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Synthesis of diverse analogues of Oenostacin and their antibacterial activities☆

Vandana Srivastava, Mahendra P. Darokar, Atiya Fatima, J. K. Kumar, Chinmay Chowdhury, Hari Om Saxena, Gaurav R. Dwivedi, Kunal Shrivastava, Vivek Gupta, S. K. Chattopadhyay, Suaib Luqman, M. M. Gupta, Arvind S. Negi* and Suman P. S. Khanuja

Central Institute of Medicinal and Aromatic Plants (CIMAP), PO CIMAP, Kukrail Picnic Spot Road, Lucknow 226 015, India

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Abstract—Several diverse analogues of Oenostacin, a naturally occurring potent antibacterial phenolic acid derivative, have been synthesized. A small library with more than forty analogues having different aromatic rings and varied side chains has been achieved through solution phase synthesis. Some of these analogues, that is, **22**, **23** and **42**, possessed potent antibacterial activities against *Staphylococcus epidermidis* and *Staphylococcus aureus* having EC_{50} ranging from 0.49 to 0.67 μM as compared to Oenostacin (EC_{50} = 0.12 μM).

1. Introduction

Multidrug-resistant gram-positive bacteria have continued to pose challenges to medicinal research community.[1] *Oenothera biennis* (Oenagraceae) commonly known as 'Evening Primarose' is cultivated in Indian gardens.[2] A number of compounds have been isolated from its aerial parts.[3] A potent antibacterial compound Oenostacin[4] was isolated during the systematic investigation of its roots along with several other compounds[5] in the recent past at this institute. Being a phenolic acid derivative with an aliphatic ester chain Oenostacin, has shown potent antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. It is known that *S. aureus*, one of the most successful opportunistic human Gram-positive pathogens, is responsible for postoperative wound infections, bacteraemia, pneumonia, osteomyelitis, mastitis, acute endocarditis, and deep abscesses in various organs.[6] In contrast to *S. aureus*, infections caused by *S. epidermidis* are less acute in nature. However, *S. epidermidis* is now recognized as an important human pathogen and is the predominant cause of infections associated with indwelling medical devices, as well as the primary cause of many nosocomial infections.[7]

In view of promising antibacterial activities[4] of Oenostacin **1**, and also in continuation to our research interest on the synthesis of biologically active gallic acid derivatives,[8] we became curious to study the efficacy of various analogues of **1**. Also, this could give us some possible antibacterial lead compounds of pharmacological spectrum of Oenostacin analogues and related compounds to evaluate their biological activities against *S. epidermidis* and *S. aureus*. Some of the synthesized compounds exhibited very good antibacterial activities as given in Figure 1 with their EC_{50} values in parentheses.

2. Results and discussion

2.1. Chemistry

Different analogues were synthesized as described in Schemes 1–5. In Scheme 1, orcinol **2**, used as starting material, underwent Reimer–Tiemann reaction with $CHCl_3$–aqueous alkali system to yield the aldehyde **4**, which upon methylation with dimethyl sulfate in acetone gave 2,6-dimethoxy-4-methyl benzaldehyde (**5**).

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* Corresponding author. Tel.: +91 522 2717529x327; fax: +91 522 2342666; e-mail: arvindcimap@rediffmail.com

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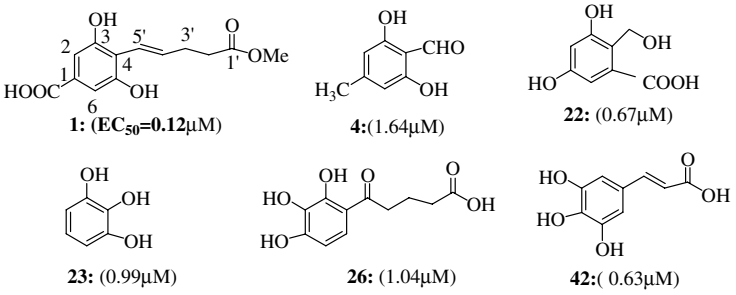
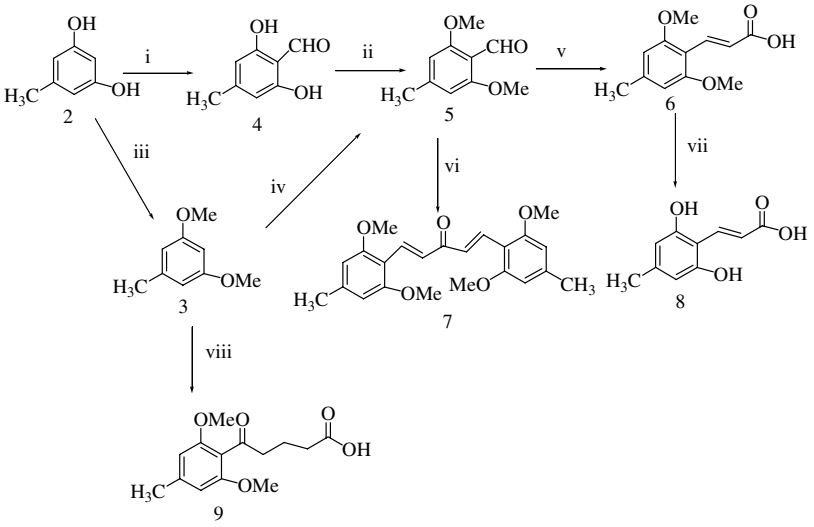


Figure 1. Oenostacin and some of the active analogues against *S. epidermidis*.



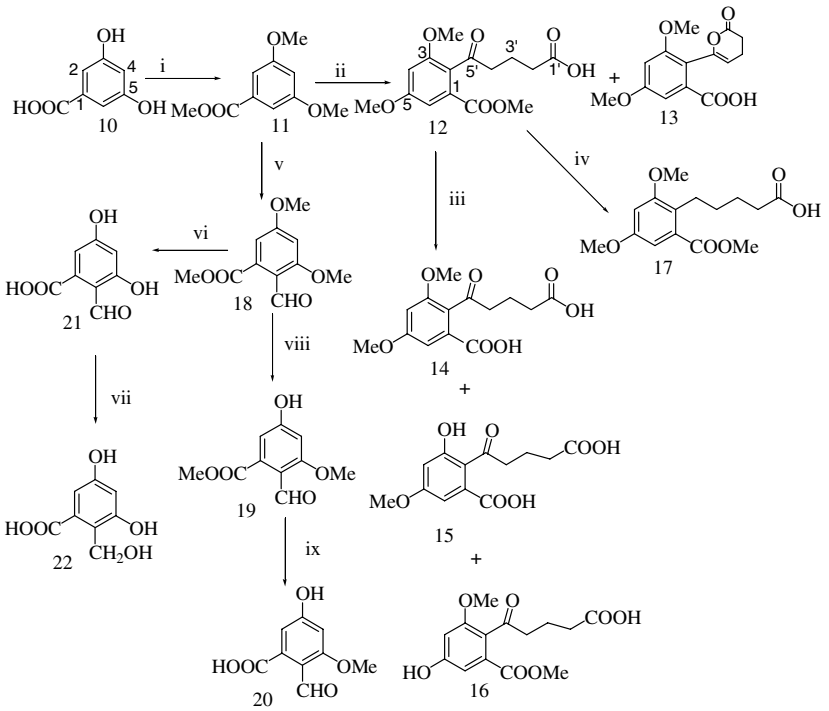
Scheme 1. Reagents and conditions: (i) $CHCl_3$–aq KOH (30%), reflux, 4 h, 14%; (ii) Me_2SO_4, K_2CO_3, dry acetone, reflux, 3 h, 87%; (iii) Me_2SO_4, K_2CO_3, acetone, reflux, 3 h, 93%; (iv) DMF, $POCl_3$, 0 °C for 30 min then rt for 3 h, 62%; (v) DMSO, NaOH, $(Ph_3P^+{=}CHCOOC_2H_5)Br^-$, rt, overnight, 32%; (vi) 1% ethanolic KOH, acetone, rt, 50–60 h, 62%; (vii) BBr_3, DCM, −78 °C for 1 h then rt, 42%; (viii) glutaric anhydride, DCM, $AlCl_3$, rt, overnight, 24%.

Due to poor yields in this approach, the reaction pathway was modified through methylation of orcinol followed by Vilsmeier–Haack formylation[9] using dimethylformamide and phosphorus oxychloride (DMF–$POCl_3$) to obtain **5** in good yield (62%). Wittig reaction of **5** with triphenyl phosphonium ethyl acetate bromide in DMSO and sodium hydroxide at room temperature yielded the corresponding cinnamic acid derivative **6**. Later, it was demethylated by stirring with boron tribromide in dichloromethane to get **8** in 42% yield.

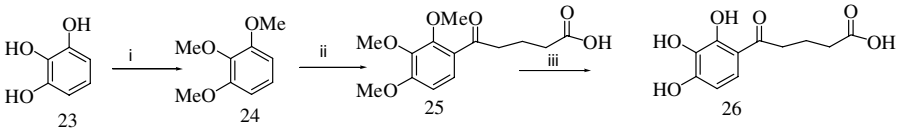
Aldehyde **5** on stirring overnight with acetone at room temperature in 1% alcoholic KOH yielded an aldol product **7**. However, compound **3** underwent Friedel-Craft's acylation with glutaric anhydride in dichloromethane and aluminium chloride at room temperature to give **9** having some similarity with the C5 side chain of Oenostacin **1**.[10]

In Scheme 2, the starting compound, 3,5-dihydroxy benzoic acid (**10**) was first methylated with dimethyl sulfate in acetone to obtain trimethylated product **11** in 93% yield. Interestingly, compound **11** on Friedel-Crafts acylation with glutaric anhydride in presence of anhydrous aluminium chloride at room temperature in dichloromethane gave a mixture of products keto (**12**) and enol lactone (**13**), which were carefully separated by silica gel column chromatography. Compound **12** was demethylated with anhydrous aluminium chloride in dichloromethane affording deprotected products **14**, **15** and **16** in 2:3:1 ratio. The keto acid **12** on reacting with sodium borohydride in trifluoroacetic acid (TFA) yielded a saturated C5 fatty acid chain containing compound **17** in 42% yield.[11] The fully protected ester **11**, on Vilsmeier–Haack reaction with DMF and $POCl_3$, yielded the aldehyde **18**, where the aldehydic group was unexpectedly introduced in the ring between one of the methoxyl and carboxylic ester groups. The methoxy groups of **18** were deprotected with boron tribromide in dichloromethane to furnish fully deprotected product **21**, which on borohydride reduction in methanol yielded corresponding alcohol **22**. On the other hand, compound **18** was selectively demethylated with $AlCl_3$–CH_2Cl_2 system[12] to get mono demethylated ester **19**, which on further hydrolysis yielded acid **20**.

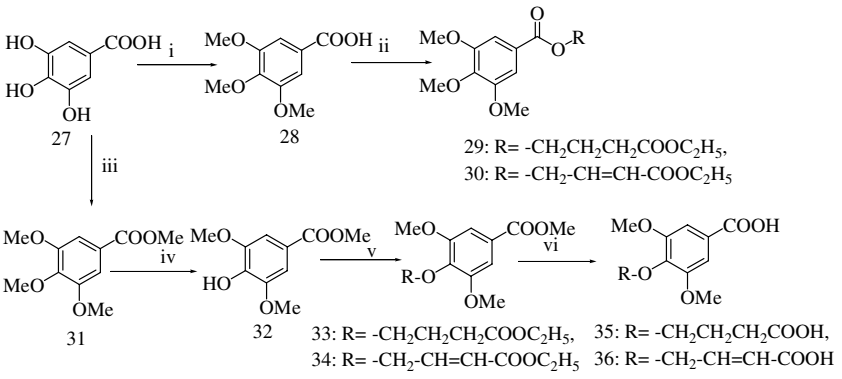
In Schemes 1 and 2, for the attachment of the required C5 aliphatic chain at the aromatic ring, as in Oenostacin **1**, we attempted Friedel-Crafts reaction on **3** and **11** with glutaric anhydride using anhydrous aluminium chloride



Scheme 2. Reagents and conditions: (i) Me_2SO_4, K_2CO_3, acetone, reflux, 3 h, 92%; (ii) glutaric anhydride, DCM, $AlCl_3$, rt, overnight, **12**: 45%, **13**: 18%; (iii) $AlCl_3$, DCM, rt,overnight 78% **14/15/16** = 2:3:1; (iv) TFA, $NaBH_4$, 0 °C for 4 h then rt, 42%; (v) DMF, $POCl_3$, 0 °C for 30 min then rt for 4 h, 42%; (vi) BBr_3, DCM, −78 °C for 30 min then RT overnight, 24%; (vii) $NaBH_4$, MeOH, rt, 40 min, 79%; (viii) $AlCl_3$-DCM, rt, overnight, 78%; (ix) 5% NaOH in MeOH/water (3:1), 50 °C, 1 h, 52%.

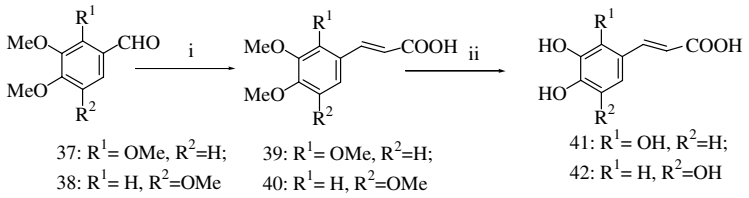


Scheme 3. Reagents and conditions: (i) Me_2SO_4, K_2CO_3, acetone, reflux, 3 h, 89%; (ii) glutaric anhydride, DCM, $AlCl_3$, rt, overnight, 22%; (iii) BBr_3, DCM, −78 °C for 1 h then rt, 58%.



Scheme 4. Reagents and conditions: (i) Me_2SO_4, 30% aqueous KOH, 10 °C for 1 h then reflux for 3 h, 62%; (ii) ethyl bromo butyrate/ethyl bromo crotonate, K_2CO_3, acetone, reflux, 4 h, 53%; (iii) Me_2SO_4, acetone, K_2CO_3, reflux, 4 h, 89%; (iv) $AlCl_3$, DCM, rt, 4 h, 69%; (v) ethyl bromoesters, acetone, K_2CO_3, reflux, 3–4 h; **33**: 64%, **34**: 71%; (vi) 5% KOH in MeOH/water (3:1), 50 °C, 1 h, **35**: 61%, **36**: 67%.

in CH_2Cl_2 at room temperature. In the above cases, we expected the attachment of the keto acid unit between both the methoxyl groups (i.e., at C4 position). Indeed, in case of **3** the attachment of the side chain was as expected to give **9** but surprisingly in **11** it was at C2-position (i.e., *ortho* to COOMe group), leading to a



Scheme 5. Reagents and conditions: (i) DMSO, KOH, $(Ph_3P^+{=}CH\text{-}COOC_2H_5)Br^-$, overnight, **39**: 48%, **40**: 54%; (ii) BBr_3, DCM, −78 °C for 30 min then rt, **41**: 49%, **42**: 62%.

mixture of products **12** and **13**. All these structural assignments have been unambiguously confirmed by various NMR experiments and mass spectral data.

Different 1D/2D-NMR experiments on **12** and **13** have confirmed the side-chain attachment at C2 position on the aromatic ring. ^{1}H NMR of **13** revealed two distinct singlet resonances in the aromatic region at δ 6.89 and 7.12 (ppm) due to two non-identical protons indicating the possible attachment of side chain at C2 position only. All the carbon resonances of ^{13}C NMR coupled with DEPT editing experiments are well in agreement with the proposed structure for **13**. Also, the appearance of only one long range correlation, in the ^{1}H–^{13}C inverse correlated HMBC experiment, between a carbonyl carbon of acid group attached to benzene ring of **13** and one of its aromatic protons attached to C6 position confirmed the side-chain attachment unambiguously at C2 position of aromatic ring. Similarly, a two-bond correlation between C3 and a proton attached to C4 (as well as two such similar correlations between C5 and protons attached to C4 and C6, respectively) further supported the proposed side-chain attachment of **13**. All the important long-range correlations of **13** are shown in Figure 2. A similar long-range HMBC correlation found in compound **12** confirms the same type of attachment as proposed in **13**.

In ^{13}C NMR of **13**, the two resonances at δ 167.2 and 175.3 ppm are due to two carbonyl functions. The former being more upfield indicates the side-chain acid was possibly cyclised at enol site to form a six-membered lactone ring. Further evidence to the proposed lactone moiety in **13** came from IR (a strong band at 1768 cm^{-1}, typical for a lactone carbonyl) and Mass spectral data. ESI-MS showed mass peaks at 279.2 (M+H) and 301.1 (M+Na) indicating the unambiguous assignment of **13** as a lactone.

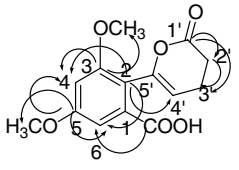


Figure 2. ^{1}H–^{13}C HMBC correlations of **13**.

In case of compound **9** (Scheme 1) the ^{1}H NMR showed a singlet resonance at δ 6.28 ppm integrating for two protons indicating the side-chain attachment at C4 position, that is, between two aromatic methoxyl groups (whereas in **12** and **13** two distinct singlet resonances appeared indicating their non-identical nature). Further confirmation of the structure proposed to compound **9** came from the ^{1}H–^{13}C inverse correlated HMBC experiment.

In Scheme 3, the phenolic hydroxy groups of pyrogallol **23** were protected through methylation with dimethyl sulfate as described earlier. Subsequent Friedel-Crafts reaction with glutaric anhydride yielded the keto derivative **25**, which was further demethylated with boron tribromide to furnish the phenolic keto acid **26**.

Several derivatives of gallic acid were also synthesized, as shown in Scheme 4, in which both ester chain derivatives (**29** and **30**) and phenolic ether derivatives (**33**–**36**) were prepared. In this approach, gallic acid was methylated with dimethyl sulfate in aqueous alkali to get trimethoxy benzoic acid **28**, which on reaction with ethyl bromo butyrate and ethyl bromo crotonate yielded **29** and **30**, respectively. On the other hand, gallic acid on methylation with dimethyl sulfate in acetone–K_2CO_3 yielded the fully protected ester **31**, which on selective demethylation with $AlCl_3$–CH_2Cl_2 system[12] yielded phenolic ester **32**. Compound **32** reacted with bromo esters

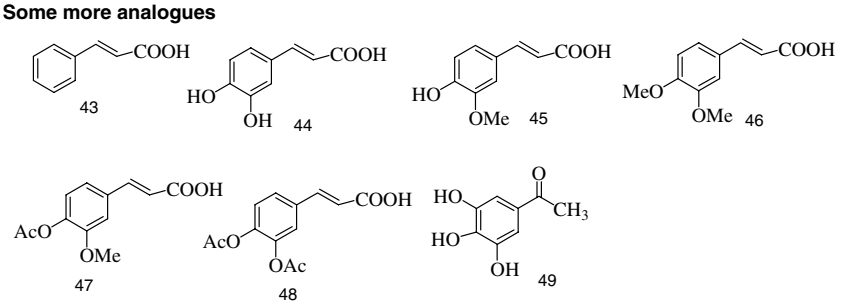


Figure 3. Some more analogues of Oenostacin.

(ethyl bromo butyrate/ethyl bromo crotonate) to afford the desired products **33** and **34**, respectively. Basic hydrolysis treatment on esters **33** and **34** afforded the acids **35** and **36**, respectively.

In Scheme 5, 2,3,4-trimethoxy benzaldehyde (**37**) and 3,4,5-trimethoxy benzaldehyde (**38**) underwent Wittig reaction using the similar reaction conditions as described earlier in Scheme 1 to furnish the product 2,3,4-trimethoxy cinnamic acid (**39**) and 3,4,5-trimethoxy cinnamic acid (**40**), respectively. Compounds **39** and **40** were demethylated in boron tribromide–CH_2Cl_2 system to get corresponding trihydroxy cinnamic acid derivatives **41** and **42**, respectively.

Besides the above derivatives, a few more compounds as shown in Figure 3 (few are commercial samples and others are their derivatives)[14a,b] were used for biological activity studies to establish structure and activity relationship of Oenostacin **1**.

2.2. Biological evaluation

All the analogues were screened for in vitro antibacterial activity against *S. epidermidis* and *S. aureus* following the method described by Petersdorf et al.[13] Compounds having EC_{50} values beyond 5 μM concentrations were considered inactive. The EC_{50} values were means of three experiments in triplicate. Only active analogues have been shown in Table 1. Amongst these **22**, **23** and **42** possessed higher level of antibacterial activity against both the strains and hence are best out of all the analogues of Oenostacin **1**. Some of the analogues **4**, **21**, **26** and **49** possessed moderate level of activity, while others **2**, **19**, **20** and **44** showed low level of antibacterial activities. However, none of these analogues was found equipotent to Oenostacin **1**.

3. Summary and conclusion

More than forty analogues have been synthesized and evaluated for antibacterial activities. From these studies, it revealed that the presence of a free phenolic group is an essential requisite for showing activity, while their protection renders it inactive. When an aldehyde group was introduced in the aromatic ring, antibacterial activity was increased. On reducing aldehyde to corresponding alcohol, bioactivity was further enhanced. The position of the phenolic hydroxyl in the ring has no effect on biological activity. Replacement of methyl group in the ring with a carboxylic group had no significant impact on the bioactivity. Increasing the side-chain length from C1 to C3 did not show much effect on the biological activity. Several analogues possessed very good activity but none of the analogues was found as active as Oenostacin **1**.

In conclusion, inspired by fascinating chemical structure and potent antibacterial activity of Oenostacin **1**, we developed a new family of non-natural analogues. The present study provided some insight into the essential structural features of Oenostacin **1**. The above studies will be helpful for further lead optimization of Oenostacin.

Table 1. Antibacterial activity of some of the active analogues of Oenostacin against *S. epidermidis* and *S. aureus*

S. No.	Compound	*S. epidermidis* EC_{50} (μM)	*S. aureus* EC_{50}(μM)
1.	**1**, Oenostacin	0.12	0.12
2.	**2**	4.03	4.03
3.	**4**	1.64	1.64
4.	**19**	2.38	4.76
5.	**20**	2.55	Inactive
6.	**21**	1.37	Inactive
7.	**22**	0.67	0.67
8.	**23**	0.99	0.49
9.	**26**	1.04	2.08
10.	**42**	0.63	1.26
11.	**44**	2.78	2.78
12.	**49**	1.48	2.96

*Analogues possessing antibacterial activity higher than 5 μM considered being inactive.

4. Experimental

4.1. Materials and methods

Melting points (mp) were determined on a JSGW melting point apparatus and are uncorrected. FT-IR spectra were recorded in KBr on Perkin-Elmer AC-1 spectrometer. ^1H NMR and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz, respectively, on a Bruker Avance DRX-300 spectrometer. Chemical shifts are given in δ values, downfield from the TMS as internal standard. All the ^1H and a few ^{13}C spectra are reported. Coupling constant (J) values are given in Hz. Electrospray mass spectra were recorded on API-3000, LC-Ms/Ms (Applied Biosystem) after dissolving the compounds in acetonitrile. FAB mass was done on a JEOL SX 102/DA-6000 Mass spectrometer using argon as the FAB gas and *m*-Nitrobenzyl alcohol as the matrix. All the solvents and reagents were of LR/AR grade. Dry solvents were prepared as per standard methods. Reactions were monitored on Merck aluminium thin layer chromatography (TLC, UV_{254nm}) plates. Visualization was accomplished either on UV chamber (254 nm and 320 nm) or by spraying TLC plates with 2% ceric sulfate in 10% aqueous sulfuric acid solution and charring them at higher temperatures (100–120 °C). Column chromatography was carried out on silica gel (60–120 mesh, Merck chemicals). Elemental analysis was carried out in Heraus CHN analyzer.

4.2. Syntheses

4.2.1. General procedure for the synthesis of compounds 5 and 18

4.2.1.1. Synthesis of 4-methyl, 2,6-dimethoxy benzaldehyde (5). In a 25 ml round-bottomed-flask 3,5 dimethoxy toluene **3** (100 mg, 0.65 mmol) was taken in dry DMF (0.1 ml, 1.29 mmol). The reaction flask was kept in an ice-bath (0–10 °C). To this stirred reaction mixture, phosphorus oxychloride (0.1 ml, 1.07 mmol) was

added dropwise. The reaction mixture was further kept for 30 min in the cooling bath and then heated at 80 °C for 3 h. On completion, the reaction mixture was slowly poured into ice-cold water and then it was made alkaline with 10% aqueous NaOH to precipitate the desired aldehyde. It was filtered and recrystallized from chloroform–hexane (1:3 v/v) to get **5** as creamish white solid (62% yield).

4.2.2. General procedure for the synthesis of compounds 6, 39 and 40. All the Wittig salts were prepared by taking the corresponding alkyl halide with triphenyl phosphine in dry toluene under refluxing condition for 1–2 h.

4.2.2.1. Synthesis of 3-(2,6-dimethoxy-4-methylphenyl)-acrylic acid (6). In a 25 ml round-bottomed-flask 4-methyl, 2, 6-dihydroxy benzaldehyde **5** (300 mg, 1.66 mmol), dry DMSO (2 ml) and sodium hydroxide (250 mg, 6.25 mmol) were stirred at room temperature. After 20 min of stirring, Wittig salt was added and the reaction mixture was further stirred overnight (~16 h). Later the reaction mixture was poured into water, acidified with 10% dil HCl and extracted with ethyl acetate. The organic layer was washed with water and dried over anhydrous sodium sulfate. The solvent was evaporated get a crude residue. Further purification was done on a silica gel column eluting with chloroform–methanol to get the desired compound **6** as white crystalline solid.

Compound **6**: Yield = 32%; mp = 164–165 °C, IR (KBr, cm^{-1}): 2952, 2595, 1654, 1508; ^1H NMR (300 MHz, $CDCl_3$): δ 2.38 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 6.28 (d, 1H, aromatic, $J = 2.17$ Hz), 6.32 (d, 1H, aromatic, $J = 2.10$ Hz), 6.56–6.61 (d, 1H, =C*H*-CO, $J = 16.05$ Hz), 7.88–7.93 (d, 1H, C*H*=, $J = 16.02$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.48, 55.48, 55.54, 96.59, 108.11, 115.53, 119.15, 139.68, 141.85, 161.44, 161.68, 171.25; Electrospray Mass (CH_3CN): 223.1 $[M+H]^+$; Elemental analysis Calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Obsd: C, 65.12; H, 6.23.

Compound **39**: Yield = 48%; mp = 162–164 °C, IR (KBr, cm^{-1}): 2570, 1694, 1619, 1498, 1590; ^1H NMR (300 MHz, $CDCl_3$): δ 3.88 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 6.41–6.46 (d, 1H, =C*H*CO, $J = 16.08$ Hz), 6.69–6.72 (d, 1H, aromatic, $J = 8.79$ Hz), 7.28–7.31 (d, 1H, aromatic, $J = 8.79$ Hz), 7.95–8.01 (d, 1H, C*H*=, $J = 16.08$ Hz); Electrospray Mass (CH_3CN): 239.1 $[M+H]^+$, 261.2 $[M+Na]^+$; Elemental analysis Calcd for $C_{12}H_{14}O_5$: C, 60.50; H, 5.92. Obsd: C, 60.72; H, 6.23.

4.2.3. Synthesis of 1,5 Di [(2,6-dimethoxy, 4-methyl phenyl)]-penta 1,4-dien, 3-one (7). In a 100 ml round-bottomed-flask 2,6-dimethoxy, 4-methyl benzaldehyde (**5**, 100 mg, 0.56 mmol) was taken in ethanol (2 ml). To this stirred solution sodium hydroxide (4 mg, 0.1 mmol) and acetone (20 mg, 0.34 mmol) were added and further stirred at room temperature for 72 h. On completion of the reaction a yellow solid mass was precipitated in the reaction mixture. It was filtered, washed with alcohol and recrystallized with chloroform–acetone to get **7** as an oil.

Yield = 62%; oil, ^1H NMR (300 MHz, $CDCl_3$): δ 2.44 (s, 6H, 2× CH_3), 3.83 (s, 6H, 2× OCH_3), 3.89 (s, 6H, 2× OCH_3), 6.37–6.40 (d, 4H, aromatic, $J = 9$Hz), 7.23–7.28 (d, 2H, 2×=C*H*CO, $J = 16.2$ Hz), 7.91–7.97 (d, 2H, 2× C*H*=, $J = 16.2$ Hz); Electrospray Mass (CH_3CN): 383.4 $[M+H]^+$, 405.3 $[M+Na]^+$, 421.2 $[M+K]^+$, 765.5 $[2M+H]^+$, 803.6 $[2M+K]^+$; Elemental analysis Calcd for $C_{23}H_{26}O_5$: C, 72.23; H, 6.85. Obsd: C, 72.02; H, 6.93.

Compound **22**: Yield = 79%; oil, ^1H NMR (300 MHz, acetone d_6): δ 2.77 (s, 2H, CH_2OH), 6.57 (d, 1H, aromatic, $J = 1.87$ Hz), 6.63 (d, 1H, aromatic, $J = 1.88$ Hz), 8.82 (s, 1H, exchangeable, phenolic O*H*), 9.13 (s, 1H, exchangeable, phenolic O*H*); Electrospray Mass (CH_3CN): 184.2 $[M]^+$, 369.0 $[2M+H]^+$.

4.2.4. General procedure for the synthesis of compounds 9, 12, 13 and 25

4.2.4.1. Synthesis of 5-(2,6-Dimethoxy-4-methyl-phenyl)-5-oxo-pentanoic acid (9). In a 25 ml round-bottomed-flask glutaric anhydride (506 mg, 3.84 mmol) was stirred with dichloromethane (5 ml.) and anhydrous aluminium chloride (506 mg, 3.79 mmol). It was stirred for 20 min and then to this 1,3-dimethoxy 5-methyl benzene **3** (500 mg, 3.29 mmol) was added in portions. The reaction mixture was stirred overnight (18 h) at room temperature. To this 5% dil HCl (5 ml) was added and extracted with dichloromethane (3× 25 ml). Organic layer was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The residue thus obtained was purified through silica column and eluted with chloroform–acetone. Compound **9** was obtained at 3% acetone–$CHCl_3$ (v/v) as an oil.

Yield = 32%; oil, ^1H NMR (300 MHz, $CDCl_3$): δ 1.88-1.97 (quintet, 2H, CH_2CH_2COOH, $J = 7.23$ Hz), 2.27 (s, 3H, CH_3), 2.30–2.36 (t, 2H, CH_2COOH), 2.69–2.74 (t, 2H, –CO–CH_2–, $J = 7.04$ Hz), 3.69 (s, 3H, 2× OCH_3), 6.28 (s, 2H, aromatic protons); Electrospray Mass (CH_3CN): 289.1 $[M+Na]^+$; Elemental analysis Calcd for $C_{14}H_{18}O_5$: C, 63.16; H, 6.77. Obsd: C, 62.72; H, 7.13.

4.2.4.2. Synthesis of 2-(4-Carboxy butyryl)-3,5-dimethoxy-benzoic acid methyl ester (12) and 2-(4-Carboxy-1-hydroxy-but-1-enyl)-3,5-dimethoxy-benzoic acid (13). In a 25 ml round-bottomed-flask glutaric anhydride (175 mg, 1.54 mmol) was stirred with dichloromethane (5 ml) and anhydrous aluminium chloride (210 mg, 1.57 mmol). It was stirred for 20 min and then to this 3,5-dimethoxy benzoic acid methyl ester **11** (250 mg, 1.28 mmol) was added in portions. The reaction mixture was stirred overnight (16–18 h) at room temperature. To this 5% dil HCl (5 ml) was added and extracted with dichloromethane (3× 25 ml). Organic layer was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The residue thus obtained was purified through silica column and eluted with chloroform–acetone. Compound **12** was obtained at 1% acetone–$CHCl_3$ and **13** was obtained at 2% acetone–$CHCl_3$ to get both crystalline solids.

Compound **12**: Yield = 45%; mp = 107–109 °C, IR (KBr, cm^{-1}): 2954, 2843, 1689, 1712, 1508; ^1H NMR

(300 MHz, $CDCl_3$): δ 2.03–2.10 (distorted quintet, 2H, CH_2–CH_2COOH), 2.49–2.54 (t, 2H, CH_2COOH, J = 7.08 Hz), 2.84–2.89 (t, 2H, CH_2 benzylic, J = 6.81 Hz), 3.78 (s, 3H, COOCH_3), 3.84 (s, 6H, 2× OCH_3), 6.63 (s, 1H, aromatic proton), 7.02 (s, 1H, aromatic proton); ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.1, 33.3, 43.19, 52.58, 55.99, 56.41, 103.45, 106.51, 127.08, 130.32, 157.78, 161.36, 166.69, 178.81, 204.34; Electrospray Mass (CH_3CN): 311.2 $[M+H]^+$, 333.3 $[M+Na]^+$, 349.1 $[M+K]^+$; Elemental analysis Calcd for $C_{15}H_{18}O_7$: C, 58.06; H, 5.85. Obsd: C, 57.86; H, 6.03.

Compound **13**: Yield = 18%; mp = 148–152 °C, IR(KBr, cm^{-1}): 3215, 2950, 1719, 1653, 1605, 1507; ^{1}H NMR (300 MHz, pyridine d_5): δ 2.57–2.62 (t, 2H, CH_2-COOH of lactone ring, J = 7.2 Hz), 2.73–2.80 (m, 2H, CH_2CH$_2$COOH of lactone ring, J = 7.3 Hz), 3.87 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 5.78–5.83 (t, 1H, =CH–CH$_2$ lactone ring), 6.69 (d, 1H, aromatic proton, J = 1.64 Hz), 6.71 (d, 1H, aromatic proton, J = 1.66 Hz); ^{13}C NMR (75 MHz, pyridine d_5): δ 22.73, 34.79, 49.84, 56.23, 56.30, 99.58, 105.89, 110.37, 121.71, 127.91, 145.34, 156.29, 163.32, 167.23, 175.28; Electrospray Mass (CH_3CN): 279.2 $[M+H]^+$, 301.1 $[M+Na]^+$, 317.0 $[M+K]^+$. Elemental analysis Calcd for $C_{14}H_{14}O_6$: C, 60.43; H, 5.04. Obsd: C, 60.03; H, 5.26.

Compound **25**: Yield = 22%; oil, IR (neat, cm^{-1}): 2940, 1735, 1654, 1508, 1631; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.03–2.13 (m, 2H,CH_2CH$_2$COOH), 2.49–2.53 (t, 2H, CH_2COOH, J = 7.00 Hz), 3.02–3.06 (t, 2H, –COCH_2-CH$_2$, J = 7.08 Hz), 3.89 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 6.48–6.51(d, 1H, aromatic, J = 9.03 Hz), 7.52–7.55 (d, 1H, aromatic, J = 9.03 Hz); Electrospray Mass (CH_3CN): 291.2 $[M+K]^+$.

4.2.5. Synthesis of 2-(4-carboxy-butyl)-3,5-dimethoxy-benzoic acid methyl ester (17). In a 25 ml round-bottomed-flask 2-(4-carboxy butyryl)-3,5-dimethoxy-benzoic acid methyl ester **12** (100 mg, 0.34 mmol) was taken in 2 ml trifluoroacetic acid (TFA). The reaction mixture was kept in an ice-bath (0–10 °C). To this stirred reaction mixture, sodium borohydride (40 mg, 1.05 mmol) was added in portions to avoid excessive heat. It was stirred for 4 h at this temperature and then at room temperature for an hour. The reaction mixture was slowly poured into ice and acidified (5% HCl). It was then extracted with ethyl acetate (3× 25 ml) and washed with water. The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue thus obtained was purified through column chromatography over silica gel and eluted with chloroform–acetone and chloroform–methanol. The desired product **17** was obtained at 5% acetone–$CHCl_3$ as a white crystalline solid.

Yield = 42%; mp = 125–130 °C, IR (KBr, cm^{-1}): 3420, 2948, 1773, 1713, 1503, 1620; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.73–1.81 (m, 2H, CH_2CH$_2$COOH), 1.85–1.95 (m, 2H, CH_2CH$_2$CH$_2$COOH), 2.57–2.62 (t, 2H, CH_2COOH, J = 7.45 Hz), 3.00–3.05 (t, 2H, benzylic, J = 7.62 Hz), 4.00 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 4.08 (s, 3H, COOCH_3), 6.77 (d, 1H, Aromatic, J = 2.38 Hz), 7.08 (d, 1H, aromatic, J = 2.46 Hz); ^{13}C NMR(75 MHz, $CDCl_3$): δ 25.2, 26.07, 30.11, 34.36, 52.29, 55.79, 56.10, 102.56, 105.69, 125.54, 132.12, 158.64, 159.31, 168.83, 179.97; Electrospray Mass (CH_3CN): 297.1 $[M+H]^+$; Elemental analysis Calcd for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80. Obsd: C, 61.02; H, 6.63.

4.2.6. General procedure for the synthesis of compounds 14, 15, 16 and 19

4.2.6.1. Synthesis of 2-(4-carboxy-butyryl)-3,5-dimethoxy-benzoic acid (14), 2-(4-carboxy-butyryl)-3-hydroxy,5-methoxy-benzoic acid (15) and 2-(4-carboxy-butyryl)-5-hydroxy, 3-methoxy benzoic acid (16). In a 25 ml round-bottomed-flask **12** (200 mg, 0.64 mmol) was taken in dry dichloromethane (10 ml). To this stirring solution anhydrous aluminium chloride (800 mg, 5.99 mmol) was added and stirred overnight at room temperature. On completion of reaction dil HCl was added dropwise to it and stirred for 10 min. It was extracted with dichloromethane and washed with water. The organic layer was dried over anhydrous sodium sulfate and evaporated to get a residue. The residue thus obtained was purified through a column of silica gel to get **14**, **15** and **16** at 2% methanol–$CHCl_3$ one by one in the ratio 2:3:1, respectively.

Compound **14**: Yield = 23%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.84–1.94 (quintet, 2H, CH_2CH$_2$COOH, J = 7.16 Hz), 2.32–2.37 (t, 2H, CH_2COOH, J = 7.33 Hz), 2.76–2.81 (t, 2H, CH_2CO–, J = 7.0 Hz), 3.73 (s, 3H, OCH_3), 3.75(s, 3H, OCH_3), 6.61 (d, 1H, aromatic, J = 1.0 Hz), 6.83 (d, 1H, aromatic, J = 1.0 Hz); Electrospray Mass (CH_3CN): 297.1 $[M+H]^+$; Elemental analysis Calcd for $C_{14}H_{16}O_7$: C, 56.76; H, 5.40. Obsd: C, 57.02; H, 5.88.

Compound **15**: Yield = 38%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.86–1.93 (quintet, 2H, CH_2CH$_2$COOH, J = 7.16 Hz), 2.28–2.33 (t, 2H, CH_2COOH, J = 7.37 Hz), 2.78–2.83 (t, 2H, CH_2CO–, J = 7.03 Hz), 3.72 (s, 3H, OCH_3), 6.39 (s, 1H, aromatic), 6.65 (s, 1H, aromatic); Electrospray Mass (CH_3CN): 283.1 $[M+H]^+$; Elemental analysis Calcd for $C_{13}H_{14}O_7$: C, 55.32; H, 4.96. Obsd: C, 54.89; H, 5.36.

Compound **16**: Yield = 12%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.99–2.04 (broad triplet, 2H, CH_2CH$_2$COOH), 2.39–2.44 (t, 2H, CH_2COOH), 2.74–2.79 (t, 2H, CH_2CO–), 3.83 (s, 3H, OCH_3), 3.91(s, 3H, –COOCH_3), 6.52 (s, 1H, aromatic), 6.68 (s, 1H, aromatic); Electrospray Mass (CH_3CN): 297.1 $[M+H]^+$; Elemental analysis Calcd for $C_{14}H_{16}O_7$: C, 56.76; H, 5.40. Obsd: C, 56.44; H, 5.89.

4.2.6.2. Synthesis of 2-formyl, 3-methoxy, 4-hydroxy benzoic acid methyl ester (19). In a 25 ml round-bottomed-flask 2-formyl, 3,5-dimethoxy-benzoic acid methyl ester (**18**, 100 mg, 0.45 mmol) was taken in dry dichloromethane (6 ml). To this stirring solution anhydrous aluminium chloride (200 mg, 1.6 mmol) was added and stirred overnight at room temperature. On completion of reaction dil HCl was added dropwise to it and stirred for 10 min. It was extracted with dichloromethane and washed with water. The organic layer was dried over anhydrous sodium sulfate and evaporated to

get a residue. It was passed through a small column of silica gel to get **19** at hexane/$CHCl_3$ (1:1 v/v) as creamish white solid.

4.2.7. General procedure of the synthesis of compounds 8, 21, 26, 41 and 42

4.2.7.1. Synthesis of 3-(3,4,5-trihydoxyphenyl)-acrylic acid (42). In a 25 ml round-bottomed-flask 3,4,5-trimethoxy cinnamic acid **40** (50 mg, 0.21 mmol) was taken in dry dichloromethane (5 ml). The reaction mixture was kept in acetone bath and cooled to −78 °C with liquid nitrogen. To the chilled reaction mixture boron tribromide (0.12 ml, 12 mmol) was added dropwise and further stirred for 2 h at this temperature. Then the reaction mixture was stirred overnight at room temperature (16 h). On completion, the reaction mixture was poured into water and dil HCl (10%, 5 ml) was added to it. It was extracted with chloroform, organic layer was washed with water, dried over anhydrous sodium sulfate and evaporated. The residue thus obtained was recrystallized with $CHCl_3$–MeOH (3:1 v/v) to get desired demethylated product **42** as light brown crystalline solid.

Yield = 62%; mp = 180–182 °C, IR (KBr, cm^{-1}): 3277, 1702, 1641, 1613, 1539; ^1H NMR (300 MHz, CD_3OD): δ 6.14–6.19 (d, 1H, =C*H*CO, J = 15.81 Hz), 6.59 (s, 2H, aromatic), 7.41–7.46 (d, 1H, C*H* = , J = 15.81 Hz); Electrospray Mass (CH_3CN): 197.0 $[M+H]^+$, 415.2 $[2M+Na]^+$; Elemental analysis Calcd for $C_9H_8O_5$: C, 55.11; H, 4.11; Obsd: C, 54.92; H, 4.23.

4.3. Biological evaluation

4.3.1. Antibacterial bioassay

4.3.1.1. Estimation of minimum inhibitory concentration (MIC). Twofold serial dilution technique was used to assess the minimal inhibitory concentration (MIC) of a test compound against the bacterial strains. In a series of eight tubes serial dilutions was made. In first tube, 2 ml of nutrient broth was taken and in subsequent tube 1 ml of broth was taken after that, test compound of known concentration was added in first tube and mix properly. From the first tube 1 ml of broth containing antibiotic was taken and added to the second tube and mixed properly. This was repeated until the seventh tube. 1 ml of mixture was expelled out from the last tube. Only broth culture was used as a control. To each of this tube, 10 μl of properly diluted log phase culture of test organism with a titre of 10^4 cfu/ml was added. The tubes were incubated at 37 °C and examined by turbidity measurement. The MIC was the lowest concentration of test compound inhibiting the development of visible growth.

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