

RESEARCH ARTICLE


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Glabridin-chalcone hybrid molecules: drug resistance reversal agent against clinical isolates of methicillin-resistant *Staphylococcus aureus*^{†‡}

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A novel series of glabridin-chalcone hybrid molecules (GCHMs) were synthesized and evaluated for their antibacterial and resistance reversal activity against clinical isolates of a 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 of 12.5 $\mu\text{g mL}^{-1}$. However, all its synthesized derivatives displayed moderate to weak activity (MICs ranging from 12.5 to $>100 \mu\text{g mL}^{-1}$). 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 and up to 16-fold reduction in MICs with norfloxacin (FICI range from 0.312 to 0.375). In a 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 the treatment of infection caused by clinical MRSA isolates.

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Introduction

Bacterial infections particularly those caused by *Staphylococcus aureus* remain 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 is often associated with an array of life-threatening infections including surgical site infections, bacteremia, pneumonia and catheter-associated infections.^{2,3} Nowadays, most of the MRSA strains are resistant to many of the clinically used antibiotics such as penicillin and β -lactams, macrolides, quinolones and even glycopeptides 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 their derivatives are always a good source of lead compounds. Glabridin **1** is a prenylated isoflavan found in the roots of *Glycyrrhiza glabra*.⁶ It is a major phenolic compound isolated from the hydrophobic fraction of licorice. It has a wide range of biological activities such as antimicrobial, antioxidant, anti-inflammatory, estrogenic, anticancer, anti-neuroprotective, anti-osteoporotic, etc.^{7–16}

Chalcones, bioprecursors of flavonoids in plants, are open-chain flavonoids in which two aromatic rings are joined by the α , β -unsaturated carbonyl system. Chalcones are versatile molecules having diverse biological activities including antimicrobial,¹⁷ anticancer,¹⁸ antioxidant,¹⁹ anti-inflammatory,²⁰ antimalarial,²¹ hepatoprotective²² and many more. In view of these biological activities of both molecules, it was envisaged to synthesize hybrid molecules where both the chalcone and the 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 fluoroquinolone antibiotics which mainly act as a substrate for efflux pumps and is mainly responsible for multidrug resistance

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[‡] 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/c5md00527b

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(MDR) generation. The main aspect of this study may lead to the discovery of novel GCHMs as efflux pump inhibitors.

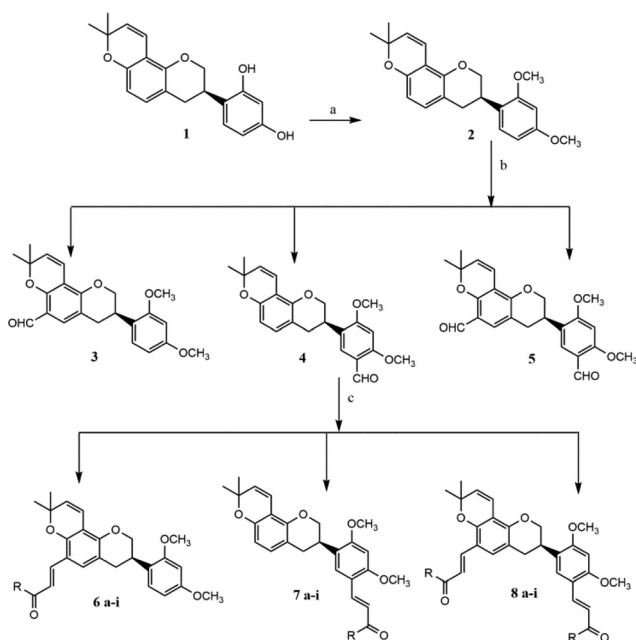
Results and discussion

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

The emergence of multidrug resistant (MDR) strains of *S. aureus* is now the major challenge for clinicians to manage MRSA infections and also threatens populations worldwide. Therefore, investigators continue to search for novel effective antimicrobial compounds or their combinations.²⁴ The interaction of plant compounds with antibiotics 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 on combinations of natural products with antibiotics against infectious diseases.²⁶ Reuk-ngam *et al.* reported the antimicrobial activity of coronarin D and its synergistic potential with antibiotics.²⁷ Similarly, Mun *et al.* reported the synergistic antibacterial effect of curcumin against MRSA.²⁸ These findings may encourage us to study *in vitro* the antibacterial efficacy of GCHMs alone as well as in combination with fluoroquinolone antibiotics against clinical MRSA strains. All the clinical

isolates of *S. aureus* (MRSA) were characterized by using various biochemical parameters including an *S. aureus* identification test kit (Microexpress, India). Based on these tests, it was confirmed that all the clinical isolates were *S. aureus* (Table S1†). Further PCR amplification of the *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 the anti-MRSA activity of fluoroquinolone antibiotics, norfloxacin (MIC ranges from 250 to 500 µg ml⁻¹) and GCHMs alone as well as in combinations. The results of MIC assay showed that free hydroxyl groups on glabridin are 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 values ranging from 12.5 to 25 µg ml⁻¹ and fourteen compounds (**6b**, **8e**, **7g**, **6c**, **8i**, **7i**, **7h**, **8h**, **6a**, **7a**, **6g**, **6f**, **7c** and **8a**) exhibited MICs ranging from 25 to 50 µg ml⁻¹, while in the case of glabridin alone the MIC ranges from 3.125 to 12.5 µg ml⁻¹. Norfloxacin, as a positive control, exhibited MIC in the range 125–500 µg ml⁻¹ against all the tested clinical isolates of MRSA (Table 1).

Norfloxacin was further selected for combination study with most active GCHMs (**6h** and **8f**), two moderate active GCHMs (**6f** and **8h**) and glabridin with norfloxacin were evaluated through checkerboard assay because it mainly acts as a substrate of efflux pump, which is mainly responsible for MDR generation. The result of the combination study showed that **8h** exhibits the strongest synergistic interaction with 8–16-fold 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⁻¹, respectively, against all the tested clinical isolates of MRSA. Similarly, the MIC of **8h** also reduced up to fourfold (Table 2). The combination of **6h** with norfloxacin showed a synergistic interaction (4–8-fold reduction in MIC of norfloxacin and 4-fold reduction in MIC of **6h**). The MIC of norfloxacin reduced from 500–250 to 62.5–31.25 µg ml⁻¹ against four clinical isolates (MRSA-ST2071, P4423, ST10760 and ST10342) with FICI ranging from 0.375 to 0.50 similarly. The combination of **8f** with norfloxacin exhibited a synergistic interaction (4–8-fold 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 a synergistic interaction (4–8-fold reduction in the MIC of norfloxacin, 500–125 to 125–31.25 µg ml⁻¹, respectively) against



Scheme 1 Reagents and conditions: a) CH₃I, K₂CO₃, acetone, reflux 4 hours. b) DMF, POCl₃ in acetonitrile, rt, overnight. c) RCOCH₃, KOH in MeOH, rt, 4–8 hours.

Table 1 Antibacterial activity of glabridin and its derivatives against clinical isolates of *S. aureus* (MRSA). Minimum inhibitory concentration ($\mu\text{g mL}^{-1}$)




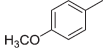
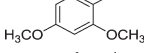
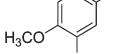
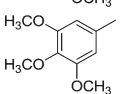



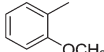

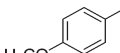
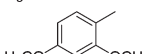
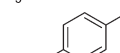
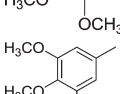



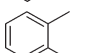

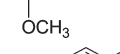
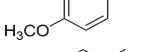
Comp	R	SA	MRSA-2071	MRSA-4629	MRSA-4423	MRSA-10 760	MRSA-10 342	MRSA-4650
6a		25	25	25	25	25	25	25
6b		25	50	25	50	50	50	25
6c		25	25	25	25	25	25	25
6d		>100	>100	>100	>100	>100	>100	>100
6e		>100	>100	>100	>100	>100	>100	>100
6f		25	50	50	50	50	50	50
6g		25	25	25	25	25	25	25
6h		12.5	25	12.5	12.5	25	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

Table 1 (continued)

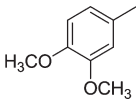
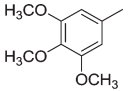


Comp	R	SA	MRSA-2071	MRSA-4629	MRSA-4423	MRSA-10 760	MRSA-10 342	MRSA-4650
8f		12.5	25	12.5	25	25	12.5	12.5
8g		50	100	100	100	100	100	100
8h		25	50	25	50	50	50	25
8i		50	50	50	50	50	50	50
1 (Glabridin)		3.125	12.5	12.5	12.5	12.5	12.5	12.5
2 (2',4'-Dimethoxyglabridin)		>100	>100	>100	>100	>100	>100	>100
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 isolates of methicillin-resistant *S. aureus* (MRSA)

Strains	MIC of NOR alone ($\mu\text{g ml}^{-1}$)	MIC of NOR in the presence of 1 ($\mu\text{g ml}^{-1}$)			MIC of NOR in the presence of 6h ($\mu\text{g ml}^{-1}$)			MIC of NOR in the presence of 8h ($\mu\text{g ml}^{-1}$)			MIC of NOR in the presence of 6f ($\mu\text{g ml}^{-1}$)			MIC of NOR in the presence of 8f ($\mu\text{g ml}^{-1}$)		
		MIC of NOR	FICI	FR ^a	MIC of NOR	FICI	FR ^a	MIC of NOR	FICI	FR ^a	MIC of NOR	FICI	FR ^a	MIC of NOR	FICI	FR ^a
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-10 760	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-10 342	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 concentration index; FR, fold reduction; MIC, minimum inhibitory concentration; NOR, norfloxacin.

^a FR = fold reduction.

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-fold. The combination of glabridin with norfloxacin exhibited a synergistic interaction (2–4-fold reduction in the MIC of norfloxacin and 4-fold reduction in the MIC of glabridin) against two clinical isolates (MRSA 4629 and 4650) only. Although in the combination study the GCHMs reduced the MIC of norfloxacin up to 16-fold, which is still quite high from the clinical point of view, nevertheless 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 further exploiting the plant-derived molecule glabridin for derivatization of its novel derivatives with clinical value. The time kill

curve represents a useful method for evaluating the kinetic interactions between bacterial cells and antimicrobial agents. A time kill kinetics study of glabridin and its derivatives (6h, 8h, 6f and 8f) with norfloxacin alone as well as in combination was evaluated at two different MIC concentrations. At 1/2 MIC and MIC concentration, glabridin and GCHMs derivatives diminish the viability of bacterial cells up to 1.52- and 2-fold, respectively, after 24 h of incubation. In a combination study, the combination of norfloxacin with glabridin, 6h and 8f at 1/2 MIC concentration diminished the viability of bacterial cells up to 3-fold only after 24 h of incubation. The combination of 6f with norfloxacin at 1/2 MIC concentration diminished the viability of bacterial cells up to 4-fold after 24 h of incubation, while the combination of derivative 8h

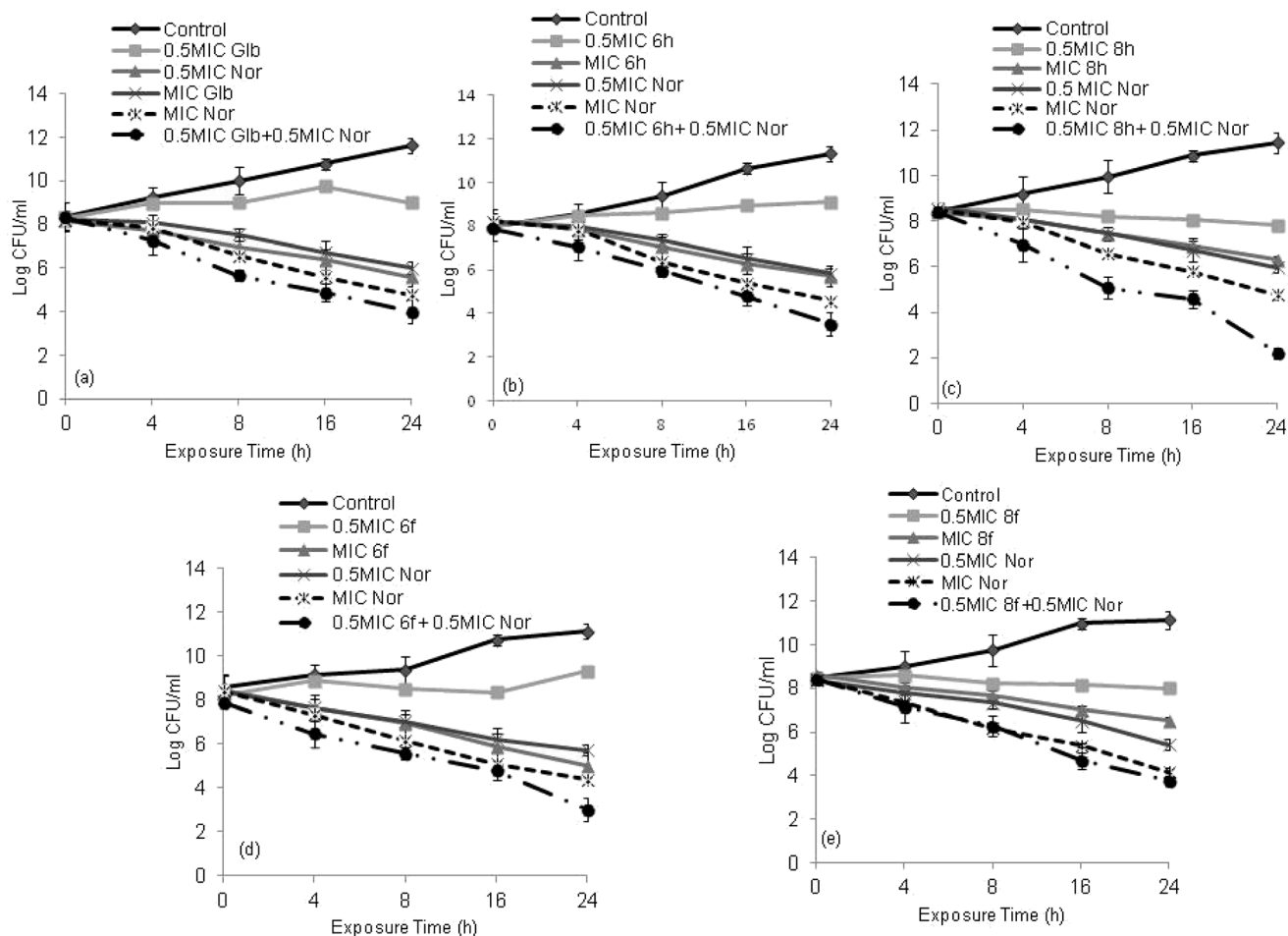


Fig. 1 Time kill kinetics study of glabridin (Glb) (a) and its derivatives 6h (b), 8h (c), 6f (d), and 8f (e) alone as well as in combination with norfloxacin (Nor) at different MIC concentrations against clinical isolate MRSA ST-2071. Data expressed as means \pm SDs.

with norfloxacin at 1/2 MIC concentration diminished the viability of bacterial cells up to 6-fold (Fig. 1). Since the maximum synergy (up to 16-fold) of derivative 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 the case of GCHMs 6a-i, having

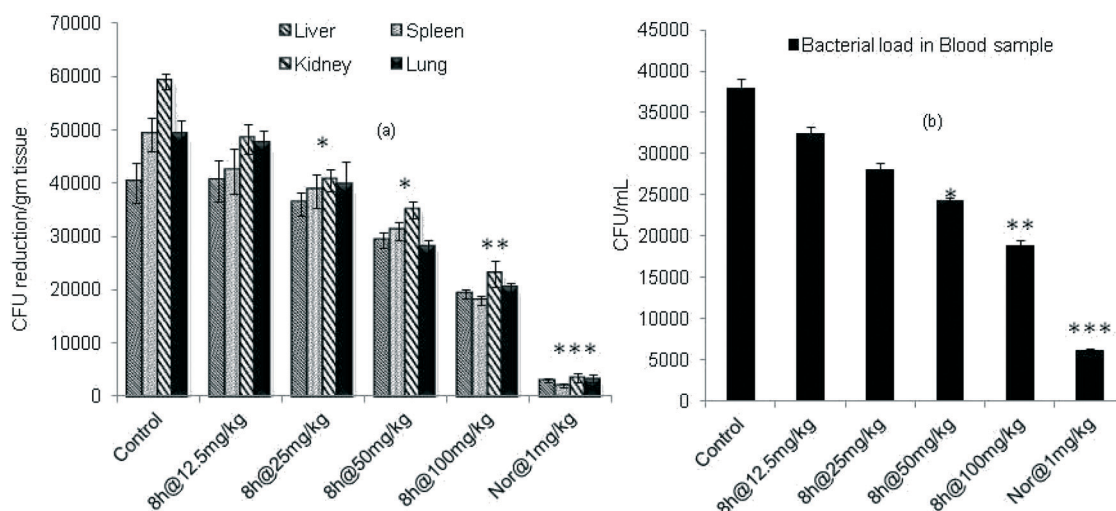


Fig. 2 Efficacy of glabridin derivative (8h) at various doses in terms of reduction of bacterial burden (*S. aureus* MTCC-96) in multiple organs (a) and blood (b). The infection was induced through the intravenous injection of 0.2 mL (10^6 CFU) of *S. aureus* in a volume of 0.2 mL. Data are expressed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

electron-donating groups ($-\text{OCH}_3$) at the *ortho* and *para* positions of the phenyl ring of the chalcone counterpart, they were less active as compared to the unsubstituted derivative (**6a**). Introduction of the $-\text{OCH}_3$ group at the *meta* position had no effect on the activity. Substitution of the phenyl ring by furan led to an increase in anti-MRSA activity. In the case of GCHMs **7a-i**, introduction of the OCH_3 group at the *ortho* and *para* positions reduced its anti-MRSA activity, and at the *meta* position resulted in no change or slight reduction in activity. In the case of compounds having a dichalcone moiety, introduction of the OCH_3 group at the *ortho*, *meta* and *para* positions led to a decrease in activity. In a combination study, compounds having a furan ring in place of a phenyl ring showed the highest reduction in MIC of norfloxacin (8–16-fold) as compared to that of the phenyl substituent.

During *in vivo* study through the infectious model, no 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⁻¹ body weight were evaluated and are 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 a methicillin-resistant strain of *Staphylococcus aureus* (MRSA) alone and in combination with norfloxacin. Results of the *in vitro* combination study showed that GCHMs exhibited synergistic and additive interactions (2–16-fold reduction in MICs) of norfloxacin against all tested clinical isolates of MRSA. Compound **8h** exhibited marked synergism with up to 16-fold reduction in MICs of norfloxacin (FICI range from 0.312 to 0.375). Though the MIC of norfloxacin is still high in combination for its clinical use, glabridin can be exploited to prepare better derivatives to increase the efficacy of norfloxacin against resistant bacterial strains. Thus we conclude from this study that GCHMs are promising templates for the development of new drugs to treat MRSA infections.

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, CDCl₃ 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 a Perkin-Elmer Turbo Mass/Shimadzu LC-MS and LC-MS-MS APC3000 (Applied Biosystems). HRMS spectra were recorded on a JEOL-AccuTOF JMS-T100LC mass spectrometer 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 I₂ 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 was used in the column chromatography for the purification of metabolites. The compounds were identified by their spectral IR, ID (¹H, ¹³C, 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 a 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 an oven at a temperature of 35 °C and powdered. The dried and powdered roots of *G. glabra* (10 kg) were extracted three times with ethyl acetate. The combined ethyl acetate extract was concentrated on a rotavapour and the residue obtained (268 grams) was subjected to column chromatography on 60–120 mesh size silica gel, eluting with a hexane/EtOAc solvent system. The glabridin-rich fraction (47.6 g) was eluted in a 70:30 hexane-EAA solvent system and was further purified by vacuum liquid chromatography and crystallized in benzene to afford pure white crystals of glabridin (11.6 g). The structure of glabridin was confirmed by comparison of its spectral data (IR, mass, NMR) with those reported in the literature.²⁹

Formylation of glabridin dimethyl ether. To a solution of 2',4'-dimethoxyglabridin **2** (1 mmol) in acetonitrile, DMF (4.5 mmol) and POCl₃ (4.5 mmol) were added and the resulting mixture was stirred at room temperature overnight. After completion of the reaction, water was added and the reaction mixture was extracted with ethyl acetate (3 × 100 ml). The organic layer was washed with water, dried over anhydrous sodium sulfate and concentrated *in vacuo*. 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 °C. IR ν^{max} (KBr): 2849, 1674 (C=O), 1585, 1467, 1289, 1157, 1131, 1032 cm⁻¹; ¹H NMR (300 Hz, acetone-*d*₆): 1.47 (6H, s, H₃-5'', H₃-6''), 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 × OCH₃), 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 ($2 \times \text{OCH}_3$), 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 $[\text{M} + \text{H}]^+$; HRMS (ESI) calcd for $\text{C}_{23}\text{H}_{24}\text{O}_5$ $[\text{M} + \text{H}]^+$ 381.1696, found 381.1697.

4. White powder, 14.5% yield, mp 85 °C. IR ν^{max} (KBr): 2850, 1670 (C=O), 1475, 1277, 1211, 1155, 1115, 1090 cm^{-1} ; ^1H NMR (300 Hz, acetone- d_6): 1.35, 1.36 (3H each, s, H₃-5'', H₃-6''), 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, OCH₃), 4.00 (3H, s, OCH₃), 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, $J = 8.1$ Hz, 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 ($2 \times \text{OCH}_3$), 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 $[\text{M} + \text{H}]^+$; HRMS (ESI) calcd for $\text{C}_{23}\text{H}_{24}\text{O}_5$ $[\text{M} + \text{H}]^+$ 381.1696, found 381.1697.

5. White powder, 60.2% yield, mp 75–76 °C. IR ν^{max} (KBr): 2847, 1673 (C=O), 1582, 1462, 1278, 1209, 1132, 1115, 1025 cm^{-1} ; ^1H NMR (300 Hz, acetone- d_6): 1.47, 1.52 (3H each, s, H₃-5'', H₃-6''), 2.87 (1H, m, H-4e), 3.05 (1H, dd, $J = 15.6, 11.1$ Hz, H-4a), 3.48 (1H, m, H-3a), 3.99, 4.05 (3H each, s, $2 \times \text{OCH}_3$), 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, $2 \times \text{CHO}$); ^{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 ($2 \times \text{OCH}_3$), 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 ($2 \times \text{CHO}$); ESI-MS, (positive): m/z 409 $[\text{M} + \text{H}]^+$; HRMS (ESI) calcd for $\text{C}_{24}\text{H}_{24}\text{O}_6$ $[\text{M} + \text{H}]^+$ 409.1645, found 409.1646.

Synthesis of glabridin-chalcone hybrid molecules

General procedure. Chalcones were prepared by Claisen-Schmidt condensation reaction of mono/diformyl glabridin and different substituted acetophenone derivatives. In the case of monoformyl glabridin (3 & 4), 1 mmol of monoformyl glabridin and 1 mmol of acetophenone derivative were dissolved in methanol with stirring. KOH (10 mmol) was added. The resulting solution was stirred for 4–8 hours. Then the reaction mixture was poured into ice-cold water and acidified by the addition of 1 N HCl. The crude product was extracted with ethyl acetate (50 ml \times 3) and the organic phase

was washed with water, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The resulting solid was subjected to chromatography on a 100–200 mesh size silica gel column. In the case of diformyl glabridin (5), 2 mmol of acetophenone derivatives were used.

6a. Yellow powder, 70% yield, mp 55–58 °C. IR ν^{max} (KBr): 1656 (C=O), 1584, 1463, 1280, 1175, 1129, 1039 cm^{-1} ; ^1H NMR (300 Hz, acetone- d_6): 1.48, 1.49 (3H each, s, H₃-5'', H₃-6''), 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, $2 \times \text{OCH}_3$), 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 ($2 \times \text{OCH}_3$), 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 $[\text{M} + \text{H}]^+$; HRMS (ESI) calcd for $\text{C}_{31}\text{H}_{30}\text{O}_5$ $[\text{M} + \text{H}]^+$ 483.2166, found 483.2167.

6b. Yellow powder, 62% yield, mp 58–60 °C. IR ν^{max} (KBr): 1649, 1599, 1462, 1286, 1158, 1130, 1034 cm^{-1} . ^1H NMR (300 Hz, acetone- d_6): 1.44, 1.45 (3H each, s, H₃-5'', H₃-6''), 2.84 (1H, m, H-4e), 3.01 (1H, dd, $J = 15.3, 10.8$ Hz, H-4a), 3.48 (1H, m, H-3a), 3.77, 3.86, 3.90 (3H each, s, $3 \times \text{OCH}_3$), 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, $J = 7.5$ Hz, 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, $J = 7.2, 1.8$ Hz, 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 ($3 \times \text{OCH}_3$), 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 $[\text{M} + \text{H}]^+$; HRMS (ESI) calcd for $\text{C}_{32}\text{H}_{32}\text{O}_6$ $[\text{M} + \text{H}]^+$ 513.2271, found 513.2273.

6c. Yellow powder, 61% yield, mp 50–52 °C. IR ν^{max} (KBr): 1656, 1576, 1464, 1210, 1161, 1127, 1033 cm^{-1} ; ^1H NMR (300 Hz, acetone- d_6): 1.49, 1.51 (3H each, s, H₃-5'', H₃-6''), 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, $3 \times \text{OCH}_3$), 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, $J = 1.8$ Hz, 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, ($3 \times \text{OCH}_3$), 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 [$\text{M} + \text{H}$] $^+$; HRMS (ESI) calcd for $\text{C}_{32}\text{H}_{32}\text{O}_6$ [$\text{M} + \text{H}$] $^+$ 513.2271, found 513.2274.

6d. Yellow powder, 64% yield, mp 88–90 °C. IR ν^{max} (KBr): 1649, 1561, 1463, 1279, 1166, 1130, 1024 cm^{-1} ; ^1H NMR (300 Hz, acetone- d_6): 1.49, 1.50 (3H each, s, H₃-5'', H₃-6''), 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, $3 \times \text{OCH}_3$), 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, ($3 \times \text{OCH}_3$), 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 [$\text{M} + \text{H}$] $^+$; HRMS (ESI) calcd for $\text{C}_{32}\text{H}_{32}\text{O}_6$ [$\text{M} + \text{H}$] $^+$ 513.2271, found 513.2272.

6e. Yellow powder, 67% yield, mp 63–65 °C. IR ν^{max} (KBr): 1645, 1578, 1462, 1279, 1159, 1128, 1028 cm^{-1} ; ^1H NMR (300 Hz, acetone- d_6): 1.46, 1.48 (3H each, s, H₃-5'', H₃-6''), 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, $4 \times \text{OCH}_3$), 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 ($4 \times \text{OCH}_3$), 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 [$\text{M} + \text{H}$] $^+$.

6f. Yellow powder, 68% yield, mp 64–65 °C. IR ν^{max} (KBr): 1649, 1569, 1462, 1264, 1207, 1161, 1124, 1024 cm^{-1} ; ^1H NMR (300 Hz, acetone- d_6): 1.49, 1.51 (3H each, s, H₃-5'', H₃-6''), 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, $4 \times \text{OCH}_3$), 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 (1H, 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 ($4 \times \text{OCH}_3$), 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 [$\text{M} + \text{H}$] $^+$; HRMS (ESI) calcd for $\text{C}_{33}\text{H}_{34}\text{O}_7$ [$\text{M} + \text{H}$] $^+$ 543.2377, found 543.2372.

6g. Yellow powder, 73% yield, mp 64–65 °C. IR ν^{max} (KBr): 1649, 1576, 1464, 1210, 1161, 1127, 1033 cm^{-1} ; ^1H NMR (300 Hz, acetone- d_6): 1.49, 1.51 (3H each, s, H₃-5'', H₃-6''), 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, $5 \times \text{OCH}_3$), 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 (1H, 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 ($5 \times \text{OCH}_3$), 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 [$\text{M} + \text{H}$] $^+$; HRMS (ESI) calcd for $\text{C}_{34}\text{H}_{36}\text{O}_8$ [$\text{M} + \text{H}$] $^+$ 573.2482, found 573.2481.

6h. Yellow powder, 72% yield, mp 90–92 °C. IR ν^{max} (KBr): 1649, 1576, 1465, 1284, 1159, 1129, 1040 cm^{-1} ; ^1H NMR (300 Hz, acetone- d_6): 1.48, 1.50 (3H each, s, H₃-5'', H₃-6''), 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, $2 \times \text{OCH}_3$), 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 (1H, 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 ($2 \times \text{OCH}_3$), 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 [$\text{M} + \text{H}$] $^+$; HRMS (ESI) calcd for $\text{C}_{29}\text{H}_{28}\text{O}_6$ [$\text{M} + \text{H}$] $^+$ 473.1958, found 473.1958.

6i. Yellow powder, 67% yield, mp 85–87 °C. IR ν^{\max} (KBr): 3244, 1637, 1565, 1474, 1290, 1208, 1157, 1128, 1054 cm^{-1} ; ^1H NMR (300 Hz, pyridine- d_5): 1.42 (6H, s, $\text{H}_{3-5''}$, $\text{H}_{3-6''}$), 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, $2 \times \text{OCH}_3$), 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 ($2 \times \text{OCH}_3$), 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 [$\text{M} + \text{H}$] $^+$; HRMS (ESI) calcd for $\text{C}_{29}\text{H}_{29}\text{NO}_5$ [$\text{M} + \text{H}$] $^+$ 472.2118, found 472.2117.

7a. Yellow powder, 63% yield, mp 50–52 °C. IR ν^{\max} (KBr): 1657 (C=O), 1585, 1464, 1290, 1210, 1155, 1115, 1033 cm^{-1} ; ^1H NMR (300 Hz, acetone- d_6): 1.34, 1.36 (3H each, s, $\text{H}_{3-5''}$, $\text{H}_{3-6''}$), 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, $2 \times \text{OCH}_3$), 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 ($2 \times \text{OCH}_3$), 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 [$\text{M} + \text{H}$] $^+$; HRMS (ESI) calcd for $\text{C}_{31}\text{H}_{30}\text{O}_5$ [$\text{M} + \text{H}$] $^+$ 483.2166, found 483.2167.

7b. Yellow powder, 68% yield, mp 52–55 °C. IR ν^{\max} (KBr): 1650, 1583, 1464, 1289, 1156, 1114, 1029 cm^{-1} ; ^1H NMR (300 Hz, acetone- d_6): 1.34, 1.35 (3H each, s, $\text{H}_{3-5''}$, $\text{H}_{3-6''}$), 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, $3 \times \text{OCH}_3$), 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 ($3 \times \text{OCH}_3$), 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 [$\text{M} + \text{H}$] $^+$.

7c. Yellow powder, 62% yield, mp 58–60 °C. IR ν^{\max} (KBr): 1657, 1579, 1469, 1288, 1207, 1161, 1117, 1090 Hz; ^1H NMR (300 Hz, acetone- d_6): 1.34, 1.36 (3H each, s, $\text{H}_{3-5''}$, $\text{H}_{3-6''}$), 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, $3 \times \text{OCH}_3$), 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, $J = 9.9$ Hz, 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 ($3 \times \text{OCH}_3$), 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 [$\text{M} + \text{H}$] $^+$.

7d. Yellow powder, 68% yield, mp 88–90 °C. IR ν^{\max} (KBr): 1654, 1586, 1474, 1293, 1166, 1116, 1029 cm^{-1} ; ^1H NMR (300 Hz, pyridine- d_5): 1.42 (6H, s, $\text{H}_{3-5''}$, $\text{H}_{3-6''}$), 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, $3 \times \text{OCH}_3$), 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 ($3 \times \text{OCH}_3$), 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 [$\text{M} + \text{H}$] $^+$.

7e. Yellow powder, 78% yield, mp 70–72 °C. IR ν^{\max} (KBr): 1644, 1576, 1465, 1284, 1159, 1129, 1040 cm^{-1} ; ^1H NMR (300 Hz, acetone- d_6): 1.34 (6H, s, $\text{H}_{3-5''}$, $\text{H}_{3-6''}$), 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, $4 \times \text{OCH}_3$), 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 ($4 \times \text{OCH}_3$), 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 °C. IR ν^{\max} (KBr): 1646, 1597, 1562, 1465, 1265, 1208, 1154, 1129, 1031 cm^{-1} ; 1H NMR (300 Hz, 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, $4 \times OCH_3$), 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 ($4 \times OCH_3$), 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 °C. IR ν^{\max} (KBr): 1651, 1572, 1463, 1289, 1209, 1158, 1126, 1029 Hz; 1H NMR (300 Hz, 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, $5 \times OCH_3$), 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 ($3 \times OCH_3$), 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 °C. IR ν^{\max} (KBr): 1649, 1584, 1467, 1291, 1208, 1156, 1116, 1031 cm^{-1} ; 1H NMR (300 Hz, 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, $2 \times OCH_3$), 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 ($2 \times OCH_3$), 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 °C. IR ν^{\max} (KBr): 3234, 1635, 1561, 1469, 1282, 1208, 1150, 1110, 1031 cm^{-1} ; 1H NMR (300 Hz, 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, $2 \times OCH_3$), 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 = 11.1 Hz, 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 ($2 \times OCH_3$), 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 °C. IR ν^{\max} (KBr): 1655, 1584, 1465, 1284, 1207, 1176, 1129, 1021 cm^{-1} ; 1H NMR (300 Hz, 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, $2 \times OCH_3$), 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, $2 \times H-3'''$, $2 \times H-4'''$, $2 \times H-5'''$), 7.63, 7.84 (2H, d, J = 15.6 Hz, $2 \times H-α$), 7.70 (1H, s, H-5), 7.76 (1H, s, H-6'), 8.07 (4H, m, $2 \times H-2'''$, $2 \times H-6'''$), 8.11, 8.16 (2H, d, J = 15.6 Hz, $2 \times H-β$); ^{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 ($2 \times OCH_3$), 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 ($2 \times C-3'''$, $2 \times C-5'''$), 128.96 ($2 \times C-2'''$, $2 \times C-6'''$), 129.63 (C-5, C-3''), 132.70 ($2 \times C-4'''$), 139.33 ($2 \times C-1'''$), 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 ($2 \times C=O$); 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 °C. IR ν^{\max} (KBr): 1649, 1599, 1578, 1463, 1287, 1205, 1129, 1027 cm^{-1} ; 1H NMR (300 Hz, 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, $4 \times OCH_3$), 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, $2 \times H-3'''$), 7.13 (1H each, m, $2 \times H-5'''$), 7.38 (1H, s, H-5), 7.30, 7.40 (1H each, d, J = 15.9, $2 \times H-α$), 7.46 (2H, m, $2 \times H-4'''$), 7.48 (2H,

m, 2 × H-6''), 7.58 (1H, s, H-6'), 7.78, 7.84 (1H each, d, J = 15.9, 2 × 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 × OCH₃), 70.51 (C-2), 76.84 (C-2''), 96.10 (C-3'), 110.15 (C-8), 112.38 (2 × 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 × C-5''), 127.74 (C-6'), 129.57 (C-3''), 130.01 (C-5), 130.18 (2 × C-6''), 130.61 (2 × C-1''), 132.65 (2 × C-4''), 137.95, 138.10 (2 × C-β), 152.29 (C-7), 152.29 (C-9), 158.40 (2 × C-2''), 159.78 (C-2'), 161.04 (C-4'), 192.53 (2 × C=O); ESI-MS, (positive): m/z 673 [M + H]⁺; HRMS (ESI) calcd for C₄₂H₄₀O₈ [M + H]⁺ 673.2795, found 673.2792.

8c. Yellow powder, 68% yield, mp 60–62 °C. IR ν^{max} (KBr): 1655, 1575, 1464, 1284, 1204, 1125, 1030 cm⁻¹; ^1H NMR (300 Hz, acetone- d_6): 1.32, 1.34 (3H each, s, H₃-5'', H₃-6''), 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 × OCH₃), 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 × H-4''), 7.25 (2H, m, 2 × H-5''), 7.36 (1H, s, H-5), 7.42 (2H, d, J = 1.5 Hz, 2 × H-2''), 7.51 (2H, d, J = 6.6 Hz, 2 × H-6''), 7.59 (1H, s, H-6'), 7.63 (2H, d, J = 15.9 Hz, 2 × H-α), 7.92, 7.98 (2H, d, J = 15.6 Hz, 2 × 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 × OCH₃), 70.52 (C-2), 77.00 (C-2''), 96.01 (C-3'), 110.17 (C-8), 113.30, 113.35 (2 × C-2''), 115.94 (C-10), 116.36 (C-6, C-5'), 116.86 (C-4''), 118.63, 118.73 (2 × C-4''), 119.52, 119.94 (2 × C-α), 120.56, 120.60 (2 × C-6''), 121.92 (C-1'), 127.73 (C-6'), 129.59 (C-3''), 129.79 (C-5), 130.02 (2 × C-5''), 139.44, 139.63 (2 × C-β), 140.61, 140.75 (2 × C-1''), 152.32 (C-9), 152.55 (C-7), 160.00 (C-2'), 160.41 (2 × C-3''), 161.33 (C-4'), 189.21, 189.32 (2 × C=O); ESI-MS, (positive): m/z 673 [M + H]⁺.

8d. Yellow powder, 64% yield, mp 80–82 °C. IR ν^{max} (KBr): 1652, 1582, 1462, 1256, 1207, 1169, 1131, 1027 cm⁻¹; ^1H NMR (300 Hz, acetone- d_6): 1.46, 1.48 (3H each, s, H₃-5'', H₃-6''), 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 × OCH₃), 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 × H-3''), 2 × H-5''), 7.42 (1H, s, H-5), 7.65 (2H, d, J = 15.9 Hz, 2 × H-α), 7.72 (1H, s, H-6'), 8.03 (4H, d, J = 8.7 Hz, 2 × H-2''), 2 × H-6''), 8.08 (2H, d, J = 15.9 Hz, 2 × 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 × OCH₃), 70.39 (C-2), 76.92 (C-2''), 96.25 (C-3'), 110.16 (C-8), 113.99, 114.16 (2 × C-5''), 113.99, 114.16 (2 × 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 × C-2''), 2 × C-6''), 131.98, 132.08 (2 × C-1''), 138.34, 138.58 (2 × C-β), 152.16 (C-9), 161.10 (C-2', C-4'), 163.68, 163.80 (2 × C-4''), 186.98, 187.83 (2 × C=O); ESI-MS, (positive): m/z 673 [M + H]⁺; HRMS (ESI) calcd for C₄₂H₄₀O₈ [M + H]⁺ 673.2795, found 673.2787.

8e. Yellow powder, 77% yield, mp 80–81 °C. IR ν^{max} (KBr): 1645, 1603, 1464, 1283, 1163, 1125, 1026 cm⁻¹; ^1H NMR (300

Hz, acetone- d_6): 1.47, 1.48 (3H each, s, H₃-5'', H₃-6''), 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 × OCH₃), 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 × H-3'', 2 × 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, 2 × H-α), 7.59 (1H, s, H-6'), 7.68 (2H, dd, J = 8.7, 1.5 Hz, 2 × H-6''), 7.87, 7.93 (1H each, d, J = 15.9, 15.6 Hz, 2 × 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 × OCH₃), 70.53 (C-2), 76.78 (C-2''), 96.10 (C-3'), 98.80 (2 × C-3''), 106.04 (2 × 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 × C-1''), 125.66 (2 × C-α), 127.65 (C-6'), 129.56 (C-5, C-3''), 132.47 (2 × C-6''), 136.73, 136.88 (2 × C-β), 151.98 (C-9), 152.07 (C-7), 159.68 (C-2'), 160.75 (C-4'), 164.41 (2 × C-2''), 2 × C-4''), 190.03 (2 × C=O); ESI-MS, (positive): m/z 733 [M + H]⁺.

8f. Yellow powder, 78% yield, mp 85–86 °C. IR ν^{max} (KBr): 1648, 1569, 1464, 1263, 1202, 1126, 1025 Hz; ^1H NMR (300 Hz, acetone- d_6): 1.43, 1.49 (3H each, s, H₃-5'', H₃-6''), 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 × OCH₃), 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 × H-5''), 7.55 (1H, s, H-5), 7.62, 7.86 (1H each, d, J = 15.6 Hz, 2 × H-α), 7.61 (1H each, d, J = 1.8 Hz, 2 × H-2''), 7.68 (1H, s, H-6'), 7.77 (1H × 2, dd, J = 8.4, 1.8 Hz, 2 × H-6''), 8.07, 8.11 (1H × 2, d, J = 15.6 Hz, 2 × 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 × OCH₃), 70.35 (C-2), 76.94 (C-2''), 95.99 (C-3'), 110.16 (C-8), 111.09 (2 × C-5''), 111.39 (2 × C-2''), 115.84 (C-10), 116.35 (C-6), 116.58 (C-5'), 116.90 (C-4''), 118.97, 119.78 (2 × C-α), 121.96 (C-1'), 123.00 (2 × C-6''), 127.37 (C-6'), 129.61 (C-5, C-3''), 132.18 (2 × C-1''), 138.23, 138.61 (2 × C-β), 149.84 (2 × C-3''), 152.18 (C-9), 152.43 (C-7), 153.85 (2 × C-4''), 159.75 (C-2'), 161.08 (C-4'), 187.86, 187.50, (2 × C=O); ESI-MS, (Positive): m/z 733 [M + H]⁺; HRMS (ESI) calcd for C₄₂H₄₄O₁₀ [M + H]⁺ 733.3007, found 733.3003.

8g. Yellow powder, 75% yield, mp 102–105 °C. IR ν^{max} (KBr): 1650, 1568, 1463, 1160, 1127, 1028 cm⁻¹; ^1H NMR (300 Hz, acetone- d_6): 1.53 (6H, s, H₃-5'', H₃-6''), 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 × OCH₃), 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 × H-2''), H-6''), 7.75, 7.82 (1H each, d, J = 15.6 Hz, 2 × 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 × 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 × OCH₃), 70.55 (C-2), 77.02 (C-2''), 96.11 (C-3'), 106.63, 106.40 (2 × C-2''), 2 × 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 (2 × C-α), 128.10 (C-6'), 129.58 (C-3''), 130.22 (C-5), 134.53 (2 × C-1''),

139.30 (2 × C-β), 142.93 (2 × C-4^m), 152.30 (C-7, C-9), 153.78 (2 × C-3^m, 2 × C-5^m), 161.22 (C-2', C-4'), 188.40, 188.61 (2 × C=O); ESI-MS, (positive): m/z 793 [M + H]⁺; HRMS (ESI) calcd for C₄₆H₄₈O₁₂ [M + H]⁺ 793.3218, found 793.3218.

8h. Yellow powder, 72% yield, mp 102–104 °C. IR ν^{\max} (KBr): 1651, 1585, 1465, 1286, 1166, 1130, 1028 cm⁻¹; ¹H NMR (300 Hz, acetone-*d*₆): 1.47, 1.51 (3H each, s, H₃-5'', H₃-6''), 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 × OCH₃), 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 × H-4''), 6.81 (1H, s, H-3'), 7.36 (2H, d, J = 3.3 Hz, 2 × H-3''), 7.43 (2H, m, 2 × H-5''), 7.46, 7.66 (1H each, d, J = 15.6 Hz, 2 × 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, 2 × H-β); ¹³C 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 × OCH₃), 70.46 (C-2), 77.03 (C-2''), 96.05 (C-3'), 110.17 (C-8), 112.62 (2 × C-4^m), 115.93 (C-10), 116.05 (C-6), 116.82 (C-5'), 117.09 (2 × C-3^m), 117.24 (C-4''), 119.13, 119.61, (2 × C-α), 121.97 (C-1'), 127.63 (C-6'), 129.66 (C-5, C-3''), 138.20, 138.41 (2 × C-β), 147.07 (2 × C-5^m), 152.35 (C-9), 152.64 (C-7), 154.49 (2 × C-2''), 160.00 (C-2'), 161.38 (C-4'), 177.51, 177.77 (2 × C=O); ESI-MS, (positive): m/z 593 [M + H]⁺; HRMS (ESI) calcd for C₃₆H₃₂O₈ [M + H]⁺ 593.2169, found 593.2170.

8i. Yellow powder, 72% yield, mp 118–120 °C. IR ν^{\max} (KBr): 3419, 1637, 1567, 1464, 1291, 1207, 1171, 1129, 1032 cm⁻¹; ¹H NMR (300 Hz, acetone-*d*₆): 1.48, 1.52 (3H each, s, H₃-5'', H₃-6''), 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 × OCH₃), 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 × H-4''), 6.67 (1H, d, J = 9.9 Hz, H-4''), 6.80 (1H, s, H-3'), 7.17 (1H, m, 2 × H-3'', 2 × H-5''), 7.42, 7.66 (1H each, d, J = 15.6, 15.9 Hz, 2 × 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 × H-β), 10.96, 11.00 (1H each, s, 2 × NH); ¹³C 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 × OCH₃), 70.40 (C-2), 76.84 (C-2''), 96.05 (C-3'), 110.16 (C-8), 110.34 (2 × C-4^m), 115.68 (C-10), 115.77, 116.05 (2 × C-3^m), 116.45 (C-6), 116.57 (C-5'), 116.94 (C-4''), 120.25, 120.82 (2 × C-α), 121.97 (C-1'), 125.12, 125.27 (2 × C-5^m), 127.26 (C-6'), 127.41 (C-3''), 129.58 (C-5), 134.01, 134.21 (2 × C-2''), 136.12, 136.50 (2 × C-β), 151.98 (C-9), 152.14 (C-7), 159.57 (C-2'), 160.84 (C-4'), 178.71, 178.97 (2 × C=O); ESI-MS, (positive): m/z 591 [M + H]⁺; HRMS (ESI) calcd for C₃₆H₃₄N₂O₆ [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 molecule derivatives was 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 the 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 combinations in accordance to the method described by McKay *et al.*³⁴ Each analysis was done in triplicate with a control without test sample. Time kill curves were derived by plotting log₁₀ CFU mL⁻¹ against time (h).

***In vivo* efficacy of selective GCHMs in systemically infected Swiss mice model.** The therapeutic efficacies of selective GCHMs were evaluated through the 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⁻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.^{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.

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