Chapter 4

Synthesis and Antibacterial Activity of Diverse Analogues of Oenostacin
Chapter 4  Synthesis and Antibacterial Activity of Diverse analogues of Oenostacin

4.1 Introduction

*Oenothera biennis* is a species of *Oenothera* native to eastern and central North America, from Newfoundland west to Alberta, southeast to Florida, and southwest to Texas, and widely naturalized elsewhere in temperate and subtropical regions, also cultivated in Indian gardens.\(^1\) It is also known as weedy evening-primrose, German rampion, hog weed, king's cure-all and fever-plant. A number of compounds have been isolated from its aerial parts.\(^2\) A potent antibacterial compound oenostacin\(^3\) was isolated during the systematic investigation of its roots along with several other compounds.\(^4\) Oenostacin (1), has shown potent antibacterial activity against *streptococci* and *staphylococci*, especially *Staphylococcus epidermidis* and *Staphylococcus aureus*. Multidrug-resistant gram-positive bacteria have continued to pose challenges to medicinal research fraternity.\(^5\) *S. aureus*, known as one of the most successful opportunistic human pathogens, is responsible for postoperative wound infections, bacteraemia, pneumonia, osteomyelitis, mastitis, acute endocarditic and deep abscesses in various organs.\(^6\) *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.\(^7\) Oenostacin was found active in 0.12\(\mu\)M concentration (EC\(_{50}\)) against *S. epidermidis* and *S. aureus*.

4.2 Approach of work

In view of promising antibacterial activities of oenostacin (1, Figure 1), we became curious to study the efficacy of various analogues of oenostacin and to establish its structure activity relationship (SAR). Also, this could give us some possible antibacterial lead compounds of pharmacological spectrum of oenostacin, to evaluate their biological activities against *S. epidermidis* and *S. aureus*. 179
Chapter 4  Synthesis and Antibacterial Activity of Diverse analogues of Oenostacin

In order to establish its SAR, we planned to have various modifications in the phenyl ring, positions of phenolic hydroxyls, carboxylic acid group and other possibilities. We also planned to have various chains attached to it.

Figure 1: Oenostacin (1)

4.3 Synthetic Strategies

Different analogues were synthesized starting with orcinol (2) (Scheme 1). Orcinol underwent Reimer Tiemann reaction with CHCl₃—aqueous alkali system to produce the aldehyde 4 in very low yield, which upon methylation with dimethyl sulfate in acetone gave 2,6-dimethoxy-4-methyl benzaldehyde (5). Due to poor yields in this method, the reaction pathway was modified through methylation of orcinol (2) with dimethyl sulfate, potassium carbonate and acetone under refluxing condition followed by Vilsmeier Haack formylation⁸ using dimethylformamide and phosphorus oxychloride (DMF-POCl₃) at 80°C to obtain compound 5 in 62% yield. Wittig reaction of aldehyde 5 with triphenylphosphonium ethyl acetate bromide (Wittig salt) in DMSO and sodium hydroxide at room temperature to produce the corresponding cinnamic acid derivative (6) in 32% yield. It was then demethylated by stirring with boron tribromide in dichloromethane at very low temperature to get compound 8 in 42% yield.

Aldehyde 5 on stirring with acetone in 1% alcoholic KOH at room temperature yielded compound 7 in 62% yield. Friedel Craft acylation of compound 3 with glutaric anhydride in dichloromethane and aluminium chloride at room temperature to give compound 9 in 24% yield.⁹
Scheme 1. Reagents and conditions: (i) CHCl₃-aq KOH (30%), reflux, 4h, 14%; (ii) Me₂SO₄, K₂CO₃, dry acetone, reflux, 3h, 87%; (iii) Me₂SO₄, K₂CO₃, acetone, reflux, 3h, 93%; (iv) DMF, POCI₃, 0°C for 30 min then RT for 3h, 62%; (v) DMSO, NaOH, (Ph₃P⁺=CHCOOC₂H₅)Br, RT, overnight, 32%; (vi) 1% ethanolic KOH, acetone, RT, 50-60h, 62%; (vii) BBr₃, DCM, -78°C for 1h then RT, 42%; (viii) glutaric anhydride, DCM, AlCl₃, RT, overnight, 24%.

In scheme 2, the starting compound 3,5-dihydroxybenzoic acid (10) was first methylated with dimethyl sulfate in acetone under refluxing condition to obtain trimethylated product 11 in 93% yield. Compound 11 on Friedel-Crafts acylation with glutaric anhydride in presence of anhydrous aluminium chloride at room temperature in dichloromethane gave a mixture of both tautomeric products keto (12) and enol (13) in lactone form. These were carefully separated through silica gel column chromatography in 45% and 18% yield, respectively. Compound 12 was demethylated with anhydrous aluminium chloride in dichloromethane producing deprotected products 14, 15 and 16 in 2:3:1 ratio. Keto group of compound 12 was reduced in the presence of trifluoroacetic
acid and sodium borohydride to produce compound 17 in 42% yield. Ester 11 on Vilsmeier Haack reaction with DMF and POCl₃ yielded the aldehyde 18, where the aldehydic group was unexpectedly introduced at C-2 position of the ring. Methoxy groups of compound 18 were fully deprotected with boron tribromide in dichloromethane at -78°C to furnish fully deprotected product 21 in 24% yield, which on reduction with sodium borohydride in methanol yielded corresponding alcohol (22) in 79% yield. On the other hand, compound 18 was selectively demethylated with AlCl₃ in dichloromethane to get mono demethylated ester 19, which on further hydrolysis yielded acid 20 in 52% yield.

In Friedel Crafts reaction of ester 11 with glutaric anhydride, we were expecting the attachment of keto acid chain at C-4 position (i.e., between both the methoxy group), but unexpectedly, it was attached at C-2 position leading to a mixture of compound 12, a keto acid and 13, a lactone of enol acid chain. The structural assignments have been confirmed by various NMR experiments and mass spectral data.
Scheme 2. Reagents and conditions: (i) Me$_2$SO$_4$, K$_2$CO$_3$, acetone, reflux, 3h, 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 4h then RT, 42%; (v) DMF, POCl$_3$, 0°C for 30 min then RT for 4h, 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, 1h, 52%.

Different 1D/2D-NMR experiments on compound 12 and 13 have confirmed the side-chain attachment at C-2 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 C-2 position only. All the carbon resonances of $^{13}$C NMR coupled with DEPT editing experiments
were well in agreement with the proposed structure for compound 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 compound 13 and one of its aromatic protons attached to C-6 position confirmed the side-chain attachment unambiguously at C-2 position of aromatic ring. Similarly, a two-bond correlation between C-3 and a proton attached to C-4 (as well as two such similar correlations between C-5 and protons attached to C-4 and C-6, respectively) further supported the proposed side-chain attachment of compound 13. 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 compound 13, the two peaks 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. Lactone moiety in compound 13 was further confirmed by IR (a strong band at 1768 cm$^{-1}$, typical for a lactone carbonyl) and Mass spectral data as ESI-MS showed mass peaks at 279 [M+H]$^+$ and 301 [M+Na]$^+$.

**Scheme 3**

Scheme 3. Reagents and conditions: (i) Me$_2$SO$_4$, K$_2$CO$_3$, acetone, reflux, 3h, 89%; (ii) glutaric anhydride, DCM, AlCl$_3$, RT, overnight, 22%; (iii) BBr$_3$, DCM, -78°C for 1h then RT, 58%.
Chapter 4  Synthesis and Antibacterial Activity of Diverse analogues of Oenostacin

In scheme 3, pyrogallol (23) was used as starting material. It was first methylated to trimethoxy benzene (24) in the presence of dimethyl sulphate and acetone in refluxing condition in 89% yield. Trimethoxy pyrogallol (24) on Friedel Craft reaction with glutaric anhydride in DCM and aluminium chloride furnished compound 25 in 22% yield, which was further demethylated with boron tribromide to furnish the phenolic keto acid (26).

Scheme 4

![Diagram of chemical reactions](image)

Scheme 4. Reagents and conditions: (i) DMSO, KOH, (Ph₃P⁺=CH-COOC₂H₅)Br⁻, overnight, 29: 48%, 30: 54%; (ii) BB₃, DCM, -78°C for 30 min then RT, 31: 49%, 32: 62%.

In scheme 4, 2,3,4-trimethoxy benzaldehyde (27) and 3,4,5-trimethoxy benzaldehyde (28) underwent Wittig reaction in the presence of DMSO, potassium hydroxide and Wittig salt (Ph₃P⁺=CH-COOC₂H₅ Br⁻) at room temperature to furnish 2,3,4-trimethoxy cinnamic acid (29) and 3,4,5-trimethoxy cinnamic acid (30) in 48% and 54% yield, respectively. These two trimethoxy cinnamic acids (29 & 30) were further demethylated in the presence of boron tribromide and dichloromethane to get corresponding trihydroxy cinnamic acid derivative 31 and 32, respectively.
4.4 Biological evaluation

4.4.1 Antibacterial bioassay

All the compounds were screened for in vitro antibacterial activity against S. epidermidis and S. aureus following the method described by Petersdorf et al.\textsuperscript{12} Oenostacin was taken as positive standard.

**Estimation of minimum inhibitory concentration:** Twofold serial dilution technique was used to assess the minimal inhibitory concentration (MIC) of test compounds against the bacterial strains. In a series of eight tubes, serial dilutions were made. In first tube, 2mL of nutrient broth was taken and in subsequent tube 1mL of broth was taken after that, test compound of known concentration was added in first tube and mixed properly. From the first tube 1mL of broth containing antibiotic was taken and added to the second tube and mixed properly. This was repeated until the seventh tube. 1mL of mixture was expelled out from the last tube. Only broth culture was used as a control. To each of this tube, 10μL 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 compounds inhibiting the development of visible growth. Various concentration of tested compounds were used and inhibition against these concentration were recorded, after plotting a graph 50% inhibition was calculated (EC\textsubscript{50}) which is the effective concentration for the inhibition of 50% of the bacterial growth. The EC\textsubscript{50} values were mean of three experiments in triplicate.
4.5 Results and discussion

More than thirty analogues have been synthesized and evaluated for their antibacterial activity against *S. epidermidis* and *S. aureus* (Table 1). Among these compounds 22, 23 and 32 possessed higher level of activity against both the bacterial strains and hence are best out of all the analogues of oenostacin (1). Some of the analogues 4, 21 and 26 possessed moderate level of activity, while other 2, 19 and 20 showed low level of antibacterial activities. However, none of these analogues was found equipotent to oenostacin.

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

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Compound</th>
<th><em>S. epidermidis</em> EC\textsubscript{50}(\mu M)</th>
<th><em>S. aureus</em> EC\textsubscript{50}(\mu M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1 (Oenostacin)</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>2.</td>
<td>2</td>
<td>4.03</td>
<td>4.03</td>
</tr>
<tr>
<td>3.</td>
<td>4</td>
<td>1.64</td>
<td>1.64</td>
</tr>
<tr>
<td>4.</td>
<td>19</td>
<td>2.38</td>
<td>4.76</td>
</tr>
<tr>
<td>5.</td>
<td>20</td>
<td>2.55</td>
<td>Inactive</td>
</tr>
<tr>
<td>6.</td>
<td>21</td>
<td>1.37</td>
<td>Inactive</td>
</tr>
<tr>
<td>7.</td>
<td>22</td>
<td>0.67</td>
<td>0.67</td>
</tr>
<tr>
<td>8.</td>
<td>23</td>
<td>0.99</td>
<td>0.49</td>
</tr>
<tr>
<td>9.</td>
<td>26</td>
<td>1.04</td>
<td>2.08</td>
</tr>
<tr>
<td>10.</td>
<td>32</td>
<td>0.63</td>
<td>1.26</td>
</tr>
</tbody>
</table>

*Analagues possessing antibacterial activity higher than 5\mu M considered as being inactive.*
4.5.1 Structure activity Relationship

From these studies it is revealed that the presence of a free phenolic group is an essential requirement for antibacterial 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).

4.6 Conclusion

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.
4.7 Experimental

General

All glass apparatus were oven dried prior to use. Melting points were determined on EZ-Melt Automated melting point apparatus-SRS Stanford Research System U.S.A. and are uncorrected. $^1$H NMR and $^{13}$C NMR were recorded on Bruker Avance 300MHz spectrometer (300MHz for $^1$H NMR and 75MHz for $^{13}$C NMR) using CDCl$_3$/acetone $d_6$ as solvents. Tetramethylsilane ($\delta$ 0.00 ppm) was used as an internal standard in $^1$H NMR and CDCl$_3$ ($\delta$ 77.0 ppm) was used in $^{13}$C NMR. Chemical shifts are reported in $\delta$ units. Multiplicity is reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiples), b (broad) and dis (distorted). Coupling constants are reported as $J$ value in hertz. Infrared spectra (cm$^{-1}$) were recorded on Perkin-Elmer-Spectrum BX FT-IR system. Electron Ionization Mass Spectra (EI-MS) were obtained on Perkin-Elmer Autosystem XL Gas Chromatography coupled with Turbo Mass spectrometer and ESI-MS spectra were obtained on LC-MS-2010EV (Shimadzu, Japan) hyphenated to LC system (LC-20AD, CTO-20A, SIL-10AF, SPDM20A and ABM-20A). The progress of the reaction was monitored by thin layer chromatography on silica gel 60F$_{254}$ (Merck, Germany) readymade aluminium sheets with detecting agents: ultraviolet radiation (256nm and 365nm) and/or iodine vapors and/or spraying with 2% aqueous solution of ceric sulphate in 10% sulfuric acid followed by heating at 110°C. Chromatographic purification was performed over silica gel (60-120 or 100-200 Mesh) obtained from Merck (India). All chemicals and reagents were obtained from Aldrich (USA), Lancaster (UK) or Merck (India) Pvt. Ltd. and were used without further purification.
General procedure for the synthesis of compound 5 and 18:

**Synthesis of 4-methyl-2,6-dimethoxy benzaldehyde (5):** In a 25mL round bottom flask, 3,5-dimethoxy toluene (3, 100mg, 0.65mmol) was taken in dry DMF (0.1mL, 1.29mmol). The reaction flask was kept in an ice-bath (0-10°C). To this stirred reaction mixture, phosphorus oxychloride (0.1mL, 1.07mmol) was added dropwise. The reaction mixture was further stirred for 30min in the cooling bath and then heated at 80°C for 3h. 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 compound 5.

**4-Methyl-2,6-dimethoxy benzaldehyde (5):** Oil, yield = 62% (72mg); $^1$H NMR (300 MHz, CDCl$_3$): δ 2.50 (s, 3H, -CH$_3$), 3.77 (s, 3H, -OCH$_3$), 3.79 (s, 3H, -OCH$_3$), 6.23 (s, 2H, 3 and 5 -CH), 10.40 (s, 1H, -CHO).

**2-Formyl-3,5-dimethoxy benzoic acid methyl ester (18):** Oil, yield = 42%; $^1$H NMR (300 MHz, CDCl$_3$): δ 3.81 (s, 3H, -COOCH$_3$), 3.83 (s, 6H, 2x-OMe), 6.45 (d, 1H, -CH, $J = 1.5$ Hz), 6.49 (s, 1H, -CH), 10.23 (s, 1H, CHO).

General procedure for the synthesis of compounds 6, 29 and 30.

**Preparation of Wittig salt:** Ethyl bromoacetate (0.66mL, 1.0g, 5.58mmol) was taken in a 25mL round bottom flak, to this dry toluene (15mL) and triphenylphosphine (1.8g, 6.92mmol) were added. The reaction mixture was refluxed for 2h at 120°C. After completion of the reaction white solid was appeared in the reaction mixture which was filtered with benzene through a sintered funnel to get the Wittig salt in 55% (1.3g) yield.

**Synthesis of 3-(2,6-dimethoxy-4-methylphenyl)-acrylic acid (6):** In a 25mL round bottom flask 4-methyl-2,6-dihydroxy benzaldehyde (5, 300mg, 1.66mmol), dry DMSO (2ml) and sodium hydroxide (250mg, 6.25mmol) were stirred at room temperature. After
Chapter 4 Synthesis and Antibacterial Activity of Diverse analogues of Oenostacin

20 min of stirring, Wittig salt was added and the reaction mixture was further stirred for overnight (~16h). Later the reaction mixture was poured into water, acidified with dil HCl (1N) and extracted with ethyl acetate. The organic layer was washed with water and dried over anhydrous sodium sulfate. The solvent was evaporated to 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.

3-(2,6-Dimethoxy-4-methylphenyl)-acrylic acid (6): Solid; yield = 32% (117mg); mp. 164-165°C, IR (KBr, cm⁻¹): 2952, 2595, 1654, 1508; ¹H NMR (300 MHz, CDCl₃): δ 2.38 (s, 3H, -CH₃), 3.81 (s, 3H, -OCH₃), 3.89 (s, 3H, -OCH₃), 6.28 (d, 1H; aromatic, J = 2.17 Hz), 6.32 (d, 1H, aromatic, J = 2.10 Hz), 6.56-6.61 (d, 1H, =CH-CO, J = 16.05 Hz), 7.88-7.93 (d, 1H, CH=, J = 16.02 Hz); ¹³C NMR (75 MHz, CDCl₃): δ 21.48, 55.48, 55.54, 96.59, 108.11, 15.53, 119.15, 139.68, 141.85, 161.44, 161.68, 171.25; ESI-MS (CH₃CN, m/z): 223 [M+H]⁺.

3-(2,3,4-Trimethoxy)-acrylic acid (29): Solid; yield = 48%; mp. 162-164°C, IR (KBr, cm⁻¹): 2570, 1694, 1619, 1498, 1590; ¹H NMR (300 MHz, CDCl₃): δ 3.88 (s, 3H, OCH₃), 3.91 (s, 3H, OCH₃), 3.94 (s, 3H, OCH₃), 6.41-6.46 (d, 1H, =CHCO, 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, CH=, J = 16.08 Hz); ESI-MS (CH₃CN, m/z): 239 [M+H]⁺, 261 [M+Na]⁺.

3-(3,4,5-Trimethoxy)-acrylic acid (30): Oil; yield = 54%; ¹H NMR (300 MHz, CDCl₃): δ 3.90 (s, 9H, 3x-OCH₃), 6.34 (d, 1H, =CHCO, J = 15.9 Hz), 6.78 (s, 2H, 2x-CH), 7.68 (d, 1H, =CH, J = 15.9 Hz).

Synthesis of 1,5-di-[2,6-dimethoxy-4-methyl phenyl]-penta-1,4-dien,3-one (7). In a 100mL round bottom flask 2,6-dimethoxy-4-methyl benzaldehyde (5, 100mg, 0.56mmol) was taken in ethanol (2mL). To this stirred solution sodium hydroxide (4mg, 0.1mmol)
and acetone (20 mg, 0.34 mmol) were added and further stirred at room temperature for 72h. On completion of the reaction a yellow solid mass was precipitated in the reaction mixture. It was filtered and washed with alcohol and recrystallized with chloroform-acetone to get compound 7 as oil. Yield = 62% (132 mg); 1H NMR (300 MHz, CDCl3): δ 2.44 (s, 6H, 2x-CH3), 3.83 (s, 6H, 2x-OCH3), 3.89 (s, 6H, 2x-OCH3), 6.37-6.40 (d, 4H, aromatic, J = 9 Hz), 7.23-7.28 (d, 2H, 2x-CH=, J = 16.2 Hz), 7.91-7.97 (d, 2H, 2x-CH=, J = 16.2 Hz); ESI-MS (CH3CN, m/z): 383 [M+H]+, 405 [M+Na]+, 421 [M+K]+.

Synthesis of 3,5-dihydroxy-2-hydroxy-methyl benzoic acid (22): Compound 21 (100 mg, 0.54 mmol) in 25 mL round bottom flask, to this 15 mL methanol and sodium borohydride (25 mg, 0.65 mmol) were added and stirred for 1 h. After completion of the reaction methanol was evaporated, 10 mL water was added to it and extracted with ethyl acetate (3 x 10 mL). Combined organic layer was washed with water (2 x 10 mL) and dried over anhydrous Na2SO4 to obtain compound 22 as oil in 79% (78 mg) yield.

1H NMR (300 MHz, acetone d6): δ 2.77 (s, 2H, -CH2OH), 6.57 (d, 1H, aromatic, J = 1.87 Hz), 6.63 (d, 1H, aromatic, J = 1.88 Hz), 8.82 (s, 1H, exchangeable, phenolic -OH), 9.13 (s, 1H, exchangeable, phenolic -OH); ESI-MS (CH3CN, m/z): 184.2 [M]+, 369 [2M+H]+.

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

Synthesis of 5-(2,6-Dimethoxy-4-methyl-phenyl)-5-oxo-pentanoic acid (9). In a 25 mL round bottom flask glutaric anhydride (506 mg, 3.84 mmol) was stirred with anhydrous aluminium chloride (500 mg, 3.79 mmol) in dichloromethane (5 mL). 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 dil HCl (5 mL, 1 N) was added and extracted with dichloromethane (3 x 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 gel column and eluted with chloroform-acetone. Compound 9 was obtained at 3% acetone-CHCl₃ (v/v) as oil. Yield = 24% (280mg); oil, $^1$H NMR (300 MHz, CDCl₃): δ 1.88-1.97 (quintet, 2H, -CH₂CH₂COOH, $J = 7.23$ Hz), 2.27 (s, 3H, -CH₃), 2.30–2.36 (t, 2H, -CH₂COOH), 2.69–2.74 (t, 2H, –CO–CH₂–, $J = 7.04$ Hz), 3.69 (s, 3H, 2x-OCH₃), 6.28 (s, 2H, aromatic protons); ESI-MS (CH₃CN, m/z): 289 [M+Na]$^+$.

Synthesis of 2-(4-Carboxybutyryl)-3,5-dimethoxy-benzoic acid methyl ester (12), 2-(4-Carboxy-1-hydroxy-but-1-enyl)-3,5-dimethoxy-benzoic acid (13) and 5-(2,3,4-trimethoxyphenyl)-5-oxopentanoic acid (25). In a 25mL round bottom flask glutaric anhydride (175mg, 1.54mmol) was stirred with dichloromethane (5mL) and anhydrous aluminium chloride (210mg, 1.57mmol). It was stirred for 20min and then to this 3,5-dimethoxy benzoic acid methyl ester (11, 250mg, 1.28mmol) was added in portions. The reaction mixture was stirred overnight (~16-18h) at room temperature. To this dil HCl (5mL, 1N) was added and extracted with dichloromethane (3x-25mL). 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-CHCl₃ and compound 13 was obtained at 2% acetone-CHCl₃ as crystalline solids.

2-(4-Carboxybutyryl)-3,5-dimethoxy-benzoic acid methyl ester (12): Solid; yield = 45% (178mg); mp. 107-109°C, IR (KBr, cm$^{-1}$): 2954, 2843, 1689, 1712, 1508; $^1$H NMR (300 MHz, CDCl₃): δ 2.03-2.10 (distorted quintet, 2H, CH₂–CH₂COOH), 2.490-2.54 (t, 2H, -CH₂COOH, $J = 7.08$ Hz), 2.84-2.89 (t, 2H, CH₂CH₂-CH₂COOH, $J = 6.81$ Hz), 3.78 (s, 3H, -COOCH₃), 3.84 (s, 6H, 2x-OCH₃), 6.63 (s, 1H, aromatic proton), 7.02 (s, 1H, aromatic proton); $^{13}$C NMR (75 MHz, CDCl₃): δ 19.1, 33.3, 43.19, 52.58, 55.99, 56.41,
Chapter 4  Synthesis and Antibacterial Activity of Diverse analogues of Oenostacin

103.45, 106.51, 127.08, 130.32, 157.78, 161.36, 166.69, 178.81, 204.34; ESI-MS (CH₃CN, m/z): 311 [M+H]⁺, 333 [M+Na]⁺, 349 [M+K]⁺.

2-(4-Carboxy-1-hydroxy-but-1-enyl)-3,5-dimethoxy-benzoic acid (13): Solid; yield = 18% (64mg); mp. 148-152°C, IR (KBr, cm⁻¹): 3215, 2950, 1719, 1653, 1605, 1507; 1H NMR (300 MHz, pyridine d₅): δ 2.57-2.62 (t, 2H, -CH₂-CO of lactone ring, J = 7.2 Hz), 2.73-2.80 (m, 2H, -CH₂CH₂CO of lactone ring, J = 7.3 Hz), 3.87 (s, 3H, -OCH₃), 3.93 (s, 3H, -OCH₃), 5.78-5.83 (t, 1H, =CH–CH₂ lactone ring), 6.69 (d, 1H, aromatic proton, J = 1.64 Hz), 6.71 (d, 1H, aromatic proton, J = 1.66 Hz); 13C NMR (75 MHz, pyridine d₅): 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, 167.23, 175.28; ESI-MS (CH₃CN, m/z): 279 [M+H]⁺, 301 [M+Na]⁺, 317 [M+K]⁺.

5-(2,3,4-Trimethoxyphenyl)-5-oxopentanoic acid (25): Oil; Yield = 22%; IR (neat, cm⁻¹): 2940, 1735, 1654, 1508, 1631; 1H NMR (300 MHz, CDCl₃): δ 2.03-2.13 (m, 2H, -CH₂CH₂COOH), 2.49-2.53 (t, 2H, -CH₂COOH, J = 7.00 Hz), 3.02-3.06 (t, 2H, -COCH₂-CH₂, J = 7.08 Hz), 3.89 (s, 3H, -OCH₃), 3.93 (s, 3H, -OCH₃), 3.96 (s, 3H, -OCH₃), 6.48-6.51(d, 1H, aromatic, J = 9.03 Hz), 7.52-7.55 (d, 1H, aromatic, J = 9.03 Hz); ESI MS (CH₃CN, m/z): 291 [M+K]⁺.

Synthesis of 2-(4-carboxy-butyl)-3,5-dimethoxy-benzoic acid methyl ester (17): In a 25mL round bottom flask 2-(4-carboxy-butyl)-3,5-dimethoxy-benzoic acid methyl ester (12, 100mg, 0.34mmol) was taken in 2mL trifluoroacetic acid (TFA). The reaction mixture was kept in an ice-bath (0-10°C). To this stirred reaction mixture, sodium borohydride (40mg, 1.05mmol) was added in portions to avoid excessive heat. It was stirred for 4h 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 (3x25mL) and washed with water. The organic layer was dried over

194
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-ethanol. The desired product 17 was obtained at 5% acetone-CHCl₃ as a white crystalline solid. Yield = 42% (40mg); mp. 125-130°C, IR (KBr, cm⁻¹): 3420, 2948, 1773, 1713, 1503, 1620; ¹H NMR (300 MHz, CDCl₃): δ1.73-1.81 (m, 2H, -CH₂CH₂COOH), 1.85-1.95 (m, 2H, -CH₂CH₂CH₂COOH), 2.57-2.62 (t, 2H, -CH₂COOH, J = 7.45 Hz), 3.00-3.05 (t, 2H, benzylic, J = 7.62 Hz), 4.00 (s, 3H, -OCH₃), 4.01 (s, 3H, -OCH₃), 4.08 (s, 3H, -COOCH₃), 6.77 (d, 1H, aromatic, J = 2.38 Hz), 7.08 (d, 1H, aromatic, J = 2.46 Hz); ¹³C NMR (75 MHz, CDCl₃): 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; ESI-MS (CH₃CN, m/z): 297 [M+H]⁺.

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

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-carboxybutyryl)-5-hydroxy-3-methoxy benzoic acid (16). In a 25mL round bottom flask compound 12 (200mg, 0.64mmol) was taken in dry dichloromethane (10mL). To this stirring solution anhydrous aluminium chloride (800mg, 5.99mmol) was added and stirred overnight at room temperature. On completion of reaction dil HCl (1N) was added dropwise to it and stirred for 10min. 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-CHCl₃ one by one in the ratio 2:3:1, respectively.

5-(2-Carboxy-4,6-dimethoxy-phenyl)-5-oxa-pentanoic acid (14): Yield = 25% (41mg); ¹H NMR (300 MHz, CDCl₃): δ 1.84-1.94 (quintet, 2H, -CH₂CH₂COOH, J = 7.16 Hz),
Chapter 4  Synthesis and Antibacterial Activity of Diverse analogues of Oenostacin

2.32-2.37 (t, 2H, -CH₂COOH, J = 7.33 Hz), 2.76-2.81 (t, 2H, -CH₂CO, J = 7.0 Hz), 3.73 (s, 3H, -OCH₃), 3.75(s, 3H, -OCH₃), 6.61 (d, 1H, -CH, J = 1.0 Hz), 6.83 (d, 1H, -CH, J = 1.0 Hz); ESI-MS (CH₃CN, m/z): 297 [M+H]+.

5-(2-Carboxy-4-methoxy,6-hydroxy-phenyl)-5-oxa-pentanic acid (15): Oil; Yield = 40% (68mg); ¹H NMR (300 MHz, CDCl₃); δ 1.86-1.93 (quintet, 2H, -CH₂CH₂COOH, J = 7.16 Hz), 2.28-2.33 (t, 2H, -CH₂COOH, J = 7.37 Hz), 2.78-2.83 (t, 2H, -CH₂CO−, J = 7.03 Hz), 3.72 (s, 3H, -OCH₃), 6.39 (s, 1H, -CH), 6.65 (s, 1H, -CH); ESI-MS (CH₃CN, m/z): 283 [M+H]+.

2-(5-Oxapentanoic acid),3-methoxy-4-hydroxy-benzoic acid methyl ester (16): Oil; Yield = 13% (23mg); ¹H NMR (300 MHz, CDCl₃); δ 1.99-2.04 (broad triplet, 2H, -CH₂CH₂COOH), 2.39-2.44 (t, 2H, -CH₂COOH), 2.74-2.79 (t, 2H, -CH₂CO−), 3.83 (s, 3H, -OCH₃), 3.91(s, 3H, -COOCH₃), 6.52 (s, 1H, -CH), 6.68 (s, 1H, -CH); ESI-MS (CH₃CN, m/z): 297 [M+H]+.

Synthesis of 2-formyl-3-methoxy-4-hydroxy-benzoic acid methyl ester (19): In a 25 mL round bottom flask 2-formyl-3,5-dimethoxy-benzoic acid methyl ester (18, 100mg, 0.45mmol) was taken in dry dichloromethane (6mL). To this stirring solution anhydrous aluminium chloride (200mg, 1.6mmol) was added and stirred overnight at room temperature. On completion of reaction dil HCl was added dropwise to it and stirred for 10min. 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 compound 19 at hexane-CHCl₃ (1:1 v/v) as creamish white solid.
Chapter 4  Synthesis and Antibacterial Activity of Diverse analogues of Oenostacin

General procedure of the synthesis of compounds 8, 21, 26, 31 and 32

Synthesis of 3-(3,4,5-trihydroxyphenyl)-acrylic acid (32): In a 25mL round bottom flask 3,4,5-trimethoxy cinnamic acid (30, 50mg, 0.21mmol) was taken in dry dichloromethane (5mL). The reaction mixture was kept in acetone bath and cooled to -78°C with liquid nitrogen. To the chilled reaction mixture boron tribromide (0.12mL, 12mmol) was added dropwise and further stirred for 2h at this temperature. Then the reaction mixture was stirred overnight at room temperature (16h). On completion, the reaction mixture was poured into water and dil HCl (10%, 5 mL) was added to it. It was extracted with chloroform, washed with water, dried over anhydrous sodium sulfate and evaporated. The residue thus obtained was recrystallized with CHCl₃-MeOH (3:1 v/v) to get desired demethylated product 32 as light brown crystalline solid. Yield = 62% (25mg); mp. 180-182°C, IR (KBr, cm⁻¹): 3277, 1702, 1641, 1613, 1539; ¹H NMR (300 MHz, CDCl₃): δ 6.14-6.19 (d, 1H, =CHCO, J = 15.81 Hz), 6.59 (s, 2H, aromatic), 7.41-7.46 (d, 1H, -CH=, J = 15.81 Hz); ESI-MS (CH₃CN, m/z): 197.0 [M+H]+, 415 [2M+Na]+.
Chapter 4 Synthesis and Antibacterial Activity of Diverse analogues of Oenostacin

References


Chapter 4  Synthesis and Antibacterial Activity of Diverse analogues of Oenostacin


8. **Vandana srivastava** *et al.*, *Synthesis of* steroidal stilbenes as promising anticancer agents. To be communicated.


**Paper presented in Symposia/seminar**
