.05 (C-10), 116.10 (C-6), 116.86 (C-4''), 117.02 (C-3'''), 119.56 (C-α), 121.32 (C-1'), 127.95 (C-6'), 129.53 (C-3''), 129.61 (C-5), 138.45 (C-β), 147.02 (C-5'''), 152.31 (C-9), 152.64 (C-7), 154.67 (C-2'''), 158.76 (C-2'), 160.50 (C-4'), 177.74 (C=O); ESI-MS, (Positive): m/ z 473 $[M + H]^+$; HRMS (ESI) calcd for $C_{29}H_{28}O_6$ $[M + H]^+$ 473.1958, found 473.1958.

6i - Yellow powder, 67 % yield, mp 85-87 ºC. IRv^{max} (KBr): 3244, 1637, 1565, 1474, 1290, 1208, 1157, 1128, 1054 cm^{-1}; ^1H NMR (300Hz, pyridine d_5): 1.42 (6H, s, H_3-5", H_3-6"), 2.83 (1H, m, H-4e), 3.00 (1H, dd, J= 15.3, 11.1 Hz, H-4a), 3.64 (1H, m, H-3a), 3.72 (6H, s, 2 x OCH_3), 4.08 (1H, t, J= 9.9 Hz, H-2a), 4.46 (1H, m, H-2e), 5.62 (1H, d, J=9.6 Hz, H-3''), 6.47 (1H, m, H-4'''), 6.59 (1H, d, J= 8.4 Hz, H-5'), 6.67 (1H, br s , H-3'), 6.82 (1H, d, J=9.6 Hz, H-4''), 7.15 (1H each, m, H-6', H-3'''), 7.43 (1H, m, H-5'''), 8.01 (1H, d, J= 15.6 Hz, H-α) , 8.63 (1H, d, J=15.9 Hz, H-β) , 13.37 (1H , s, NH); ^{13}C NMR (75 MHz, pyridine d_5): 28.94, 29.15 (C-5''/C-6''), 31.89 (C-4), 33.04 (C-3), 56.63, 56.74 (2 x OCH_3), 72.09 (C-2), 78.14 (C-2''), 100.58 (C-3'), 106.50 (C-5'), 111.59 (C-8), 111.99 (C-4'''), 117.20 (C-10) , 117.63 (C-3''') 117.99 (C-6), 118.53 (C-4''), 122.71 (C-α), 122.79 (C-1'), 127.11 (C-5'''), 129.30 (C-6'), 130.70 (C-3''), 130.81 (C-5), 137.14 (C-2'''), 138.04 (C-β), 153.43 (C-9), 153.56 (C-7), 159.99 (C-2'), 161.77 (C-4'), 180.87 (C=O); ESI-MS, (Positive): m/ z 472 $[M + H]^+$; HRMS (ESI) calcd for $C_{29}H_{29}NO_5$ $[M + H]^+$ 472.2118, found 472.2117.

7a- Yellow powder, 63 % yield, mp 50-52 ºC. IRv^{max} (KBr): 1657 (C=O), 1585, 1464, 1290, 1210,–1155, 1115, 1033 cm^{-1}; ^1H NMR (300Hz, acetone d_6): 1.34, 1.36 (3H each, s, H_3-5", H_3-6"), 2.87 (1H, dd, J=15.6, 4.2 Hz, H-4e), 3.05 (1H, dd, J=15.6, 10.5 Hz, H-4a), 3.54 (1H, m, H-3a), 4.13 (6H, brs, 2 x OCH_3), 4.10 (1H, t, J= 9.9 Hz, H-2a), 4.33 (1H, dd, J=10.5, 2.1 Hz, H-2e), 5.61 (1H, d, J=10.2 Hz, H-3''),

6.61 (1H, d, J=9.9 Hz, H-4''), 6.80 (1H, s, H-3'), 6.84 (1H, d , J=8.4, H-5), 7.52 (3H, m H-3''', H-4''', H-5'''), 7.65 (1H, d, J= 15.6 Hz, H-α), 7.73 (1H, s, Hz, H-6'), 8.03(2H, d, J=7.2 Hz, H-2''', H-6'''), 8.12 (1H, d, J=15.6 Hz, H-β) ; ^{13}C NMR (75 MHz, acetone d-$_6$), 27.29, 27.37 (C-5''/C-6''), 30.68 (C-4), 31.50 (C-3), 55.56, 55.86 (2 x OCH_3), 70.07 (C-2), 75.59 (C-2''), 95.98 (C-3'), 108.95 (C-6), 110.05 (C-8), 114.78 C-10), 116.26 (C-5'), 117.14 (C-4''), 119.38 (C-α), 122.44 (C-1'), 127.72 (C-6'), 128.58 (C-5'''), 128.81 (C-3'''), 128.94 (C-2''', C-6'''), 129.28 (C-3''), 129.64 (C-5), 132.73 (C-4'''), 139.17 (C-1'''), 139.32 (C-β), 150.10 (C-9), 152.41 (C-7), 159.89 (C-2'), 161.32 (C-4'), 189.36 (C=O); ESI-MS, (Positive): m/ z 483 $[M + H]^+$; HRMS (ESI) calcd for $C_{31}H_{30}O_5$ $[M + H]^+$ 483.2166, found 483.2167.

7b- Yellow powder, 68 % yield, mp 52-55 ºC. IRv^{max} (KBr): 1650, 1583, 1464, 1289, 1156, 1114, 1029 cm^{-1}; ^{1}H NMR (300Hz, acetone d_6): 1.34, 1.35 (3H each, s, H_3-5", H_3-6"), 2.81 (1H, dd, J= 15.0, 4.5 Hz, H-4e), 3.03 (1H, dd, J= 15.6, 10.8 Hz, H-4a), 3.49 (1H, m, H-3a), 3.92, 3.93, 3.95 (3H each, s, 3 x OCH_3), 4.07 (1H, t, J=9.9 Hz, H-2a), 4.30 (1H, m, H-2e), 5.59 (1H, d, J=9.9 Hz, H-3''), 6.29 (1H, d, J=8.1 Hz, H-6), 6.60 (1H, d, J=9.9 Hz, H-4''), 6.74 (1H, s, H-3'), 6.82 (1H, d, J=8.1 Hz, H-5), 7.02 (1H, m, H-3'''), 7.10 (1H, m, H-5'''), 7.33 (1H, d, J=15.9 Hz, H-α), 7.43 (1H, m, H-4'''), 7.49 (1H, m, H-6'''), 7.55 (1H, s, H-6'), 7.85 (1H, d, J=16.2 Hz, H-β); ^{13}C NMR (75 MHz, acetone d-$_6$): 27.35, 27.44 (C-5''/C-6''), 30.65 (C-4), 31.79 (C-3), 55.69, 55.82 (3 x OCH_3), 70.12 (C-2), 75.58 (C-2''), 96.03 (C-3'), 108.94 (C-6), 110.04 (C-8), 112.34 (C-3'''), 114.03 (C-10), 116.39 (C-5'), 117.17 (C-4''), 120.86 (C-α), 122.30 (C-1'), 125.45 (C-5'''), 127.73 (C-6'), 129.23 (C-3''), 129.67 (C-5), 130.07 (C-6'''), 130.63 (C-1'''), 132.64 (C-4'''), 137.99 (C-β), 150.08 (C-9), 152.38 (C-7), 158.41 (C-2'''), 159.68 (C-2'), 161.04 (C-4'), 192.55 (C=O); ESI-MS, (Positive): m/ z 513 $[M + H]^+$.

7c- Yellow powder, 62 % yield, mp 58-60 ºC. IRv^{max} (KBr): 1657, 1579, 1469, 1288, 1207, 1161, 1117, 1090 Hz; ^{1}H NMR (300Hz, acetone d_6): 1.34, 1.36 (3H each, s, H_3-5", H_3-6"), 2.83 (1H, dd, J= 15.9, 4.8 Hz, H-4e), 3.06 (1H, dd, J= 15.6, 10.5 Hz, H-4a), 3.52 (1H, m, H-3a), 3.86, 3.97, 3.99 (3H each, s, 3 x OCH_3), 4.11 (1H, t, J=9.9 Hz, H-2a), 4.31 (1H, m, H-2e), 5.61 (1H, d, J=9.9 Hz, H-3''), 6.32 (1H, d, J=8.1 Hz, H-6), 6.62 (1H, d, J=9.9 H-4''), 6.77 (1H, s, H-3'), 6.85 (1H, d, J=8.1 Hz, H-5), 7.15 (1H, dd, J=8.1, 2.1 Hz, H-4'''), 7.43 (1H, dd, J=8.1, 7.5 Hz, H-5'''), 7.54 (1H, d J=1.2 Hz, H-2'''), 7.62 (1H, s, H-6'), 7.63 (1H, d, J=6.6 Hz, H-6'''), 7.69 (1H, d, J=15.3 Hz, H-α), 8.12 (1H, d, J=15.6 Hz, H-β); ^{13}C NMR (75 MHz, acetone d-$_6$), 27.34, 27.41 (C-5''/C-6''), 31.61 (C-4), 31.75 (C-3), 55.27, 55.85, 55.87 (3 x OCH_3), 70.10 (C-2), 75.59 (C-2''), 95.99 (C-3'), 108.96 (C-6), 110.06 (C-8), 113.38 (C-2'''), 114.79 (C-10), 116.32 (C-5'), 117.17 (C-4'''), 118.65 (C-4''), 119.58 (C-α), 121.05 (C-6'''), 122.44 (C-1'), 127.80 (C-6'), 129.26 (C-3''), 129.64 (C-5), 130.00 (C-5'''), 139.43 (C-β), 140.65 (C-1'''), 150.10 (C-9), 152.41 (C-7), 159.90 (C-2'), 160.43 (C-3'''), 161.32 (C-4'), 189.22 (C=O); ESI-MS, (Positive): m/ z 513 $[M + H]^+$.

7d- Yellow powder, 68 % yield, mp 88-90 ºC. IRv^{max} (KBr): 1654, 1586, 1474, 1293, 1166, 1116, 1029 cm^{-1}; ^{1}H NMR (300Hz, Pyridine d_5): 1.42 (6H, s, H_3-5", H_3-6"), 2.83 (1H, dd, J= 15.3, 4.2 Hz, H-4e), 3.07 (1H, dd, J= 15.9, 11.1 Hz, H-4a), 3.61 (1H, m, H-3a), 3.70, 3.79 (3H, 6H, s, 3 x OCH_3), 4.07 (1H, t, J=10.2 Hz, H-2a), 4.43 (1H, d, J=10.2 Hz, H-2e), 5.61 (1H, d, J=9.6 Hz, H-3''), 6.59 (1H, s, H-3'), 6.66 (1H, d, J=8.1 Hz, H-6), 6.90 (1H, d, J=10.2 Hz, H-4''), 6.94 (1H, d, J=8.4 Hz, H-5), 7.07 (2H, d, J=8.7 Hz, H-3''', H-5'''), 7.79 (1H, s, H-6'), 8.02 (1H, d, J=15.6 Hz, H-α), 8.38 (2H, d, J=8.7 Hz, H-2''', H-6'''), 8.66 (1H, d, J=15.9 Hz, H-β); ^{13}C NMR (75 MHz, Pyridine d_5): 28.92, 29.11 (C-5''/C-6''), 31.96 (C-4), 33.00 (C-3), 56.74, 57.03, 57.10 (3 x OCH_3), 71.50 (C-2), 77.13 (C-2''), 97.16 (C-3'), 110.52 (C-6), 111.60 (C-8),

115.61 (C-3''', C-5'''), 116.27 (C-10), 118.05 (C-5'), 118.79 (C-4''), 121.25 (C-α), 123.55 (C-1'), 129.09 (C-6'), 130.63 (C-3''), 131.20 (C-5), 132.51 (C-2''', C-6'''), 133.52 (C-1'''), 140.35 (C-β), 153.92 (C-7, C-9), 161.04 (C-2'), 162.29 (C-4'), 164.95 (C-4'''), 189.70 (C=O); ESI-MS, (Positive): m/ z 513 $[M + H]^+$.

7e- Yellow powder, 78% yield, mp 70-72 ºC. IRv^{max} (KBr): 1644, 1576, 1465, 1284, 1159, 1129, 1040 cm^{-1}; ^1H NMR (300Hz, acetone d_6): 1.34 (6H, s, H_3-5", H_3-6"), 2.83 (1H, dd, J= 15.0, 4.5 Hz, H-4e), 3.04 (1H, dd, J= 15.3, 10.5, H-4a), 3.50 (1H, m, H-3a), 3.89, 3.92, 3.96, (3H, 3H, 6H, s, 4 x OCH_3), 4.08 (1H, m, H-2a), 4.32 (1H, dd, J=10.8, 2.1 Hz, H-2e), 5.60 (1H, d, J=9.9 Hz, H-3''), 6.29 (1H, d, J=8.1, H-6), 6.58 (1H, d, J=2.1, H-3'''), 6.62 (2H, m, H-4'', H-5'''), 6.77 (1H, s, H-3'), 6.84 (1H, d, J=8.1 Hz, H-5), 7.54 (1H, s, H-6'), 7.48 (1H, d, J=15.9 Hz, H-α), 7.60 (1H, d, J=8.7 Hz, H-6'''), 7.88 (1H, d, J=15.9 Hz, CH β); ^{13}C NMR (75 MHz, acetone d_6): 27.30, 27.41 (C-5''/C-6''), 30.64 (C-4), 31.84 (C-3), 55.43, 55.72, 55.77 (4x OCH_3), 70.12 (C-2), 75.56 (C-2''), 96.07 (C-3'''), 98.78 (C-3'), 105.98 (C-5'''), 108.91 (C-6), 110.03 (C-8), 114.79 (C-10), 116.73 (C-5'), 117.14 (C-4''), 122.18 (C-1'), 123.14 (C-1'''), 125.64 (C-α), 127.61 (C-6'), 129.22 (C-3''), 129.65 (C-5), 132.45 (C-6'''), 136.68 (C-β), 150.09 (C-9), 152.38 (C-7), 159.58 (C-2'), 160.72 (C-4', C-2'''), 164.38 (C-4'''), 190.00 (C=O); ESI-MS, (Positive): m/ z 543 $[M + H]^+$; HRMS (ESI) calcd for $C_{33}H_{34}O_7$ $[M + H]^+$ 543.2377, found 543.2376.

7f- Yellow powder, 76 % yield, mp 132-135 ºC. IRv^{max} (KBr): 1646, 1597, 1562, 1465, 1265, 1208, 1154, 1129, 1031 cm^{-1}; ^1H NMR (300Hz, Pyridine d_5): 1.43 (6H, s, H_3-5", H_3-6"), 2.82 (1H, dd, J= 15.6, 3.9 Hz, H-4e), 3.03 (1H, dd, J= 15.6, 10.8 Hz, H-4a), 3.61 (1H, m, H-3a), 3.75, 3.77, 3.79, 3.80, (3H each, s, 4 x OCH_3), 4.05 (1H, t, J=9.9, H-2a), 4.43 (1H, d, J=9.9 Hz, H-2e), 5.62 (1H, d, J=10.2 Hz, H-3''), 6.60 (1H, s, H-3'), 6.67 (1H, d, J=8.4 Hz, H-6), 6.93 (2H, m, H-4'', H-5'''), 7.02 (1H, d, J=8.4 Hz, H-5), 7.19 (1H, s, H-2'''), 7.80 (1H, s, H-6'), 8.03 (1H, d, J=15.9 Hz, H-α), 8.07 (1H, dd, J=8.1, 3.0 Hz, H-6'''), 8.71 (1H, d, J=15.6 Hz, H-β); ^{13}C NMR (75 MHz, Pyridine d_5): 28.91, 29.12 (C-5''/C-6''), 31.96 (C-4), 33.07 (C-3), 57.04, 57.10, 57.16 (4 x OCH_3), 71.49 (C-2), 77.13 (C-2''), 97.17 (C-3'), 110.53 (C-6), 111.61 (C-8), 112.42 (C-5'''), 113.16 (C-2'''), 116.30 (C-10), 118.09 (C-5'), 118.79 (C-4''), 121.23 (C-α), 123.52 (C-1'), 125.13 (C-6'''), 129.20 (C-6'), 130.64 (C-3''), 131.21 (C-5), 133.70 (C-1'''), 140.38 (C-β), 150.82 (C-9), 151.53 (C-7), 153.92 (C-3'''), 155.23 (C-4'''), 161.07 (C-2'), 162.29 (C-4'), 189.78 (C=O); ESI-MS, (Positive): m/ z 543 $[M + H]^+$.

7g- Yellow powder, 72 % yield, mp 75-76 ºC. IRv^{max} (KBr): 1651, 1572, 1463, 1289, 1209, 1158, 1126, 1029 Hz; ^1H NMR (300Hz, acetone d_6): 1.35 (6H, s, H_3-5", H_3-6"), 2.82 (1H, m, H-4e), 3.02 (1H, dd, J=15.6, 10.8 Hz, H-4a), 3.49 (1H, m, H-3a), 3.81, 3.89, 3.91, 3.98, 4.00 (3H each, s, 5 x OCH_3), 4.10 (1H, t, J=10.2 Hz, H-2a), 4.30 (1H, m, H-2e), 5.61 (1H, d, J=9.9 Hz, H-3''), 6.30 (1H, d, J=8.1 Hz, H-6), 6.61 (1H, d, J= 9.9 Hz, H-4''), 6.79 (1H, s, H-3'), 6.84 (1H, d, J=8.1, Hz, H-5), 7.33 (2H, s, H-2''', H-6'''), 7.68 (1H, s, H-6'), 7.68 (1H, d, J=15.6 Hz, H-α), 8.09 (1H, d, J=15.6 Hz, CH β); ^{13}C NMR (75 MHz, acetone d_6): 27.28, 27.37 (C-5''/C-6''), 30.74 (C-4), 31.86 (C-3), 55.84, 56.21, 60.20 (3 x OCH_3), 70.08 (C-2), 75.58 (C-2''), 96.02 (C-3'), 106.58 (C-2''', C-6'''), 108.94 (C-6), 110.48 (C-8), 114.81 (C-10), 116.38 (C-5'), 117.11 (C-4''), 119.65 (C-α), 122.32 (C-1'), 127.95 (C-6'), 129.30 (C-3''), 129.64 (C-5), 134.50 (C-1'''), 139.24 (C-β), 149.73 (C-4'''), 152.39 ((C-7,C-9), 153.77 (C-3''', C-5'''), 159.90 (C-2'), 161.25 (C-4'), 188.54 (C=O); ESI-MS, (Positive): m/ z 573 $[M + H]^+$; HRMS (ESI) calcd for $C_{34}H_{36}O_8$ $[M + H]^+$ 573.2482, found 573.2483.

7h- Yellow powder, 68 % yield, mp 94-95 ºC. IRv^{max} (KBr): 1649, 1584, 1467, 1291, 1208, 1156, 1116, 1031 cm^{-1}; ^1H NMR (300Hz, acetone d_6): 1.34, 1.36 (3H each, s, H_3-5", H_3-6"), 2.84 (1H, m, H-4e), 3.05 (1H, dd, J= 15.6, 10.5 Hz, H-4a), 3.52 (1H, m, H-3a), 3.98, 3.99, (3H each, s, 2 x OCH_3), 4.10 (1H, t , J=9.9 Hz, H-2a), 4.32 (1H,

dd, J=10.5, 2.1 Hz, H-2e), 5.62 (1H, d, J=9.9 Hz, H-3''), 6.32 (1H, d, J=8.1 Hz, H-6), 6.62 (1H, d, J= 9.9 Hz, H-4''), 6.66 (1H, m, H-4'''), 6.78 (1H, s, H-3'), 6.86 (1H, d, J=8.4 Hz, H-5), 7.38 (1H, d, J=3.6 Hz, H-3'''), 7.48 (1H, d, J=15.9 Hz, H-α), 7.66 (1H, s, H-6'), 7.82 (1H, s, H-5'''), 8.09 (1H, d, J=15.9 Hz, H-β); ^{13}C NMR (75 MHz, acetone d-$_6$) 27.31, 27.39 (C-5''/C-6''), 30.68 (C-4), 31.60 (C-3), 55.55, 55.85 (2 x OCH_3), 70.09 (C-2), 75.59 (C-2''), 96.00 (C-3'), 108.96 (C-6), 111.12 (C-8), 112.61 (C-4'''), 114.75 (C-10), 116.09 (C-5'), 117.16 (C-4''), 117.22 (C-3'''), 119.27 (C-α), 122.42 (C-1'), 127.80 (C-6'), 129.26 (C-3''), 129.65 (C-5), 138.30 (C-β), 147.09 (C-5'''), 150.08 (C-9), 152.42 (C-7), 154.52 (C-2'''), 159.93 (C-2'), 161.35 (C-4'), 177.59 (C=O); ESI-MS, (Positive): m/ z 473 $[M + H]^+$; HRMS (ESI) calcd for $C_{29}H_{28}O_6$ $[M + H]^+$ 473.1958, found 473.1958.

7i- Yellow powder, 72 % yield, mp 160-162 ºC. IR v^{max} (KBr): 3234, 1635, 1561, 1469, 1282, 1208, 1150, 1110, 1031cm^{-1}; ^{1}H NMR (300Hz, Pyridine d_5): 1.44 (6H, s, H_3-5", H_3-6"), 2.75 (1H, m, H-4e), 3.00 (1H, m, H-4a), 3.46 (1H, m, H-3a), 3.77 (6H, s, 2 x OCH_3), 4.07 (1H, t , J=9.9, H-2a), 4.27 (1H, dd, J=10.5, 2.1 Hz, H-2e), 5.62 (1H, d, J=9.9 Hz, H-3''), 6.46 (1H, br s, H-4'''), 6.57 (1H, s, H-3'), 6.67 (1H, d, J=8.1 Hz, H-6), 6.91 (1H, d, J= 9.6 Hz, H-4''), 6.94 (1H, d, J=7.8 Hz, H-5), 7.41 (1H, br s, H-3'''), 7.54 (1H, d, J=11.1Hz, H-5'''), 7.76 (1H, s, H-6'), 7.93 (1H, d, J=15.6 Hz, H-α), 8.70 (1H, d, J=15.6 Hz, H-β), 13.36 (1H , s, NH); ^{13}C NMR (75 MHz, Pyridine d_5): 28.95, 29.12 (C-5''/C-6''), 31.99 (C-4), 33.03 (C-3), 57.00, 57.08 (2 x OCH_3), 71.49 (C-2), 77.12 (C-2''), 97.21 (C-3'), 110.53 (C-6), 111.60 (C-8), 111.99 (C-4'''), 116.28 (C-10), 117.96 (C-3'''), 118.18 (C-5'), 118.81 (C-4''), 122.38 (C-α), 123.50 (C-1'), 127.12 (C-5'''), 128.77 (C-6'), 130.64 (C-3''), 131.21 (C-5), 137.13 (C-2'''), 138.00 (C-β), 153.93 (C-7, C-9), 160.78 (C-2'), 161.96 (C-4'), 180.75 (C=O); ESI-MS, (Positive): m/ z 472 $[M + H]^+$.

8a- Yellow powder, 64 % yield, mp 70-72 ºC. IRv^{max} (KBr): 1655, 1584, 1465, 1284, 1207, 1176, 1129, 1021 cm^{-1}; ^{1}H NMR (300Hz, acetone d_6): 1.47, 1.51 (3H each, s, H_3-5", H_3-6"), 2.99 (1H, dd, J= 15.6, 4.5 Hz, H-4e), 3.18 (1H, dd, J= 15.3, 10.2 Hz, H-4a), 3.60 (1H, m, H-3a), 3.99, 4.01 (3H each, s, 2 x OCH_3), 4.23 (1H, t, J=10.2 Hz, H-2a), 4.43 (1H, dd, J= 10.2, 1.5 Hz, H-2e), 5.75 (1H, d, J=9.9 Hz, H-3''), 6.67 (1H, d, J=9.9 Hz, H-4''), 6.80 (1H, s, H-3'), 7.57 (6H, m, 2 x H-3''', 2x H-4''', 2 X H- 5'''), 7.63, 7.84 (2H, d, J=15.6 Hz, 2x H-α), 7.70 (1H, s, H-5), 7.76 (1H, s, H-6'), 8.07 (4H, m, 2 x H-2''', 2x H-6'''), 8.11, 8.16 (2H, d, J=15.6 Hz, 2x H-β); ^{13}C NMR (75 MHz, acetone d-$_6$): 27.32, 27.53 (C-5''/C-6''), 30.38 (C-4), 31.48 (C-3), 55.89 (2 x OCH_3), 70.49 (C-2), 77.00 (C-2''), 96.05 (C-3'), 110.18 (C-8), 115.93 (C-10), 116.31 (C-6), 116.38 (C-5'), 116.86 (C-4''), 119.37 (C-α), 119.88 (C-α'), 121.99 (C-1'), 127.67 (C-6'), 128.54 (2 x C-3''',2 x C-5'''), 128.96 (2 x C-2''',2 x C-6'''), 129.63 (C-5,C-3''), 132.70 (2 x C-4'''), 139.33 (2 x C-1'''), 139.37 (C-β), 139.55 (C-β'), 152.33 (C-9), 152.58 (C-7), 159.99 (C-2'), 161.33 (C-4'), 189.41, 189.60 (2x C=O); ESI-MS, (Positive): m/ z 613 $[M + H]^+$; HRMS (ESI) calcd for $C_{40}H_{36}O_6$ $[M + H]$+ 613.2584, found 613.2584.

8b- Yellow powder, 78 % yield, mp 65-68 ºC. IRv^{max} (KBr): 1649, 1599, 1578, 1463, 1287, 1205, 1129, 1027 cm^{-1}; ^{1}H NMR (300Hz, acetone d_6): 1.42, 1.44 (3H each, s, H_3-5", H_3-6"), 2.91 (1H, dd, J= 15.6, 5.1 Hz, H-4e), 3.11 (1H, dd, J= 15.6, 10.8 Hz, H-4a), 3.55 (1H, m, H-3a), 3.88, 3.90, 3.96, 3.98 (3H each, s, 4 X OCH_3), 4.19 (1H, t, J=9.9 Hz, H-2a), 4.40 (1H, d, J= 10.5 Hz, H-2e), 5.70 (1H, d, J=9.9 Hz, H-3''), 6.63 (1H,d, J=9.9 Hz, H-4''), 6.78 (1H, s, H-3'), 7.02 (1H, m, 2 x H-3'''), 7.13 (1H each, m, 2 x H- 5'''), 7.38 (1H, s, H-5), 7.30, 7.40 (1H each, d, J=15.9, 2 x H-α), 7.46 (2H, m, 2 x H- 4'''), 7.48 (2H, m, 2 x H- 6'''), 7.58 (1H, s, H-6'), 7.78, 7.84 (1H each, d, J=15.9 , 2 x H-β); ^{13}C NMR (75 MHz, acetone d_6), 27.46, 28.58 (C-5''/C-6''), 31.70 (C-3), 30.39 (C-4), 55.69 55.75, 55.83 (3 x OCH_3), 70.51(C-2), 76.84 (C-

2''), 96.10 (C-3'), 110.15 (C-8), 112.38 (2 x C-3'''), 115.88 (C-10), 116.35 (C-6, C-5'), 116.85 (C-4''), 120.87 (C-α, C-α'), 121.85 (C-1'), 125.43 (2 x C-5'''), 127.74 (C-6'), 129.57 (C-3''), 130.01 (C-5), 130.18 (2 x C-6'''), 130.61 (2 x C-1'''), 132.65 (2 x C-4'''), 137.95, 138.10 (2 x C-β), 152.29 (C-7), 152.29 (C-9), 158.40 (2 x C-2'''), 159.78 (C-2'), 161.04 (C-4'), 192.53 (2 x C=O); ESI-MS, (Positive): m/ z 673 $[M + H]^+$; HRMS (ESI) calcd for $C_{42}H_{40}O_8$ $[M + H]^+$ 673.2795, found 673.2792.

8c- Yellow powder, 68 % yield, mp 60-62 ºC. IRv^{max} (KBr): 1655, 1575, 1464, 1284, 1204, 1125, 1030 cm^{-1}; ^1H NMR (300Hz, acetone d_6): 1.32, 1.34 (3H each, s, H_3-5", H_3-6"), 2.78 (1H, m, H-4e), 2.99 (1H, dd, J=15.6, 10.5 Hz, H-4a), 3.41 (1H, m, H-3a), 3.69, 3.71, 3.83 (4H each, s, 4 x OCH_3), 4.04 (1H, t, J=9.9 Hz, H-2a), 4.24 (1H, m, H-2e), 5.58 (1H, d, J=10.2 Hz, H-3''), 6.50 (1H, d, J=10.2 Hz, H-4''), 6.62 (1H, s, H-3'), 7.01 (2H, m, 2 x H-4'''**),** 7.25 (2H, m, 2 x H-5'''), 7.36 (1H, s, H-5), 7.42 (2H , d, J= 1.5 Hz, 2 x H-2'''), 7.51 (2H , d, J= 6.6 Hz, 2 x H-6'''), 7.59 (1H, s, H-6'), 7.63 (2H, d, J=15.9 Hz, 2 x H-α), 7.92 , 7.98 (2H, d, J=15.6Hz, 2 x H-β); ^{13}C NMR (75 MHz, acetone d_6) : 27.54, 27.58 (C-5''/C-6''), 30.41 (C-4), 31.53 (C-3), 55.25, 55.59, 55.88 (3 x OCH_3), 70.52 (C-2), 77.00 (C-2''), 96.01 (C-3'), 110.17 (C-8), 113.30, 113.35 (2 x C-2'''), 115.94 (C-10), 116.36 (C-6, C-5'), 116.86 (C-4''), 118.63, 118.73 (2 x C-4'''), 119.52, 119.94 (2xC-α), 120.56, 120.60 (2 x C-6'''), 121.92 (C-1'), 127.73 (C-6'), 129.59 (C-3''), 129.79 (C-5), 130.02 (2 x C-5'''), 139.44, 139.63 (2 x C-β), 140.61, 140.75 (2 x C-1'''), 152.32 (C-9), 152.55 (C-7), 160.00 (C-2'), 160.41 (2 x C-3'''), 161.33 (C-4'), 189.21, 189.32 (2x C=O); ESI-MS, (Positive): m/ z 673 $[M + H]^+$.

8d- Yellow powder, 64 % yield, mp 80-82 ºC. IRv^{max} (KBr): 1652, 1582, 1462, 1256, 1207, 1169, 1131, 1027 cm^{-1}; ^1H NMR (300Hz, acetone d_6): 1.46, 1.48 (3H each, s, H_3-5", H_3-6"), 2.94 (1H, dd, J= 15.6, 4.2 Hz, H-4e), 3.13 (1H, m H-4a), 3.55 (1H, m, H-3a), 3.86, 3.88, 3.99, 4.03 (3H each, s, 4 X OCH_3), 4.24 (1H, t, J=9.9 Hz, H-2a), 4.44 (1H, m, H-2e), 5.78 (1H, d, J=10.2 Hz, H-3''), 6.64 (1H, d, J=10.2 Hz, H-4''), 6.82 (1H, s, H-3'), 7.03 (1H, d, J= 8.7 Hz, 2 x H-3''', 2 x H-5'''), 7.42 (1H, s, H-5), 7.65 (2H, d, J=15.9 Hz, 2xH-α), 7.72 (1H, s, H-6'), 8.03 (4H, d, J= 8.7 Hz, 2x H-2''', 2 x H-6'''), 8.08 (2H, d, J=15.9 Hz, 2x H-β); ^{13}C NMR (75 MHz, acetone d_6), 27.48 (C-5''), 27.62 (C-6''), 31.32 (C-4), 31.69 (C-3), 55.41, 55.85 (3 x OCH_3), 70.39 (C-2),76.92 (C-2''), 96.25 (C-3'), 110.16 (C-8), 113.99, 114.16 (2 x C-5'''), 113.99, 114.16 (2 x C-3'''), 114.16 (C-10), 116.36 (C-6), (C-7), 116.48 (C-5'), 116.89 (C-4''), 19.01 (C-α),119.76 (C-α'), 121.98 (C-1'), 127.35 (C-6'), 129.60 (C-5, C-3''), 130.69, 130.79 (2 x C-2''', 2 x C-6'''), 131.98, 132.08 (2x C-1'''), 138.34, 138.58 (2xC-β), 152.16 (C-9), 161.10 (C-2', C-4'), 163.68, 163.80 (2 x C-4'''), 186.98, 187.83 (2x C=O); ESI-MS, (Positive): m/ z 673 $[M + H]^+$; HRMS (ESI) calcd for $C_{42}H_{40}O_8$ $[M + H]^+$ 673.2795, found 673.2787.

8e- Yellow powder, 77 % yield, mp 80-81 ºC. IRv^{max} (KBr): 1645, 1603, 1464, 1283, 1163, 1125, 1026 cm^{-1}; ^1H NMR (300Hz, acetone d_6): 1.47, 1.48 (3H each, s, H_3-5", H_3-6"), 2.95 (1H, dd, J= 15.6, 4.2 Hz, H-4e), 3.14 (1H, m, H-4a), 3.55 (1H, m, H-3a), 3.82, 3.83, 3.84, 3.87, 3.88, 3.89 (3H each, s, 6 x OCH_3), 4.19 (1H, t, J=9.9 Hz, H-2a), 4.42 (1H,d , J= 9.3 Hz, H-2e), 5.72 (1H, d, J=9.9 Hz, H-3''), 6.64 (5H each, m, H-4'', 2 x H-3''', 2 x H- 5'''), 6.78 (1H, s, H-3'), 7.35 (1H, s, H-5), 7.53, 7.62 (1H each, d, J=15.9, 15.6, 2x H-α), 7.59 (1H, s, H-6'), 7.68 (2H, dd, J= 8.7, 1.5 Hz, 2 x H-6'''), 7.87, 7.93 (1H each, d, J=15.9, 15.6 Hz, 2x H-β); ^{13}C NMR (75 MHz, acetone d_6), 27.49 (C-5''), 27.57 (C-6''), 30.41 (C-4), 31.82 (C-3), 55.45, 55.76, 55.84 (4 x OCH_3), 70.53 (C-2), 76.78 (C-2''), 96.10 (C-3'), 98.80 (2 x C-3'''), 106.04 (2 x C-5'''), 110.16 (C-8), 115.79 (C-10), 116.69 (C-6), 116.78 (C-5'), 116.94 (C-4''), 121.76 (C-1'), 123.10, 123.23 (2 x C-1'''), 125.66 (2 x C-α), 127.65 (C-6'), 129.56 (C-5, C-3''), 132.47 (2 x C-6'''), 136.73, 136.88 (2xC-β), 151.98 (C-9), 152.07 (C-7), 159.68 (C-2'),

160.75 (C-4'), 164.41 (2 x C-2''', 2 x C-4'''), 190.03 (2 x C=O); ESI-MS, (Positive): m/ z 733 $[M + H]^+$.

8f- Yellow powder, 78 % yield, mp 85-86 ºC. IRv^{max} (KBr): 1648, 1569, 1464, 1263, 1202, 1126, 1025 Hz; ^{1}H NMR (300Hz, acetone d_6): 1.43, 1.49 (3H each, s, H_3-5", H_3-6"), 3.02 (1H, m, H-4e), 3.10 (1H, m, H-4a), 3.57 (1H, m, H-3a), 3.84, 3.86, 3.88, 3.96, 3.99 (18H, s, 6 x OCH_3), 4.23 (1H, t, J=8.7 Hz, H-2a), 4.39 (1H, d, J= 10.2 Hz, H-2e), 5.72 (1H, d, J=10.2 Hz, H-3''), 6.63 (1H, d, J= 9.9 Hz, H-4''), 6.78 (1H, s, H-3'), 6.99, 7.04 (1H each, d, J= 8.1, 8.4 Hz, 2 x H- 5'''), 7.55 (1H, s, H-5), 7.62, 7.86 (1H each, d, J=15.6 Hz, 2x H-α), 7.61 (1H each, d, J= 1.8 Hz, 2 x H-2'''), 7.68 (1H, s, H-6'), 7.77 (1H x 2 , dd, J= 8.4. 1.8 Hz, 2 x H-6'''), 8.07, 8.11 (1H x 2 , d, J=15.6 Hz, 2 x H-β); ^{13}C NMR (75 MHz, acetone d_6 : 27.50, 27.54 (C-5'', C-6''), 30.22 (C-4), 31.34 (C-3), 55.58, 55.71, 55.63, 55.84 (4 x OCH_3), 70.35 (C-2), 76.94 (C-2''), 95.99 (C-3'), 110.16 (C-8), 111.09 (2 x C-5'''), 111.39 (2 x C-2'''), 115.84 (C-10), 116.35 (C-6), 116.58 (C-5'), 116.90 (C-4''), 118.97, 119.78 (2 x C-α), 121.96 (C-1'), 123.00 (2 x C-6'''), 127.37 (C-6'), 129.61 (C-5, C-3''), 132.18 (2 x C-1'''), 138.23, 138.61 (2 x C-β), 149.84 (2 x C-3'''), 152.18 (C-9), 152.43 (C-7), 153.85 (2 x C-4'''), 159.75 (C-2'), 161.08 (C-4'), 187.86, 187.50, (2x C=O); ESI-MS, (Positive): m/ z 733 $[M + H]^+$; HRMS (ESI) calcd for $C_{42}H_{44}O_{10}$ $[M + H]^+$ 733.3007, found 733.3003.

8g- Yellow powder, 75 % yield, mp 102-105 ºC. IRv^{max} (KBr): 1650, 1568, 1463, 1160, 1127, 1028 cm^{-1}; ^{1}H NMR (300Hz, acetone d_6): 1.53 (6H, s, H_3-5", H_3-6"), 2.89 (1H, dd, J= 15.6, 4.2 Hz, H-4e), 3.14 (1H, m, H-4a), 3.56 (1H, m, H-3a), 3.82, 3.84, 3.91, 3.95, 4.02, 4.04 (24H, s, 8 x OCH_3), 4.20 (1H, t, J=10.2 Hz, H-2a), 4.42 (1H, dd , J= 10.2 , 1.5 Hz, H-2e), 5.77 (1H, d, J=9.9 Hz, H-3''), 6.68 (1H, d, J= 9.9 Hz, H-4''), 6.83 (1H, s, H-3'), 7.38 (1H, d, J= 7.2 Hz, 2 x H-2''' H-6'''), 7.75, 7.82 (1H each, d, J=15.6 Hz, 2 x H-α), 7.44 (1H, s, H-5), 7.73 (1H, s, H-6'), 8.01, 8.12 (1H each, d, J=15.6, 2 x H-β); ^{13}C NMR (75 MHz, acetone d_6: 27.53 (C-5''), 27.65 (C-6''), 30.63 (C-4), 31.89 (C-3), 55.88, 56.13, 56.22, 60.20 (8 x OCH_3), 70.55 (C-2), 77.02 (C-2''), 96.11 (C-3'), 106.63, 106.40 (2 x C-2''', 2 x C-6'''), 110.20 (C-8), 115.89 (C-10), 115.89 (C-6), 121.82 (C-1'), 116.51 (C-5'), 116.87 (C-4''), 119.91, 120.00 (2xC-α), 128.10 (C-6'), 129.58 (C-3''), 130.22 (C-5), 134.53 (2 x C-1'''), 139.30 (2 x C-β), 142.93 (2 x C-4'''), 152.30 (C-7, C-9), 153.78 (2 x C-3''', 2 x C-5'''), 161.22 (C-2', C-4'), 188.40, 188.61 (2 x C=O); ESI-MS, (Positive): m/ z 793 $[M + H]^+$; HRMS (ESI) calcd for $C_{46}H_{48}O_{12}$ $[M + H]^+$ 793.3218, found 793.3218.

8h- Yellow powder, 72 % yield, mp 102-104 ºC. IRv^{max} (KBr): 1651, 1585, 1465, 1286, 1166, 1130, 1028 cm^{-1}; ^{1}H NMR (300Hz, acetone d_6): 1.47, 1.51 (3H each, s, H_3-5", H_3-6"), 3.01 (1H, dd, J= 15.6, 4.8 Hz, H-4e), 3.24 (1H, m, H-4a), 3.58 (1H, m, H-3a), 4.02 (6H, s, 2 x OCH_3), 4.24 (1H, t, J=9.6 Hz, H-2a), 4.44 (1H, d , J= 10.2, 1.5 Hz, H-2e), 5.76 (1H, d, J=9.9 Hz, H-3''), 6.66 (1H, d, J= 9.9 Hz, H-4''), 6.70 (2H, m, 2 x H- 4'''), 6.81 (1H, s, H-3'), 7.36 (2H, d, J= 3.3 Hz, 2x H-3'''), 7.43 (2H, m, 2 x H-5'''), 7.46, 7.66 (1H each, d, J=15.6 Hz, 2x H-α), 7.55 (1H, s, H-5), 7.69 (1H, s, H-6'), 8.09, 8.12 (1H each, d, J=15.6, 15.9 Hz, 2x H-β); ^{13}C NMR (75 MHz, acetone d_6): 27.45, 28.55 (C-5'',C-6''), 30.29 (C-4), 31.46 (C-3), 55.87 (2 x OCH_3), 70.46 (C-2), 77.03 (C-2''), 96.05 (C-3'), 110.17 (C-8), 112.62 (2 x C-4'''), 115.93 (C-10), 116.05 (C-6), 116.82 (C-5'), 117.09 (2 x C-3'''), 117.24 (C-4''), 119.13, 119.61, (2 x C-α), 121.97 (C-1'), 127.63 (C-6'), 129.66 (C-5, C-3''), 138.20, 138.41 (2 x C-β), 147.07 (2 x C-5'''), 152.35 (C-9), 152.64 (C-7), 154.49 (2 x C-2'''), 160.00 (C-2'), 161.38 (C-4'), 177.51, 177.77 (2x C=O); ESI-MS, (Positive): m/ z 593 $[M + H]^+$; HRMS (ESI) calcd for $C_{36}H_{32}O_8$ $[M + H]^+$ 593.2169, found 593.2170.

8i- Yellow powder, 72 % yield, mp 118-120 ºC. IRv^{max} (KBr): 3419, 1637, 1567, 1464, 1291, 1207, 1171, 1129, 1032 cm^{-1}; ^{1}H NMR (300Hz, acetone d_6): 1.48, 1.52 (3H each, s, H_3-5", H_3-6"), 3.01 (1H,

dd, J= 15.6, 5.1 Hz, H-4e), 3.17 (1H, dd, J= 15.6, 9.6 Hz, H-4a), 3.59 (1H, m, H-3a), 4.01 (6H each, s, 2 x OCH_3), 4.26 (1H, t, J=9.0 Hz, H-2a), 4.30 (1H, dd , J= 9.0, 1.8 Hz, H-2e), 5.76 (1H, d, J=9.9 Hz, H-3''), 6.30 (1H each, m, 2 x H- 4'''), 6.67 (1H, d, J= 9.9 Hz, H-4''), 6.80 (1H, s, H-3'), 7.17 (1H, m, 2 x H-3''', 2 x H-5'''), 7.42, 7.66 (1H each, d, J=15.6, 15.9 Hz, 2x H-α), 7.50 (1H, s, H-5), 7.70 (1H, s, H-6'), 8.03, 8.08 (1H each , d, J=15.9 Hz, 2 x H-β), 10.96, 11.00 (1H each, s, 2 x NH); ^{13}C NMR (75 MHz, acetone d-$_6$): 27.52, 28.55 (C-5'',C-6''), 30.38 (C-4), 31.47 (C-3), 55.81 (2 x OCH_3), 70.40 (C-2), 76.84 (C-2''), 96.05 (C-3'), 110.16 (C-8), 110.34 (2 x C-4'''), 115.68 (C-10), 115.77, 116.05 (2 x C-3'''), 116.45 (C-6), 116.57 (C-5'), 116.94 (C-4''), 120.25, 120.82 (2 x C-α), 121.97 (C-1'), 125.12, 125.27 (2 x C-5'''), 127.26 (C-6'), 127.41 (C-3''), 129.58 (C-5), 134.01, 134.21 (2 x C-2'''), 136.12, 136.50 (2x C-β), 151.98 (C-9), 152.14 (C-7), 159.57 (C-2'), 160.84 (C-4'), 178.71, 178.97 (2 x C=O); ESI-MS, (Positive): m/ z 591 $[M + H]^+$; HRMS (ESI) calcd for $C_{36}H_{34}N_2O_6$ $[M + H]^+$ 591.2489, found 591.2492.

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[30]. 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 glabridin and glabridin-chalcone hybrid molecules derivatives were determined by the broth microdilution assay using 96 'U'-bottom micro-titre plates as per CLSI guidelines.[31] Experimental observations were performed in triplicate to rule out any error during the procedure. An antibiotic norfloxacin was used as the positive control.

Interaction study of Glabridin-Chalcone hybrid molecules (GCHMs) with norfloxacin

The interaction studies of GCHMs with norfloxacin against clinical isolates (MRSA) were assessed through checkerboard method.[32] The synergy between GCHMs and norfloxacin was evaluated as a fractional inhibitory concentration index (FICI). The FIC was calculated as the MIC of an antibiotic or GCHMs in combination, divided by the MIC of the antibiotic or GCHMs 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.[33]

FIC A = MIC of A in combination / MIC of A alone

FIC B = MIC of B in combination / MIC of B alone

FICI = FIC (A) + FIC (B)

Bacterial-killing assay

The *in vitro* bactericidal activity of selective GCHMs 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.[34] Each analysis was done in triplicate with a control without test sample. Time kill curves were derived by plotting log10 CFU/ml against time (h).

***In vivo* efficacy of selective GCHMs in systemically infected Swiss mice model**

The therapeutic efficacies of selective GCHMs 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 (106 CFU) of *S.aureus* (SA-MTCC96). GCHMs at graded doses of 100, 50, 25 and 12.5 mg/kg 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.[30,35] 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 $p< 0.01$, $p< 0.05$.

Ethical clearance

The study was approved (Protocol number AH-2012-07) 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.

Acknowledgements

The authors are grateful to the Director, CSIR-CIMAP for providing necessary facilities for this work. Financial support from the National Medicinal Plant Board (NMPB , GAP-208), New Delhi, is duly acknowledged. D.S. Kapkoti is thankful to CSIR New Delhi for award of CSIR-SRF fellowship.

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