Electronic Supplementary Information

Design and synthesis of cenocladamide analogues and their evaluation against breast cancer cell lines

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Table of contents:  
1. Experimental section - Chemistry  
2. NMR spectra  
3. Experimental section - Biology
1. Experimental section - Chemistry

**Synthesis of methoxylated carboxylic acids**

![Chemical structure of (E)-3-(3,4,5-trimethoxyphenyl)acrylic acid]

\[
\text{O} \quad \text{H}
\]

\[
\text{MeO} \quad \text{MeO} \quad \text{MeO}
\]

\[
\text{HO} \quad \text{COO} \quad \text{COOH}
\]

\[
\text{MeO} \quad \text{MeO} \quad \text{MeO}
\]

\[
\text{O} \quad \text{H}
\]

**\((E)\)-3-(3,4,5-trimethoxyphenyl)acrylic acid**

A mixture of 3,4,5-trimethoxybenzaldehyde (1.00 g, 5.0 mmol, 1 eq.), malonic acid (1.15 g, 11.0 mmol, 2.2 eq.) and piperidine (98.8 µL, 1 mmol, 0.2 eq.) in anhydrous pyridine (2.31 mL, 28.5 mmol, 5.7 eq.) was heated at reflux for 2 h. After cooling to room temperature, an aqueous solution of HCl (5% v/v, 50 mL) was added to the reaction. The solvent was removed and the precipitate was dissolved in ethyl acetate (35 mL) and washed with water (2 x 10 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to yield the carboxylic acid.

Yield: 1.15 g, 96% as a white solid; Rf = 0.33, hexanes/ethyl acetate (50:50); mp 102-104 °C; \(\delta_H\) (250 MHz; CDCl\(_3\)) 3.90 (9H, s), 6.36 (1H, d, \(J = 15.8\) Hz), 6.78 (2H, s), 7.61 (1H, d, \(J = 15.9\) Hz), 11.43 (1H, br. s); \(\delta_C\) (62.9 MHz; CDCl\(_3\)) 56.1 (CH\(_3\)), 60.9 (CH\(_3\)), 105.5 (CH), 116.4 (CH), 129.4 (C), 140.5 (C), 147 (CH), 153.4 (C), 172.4 (C).

![Chemical structure of (E)-3-(4-acetoxy-3,5-dihydroxyphenyl)acrylic acid]

**\((E)\)-3-(4-acetoxy-3,5-dihydroxyphenyl)acrylic acid**

To sinapic acid (229 mg, 1 mmol, 1 eq.) were added pyridine (1.0 mL, 12.5 mmol, 12.5 eq.) and acetic anhydride (1.0 mL, 11 mmol, 11 eq.) at room temperature. The mixture was stirred overnight. The mixture was diluted with cold HCl (1 M, 20 mL) and ethyl acetate (30 mL). The organic phase was washed with cold H\(_2\)O (10 mL), and dried (Na\(_2\)SO\(_4\)). The crude was further purified by flash chromatography, eluting with hexanes/ethyl acetate (75:25 to 50:50).
Yield: 187 mg, 70 % as a white solid; Rf = 0.20, hexanes/ethyl acetate (50:50); mp 199-201 °C; δH (250 MHz) 2.36 (3H, s), 3.87 (6H, s), 6.40 (1H, d, J = 15.9 Hz), 6.81 (2H, s), 7.73 (1H, d, J = 15.9 Hz); δC (62.9 MHz; CDCl3) 20.4 (CH3), 56.2 (CH3), 104.9 (CH), 117.5 (CH), 130.8 (C), 132.3 (C), 146.7 (CH), 152.5 (C), 168.5 (C), 171.9 (C)
2. NMR Spectra

Figure S1. $^1$H NMR spectrum of 12 (CDCl$_3$, 250 MHz)

Figure S2. DEPT135 spectrum of 12 (CDCl$_3$, 62.9 MHz)
Figure S3. $^{13}$C NMR spectrum of 12 (CDCl$_3$, 62.9 MHz)

Figure S4. $^1$H NMR spectrum of 13 (CDCl$_3$, 250 MHz)
Figure S5. DEPT135 spectrum of 13 (CDCl₃, 62.9 MHz)

Figure S6. ¹³C NMR spectrum of 13 (CDCl₃, 62.9 MHz)
Figure S7. $^1$H NMR spectrum of 3,4,5-trimethoxycinnamic acid (CDCl$_3$, 250 MHz)

Figure S8. DEPT135 spectrum of 3,4,5-trimethoxycinnamic acid (CDCl$_3$, 62.9 MHz)
Figure S9. $^{13}$C NMR spectrum of 3,4,5-trimethoxycinnamic acid (CDCl$_3$, 62.9 MHz)

Figure S10. $^1$H NMR spectrum of 2 (CDCl$_3$, 250 MHz)
Figure S11. DEPT135 spectrum of 2 (CDCl₃, 62.9 MHz)

Figure S12. $^{13}$C NMR spectrum of 2 (CDCl₃, 62.9 MHz)
Figure S13. $^1$H NMR spectrum of 7 (CDCl$_3$, 250 MHz)

Figure S14. $^{13}$C NMR spectrum of 7 (CDCl$_3$, 62.9 MHz)
Figure S15. $^1$H NMR spectrum of 8 (CDCl$_3$, 250 MHz)

Figure S16. DEPT135 spectrum of 8 (CDCl$_3$, 62.9 MHz)
Figure S17. $^{13}$C NMR spectrum of 8 (CDCl$_3$, 62.9 MHz)

Figure S18. $^1$H NMR spectrum of 9 (CDCl$_3$, 250 MHz)
Figure S19. DEPT135 spectrum of 9 (CDCl₃, 62.9 MHz)

Figure S20. $^{13}$C NMR spectrum of 9 (CDCl₃, 62.9 MHz)
Figure S21. $^1$H NMR spectrum of 3-(3,4,5-trimethoxyphenyl)propanoic acid (CDCl$_3$, 250 MHz)

Figure S22. DEPT135 spectrum of 3-(3,4,5-trimethoxyphenyl)propanoic acid (CDCl$_3$, 62.9 MHz)
Figure S23. $^{13}$C NMR spectrum of 3-(3,4,5-trimethoxyphenyl)propanoic acid (CDCl$_3$, 62.9 MHz)

Figure S24. $^1$H NMR spectrum of 10 (CDCl$_3$, 250 MHz)
Figure S25. $^{13}$C NMR spectrum of 10 (CDCl$_3$, 62.9 MHz)

Figure S26. $^1$H NMR spectrum of 3,5-dimethoxy-4-acetoxycinnamic acid (CDCl$_3$, 250 MHz)
Figure S27. DEPT135 spectrum of 3,5-dimethoxy-4-acetoxycinnamic acid (CDCl₃, 62.9 MHz)

Figure S28. $^{13}$C NMR spectrum of 3,5-dimethoxy-4-acetoxycinnamic acid (CDCl₃, 62.9 MHz)
Figure S29. $^1$H NMR spectrum of 1 (CDCl$_3$, 250 MHz)

Figure S30. $^{13}$C NMR spectrum of 1 (CDCl$_3$, 62.9 MHz)
Figure S31. $^1$H NMR spectrum of 5 (CDCl$_3$, 250 MHz)

Figure S32. DEPT135 spectrum of 5 (CDCl$_3$, 62.9 MHz)
Figure S33. $^{13}$C NMR spectrum of 5 (CDCl$_3$, 62.9 MHz)

Figure S34. $^1$H NMR spectrum of 6 (CDCl$_3$, 250 MHz)
Figure S35. DEPT135 spectrum of 6 (CDCl₃, 62.9 MHz)

Figure S36. $^{13}$C NMR spectrum of 6 (CDCl₃, 62.9 MHz)
Figure S37. $^1$H NMR spectrum of 17 (CDCl$_3$, 250 MHz)

Figure S38. DEPT135 spectrum of 17 (CDCl$_3$, 62.9 MHz)
Figure S39. $^{13}$C NMR spectrum of 17 (CDCl$_3$, 62.9 MHz)

Figure S40. $^1$H NMR spectrum of 18 (CDCl$_3$, 250 MHz)
Figure S41. DEPT135 spectrum of 18 (CDCl₃, 62.9 MHz)

Figure S42. $^{13}$C NMR spectrum of 18 (CDCl₃, 62.9 MHz)
Figure S43. $^1$H NMR spectrum of 3 (CDCl$_3$, 250 MHz)

Figure S44. $^{13}$C NMR spectrum of 3 (CDCl$_3$, 62.9 MHz)
Figure S45. $^1$H NMR spectrum of 4 (CDCl$_3$, 400 MHz)

Figure S46. DEPT135 spectrum of 4 (CDCl$_3$, 100 MHz)
Figure S47. $^{13}$C NMR spectrum of 4 (CDCl$_3$, 100 MHz)
3. Experimental section - Biology

Proliferation assay

Table 1: Growth inhibitory effects of the cenocladamide derivatives on MDA-MB-231.

<table>
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<tr>
<th>Compound</th>
<th>Cell loss/proliferation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>-54.9 ± 10.8</td>
</tr>
<tr>
<td>2</td>
<td>43.3 ± 5.9</td>
</tr>
<tr>
<td>9</td>
<td>43.9 ± 8.8</td>
</tr>
<tr>
<td>1</td>
<td>82.9 ± 4.1</td>
</tr>
<tr>
<td>4</td>
<td>85.6 ± 5.7</td>
</tr>
<tr>
<td>8</td>
<td>94.8 ± 8.8</td>
</tr>
<tr>
<td>7</td>
<td>98.3 ± 7.4</td>
</tr>
<tr>
<td>6</td>
<td>98.7 ± 10.5</td>
</tr>
<tr>
<td>5</td>
<td>103.0 ± 10.9</td>
</tr>
<tr>
<td>10</td>
<td>103.0 ± 5.6</td>
</tr>
</tbody>
</table>

Table 2: Growth inhibitory effects of analogue 3 on several human breast cancer cell lines.

<table>
<thead>
<tr>
<th>Cell loss/proliferation (%)</th>
<th>3</th>
<th>5</th>
<th>Doxo</th>
</tr>
</thead>
<tbody>
<tr>
<td>SKBR3</td>
<td>-86.7 ± 5.9</td>
<td>105.6 ± 8.2</td>
<td>1.9 ± 10.0</td>
</tr>
<tr>
<td>Hs578T</td>
<td>-79.6 ± 4.8</td>
<td>104.9 ± 6.3</td>
<td>15.5 ± 3.3</td>
</tr>
<tr>
<td>HCC38</td>
<td>-78.0 ± 1.9</td>
<td>98.1 ± 6.2</td>
<td>0.2 ± 1.6</td>
</tr>
<tr>
<td>MDA-MB-468</td>
<td>-74.1 ± 5.9</td>
<td>96.0 ± 4.6</td>
<td>5.5 ± 1.5</td>
</tr>
<tr>
<td>BT549</td>
<td>-69.0 ± 5.6</td>
<td>98.2 ± 3.9</td>
<td>13.3 ± 0.6</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>-66.9 ± 6.15</td>
<td>101.8 ± 1.9</td>
<td>10.5 ± 1.7</td>
</tr>
<tr>
<td>MCF-10A</td>
<td>-51.3 ± 2.4</td>
<td>113.5 ± 10.5</td>
<td>9.4 ± 1.7</td>
</tr>
<tr>
<td>T47D</td>
<td>-50.5 ± 5.0</td>
<td>98.0 ± 14.0</td>
<td>0.2 ± 2.6</td>
</tr>
<tr>
<td>iHMEC</td>
<td>-39.9 ± 8.9</td>
<td>77.6 ± 9.6</td>
<td>-23.9 ± 6.8</td>
</tr>
<tr>
<td>MCF7</td>
<td>-9.3 ± 7.3</td>
<td>103.3 ± 10.1</td>
<td>49.4 ± 6.7</td>
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