Sustainable synthesis of \( p \)-hydroxycinnamic diacids through proline-mediated Knoevenagel condensation in ethanol: an access to potent phenolic UV filters and radical scavengers.

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General

All reagents were purchased from Sigma–Aldrich, TCI, Merck or VWR and used as received. Solvents were purchased from Thermo Fisher Scientific and VWR. Deuterated dimethylsulfoxide (DMSO-d6 <0.02%H2O) was purchased from Euriso-top. Evaporations were conducted under reduced pressure at temperature below 40 °C. Column chromatography was carried out with an automated flash chromatography (PuriFlash 4100, Interchim) and pre-packed INTERCHIM PF-30C18HP-HP-F0080 (30 μm silica gel) columns. UV/visible absorption spectra of diacids in ethanol were recorded using a UV/visible spectrometer Cary 60 Agilent Technologies at a concentration of 10 μM. For the photostability study, samples (10 μM, EtOH) were irradiated during one hour into a Rayonet© RPR-200 (λ = 300 nm, P = 8.32 W/m², stirring, T = 35 °C) using 14 RPR-3000A lamps (SNE Ultraviolet Co Branford Ct USA RPR-3000A). Then, UV spectra were recorded and the absorbance loss were calculated in percentage at the λmax. NMR analyses were recorded on a Bruker Fourier 300. 1H NMR spectra of samples were recorded in DMSO (residual peak at δ = 2.50 ppm) or (CD3)2CO (residual signal at δ = 2.05 ppm) at 300 MHz, chemical shifts were reported in parts per million relatives to the solvent residual peak. 13C NMR spectra of samples were recorded at 75 MHz in DMSO (residual signal at δ = 39.52 ppm) or (CD3)2CO (residual peak at δ = 206.26 and 29.84 ppm). 1H NMR crude calculation conversion was recorded by integration of diacids’ aromatic signal. Melting points were recorded on a Metler Toledo MP50 Melting Points system, Tinitial = 40 °C, heating 3 °C / minute until 200 °C with ME-18552 sample tubes. Mass spectrometry analysis were recorded on an Agilent Technologies 6545 Q-TOF LC/MS. The determination of the diacids’ antiradical activities were determined via 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. These tests involve adding potential antiradical molecule solution in ethanol at different concentration to homogeneous DPPH solution. The amount needed to reduce the initial number of DPPH free radicals by half, i.e. EC50, is provided by the crossing point of % DPPH (blue) and % reduced DPPH (green)

Figure S1. Structures and numeration of coumaric diacid (A), ferulic diacid (B), sinapic diacid (C) and caffeic diacid (D).
Ferulic diacid

**Synthesis:** Vanillin (225 mg, 1.5 mmol), malonic acid (153.9 mg, 1.5 mmol, 1 eq), proline (17.1 mg, 0.15 mmol, 0.1 eq) and ethanol (3 mL, 0.5 M) were stirred at 60 °C for 4 hours. The reaction mixture was then evaporated under vacuum. Conversion yields were determined by proton NMR. Crude mixture was then purified by column chromatography on C-18 reverse silica gel using appropriate eluent system (H₂O:methanol 80:20). 282 mg of a yellow/white powder was recovered (80%).

**¹H NMR** (300 MHz, 25°C, (CD₃)₂CO): δ (ppm) = 7.62 (1H, s, H-3), 7.35 (1H, d, J = 2.0 Hz, H-9), 7.21 (1H, dd, J = 2.0 and 8.3 Hz, H-5), 6.89 (1H, d, J = 8.3 Hz, H-6), 3.86 (3H, s, H-10).

**¹³C NMR** (75 MHz, 25°C, (CD₃)₂CO): δ (ppm) = 168.5 and 165.9 (C-1 and C-1'), 150.1 (C-7), 148.3 (C-8), 142.1 (C-3), 125.7 (C-4), 125.5 (C-2), 124.4 (C-5), 116.0 (C-6), 113.6 (C-9), 56.1 (C-10).

**Melting point:** 190-192 ± 0.1 °C.

**TOF MS ES⁺:** [M+H]+ for C₁₁H₁₁O₄: m/z 239.0556; found: m/z 239.0555.

**UV:** λ max = 328 nm.

**DPPH:** EC₅₀ = 20.72 nmol.

Cumaric diacid

**Synthesis:** 4-hydroxybenzaldehyde (181.4 mg, 1.5 mmol), malonic acid (153.9 mg, 1.5 mmol, 1 eq), proline (17.1 mg, 0.15 mmol, 0.1 eq) and ethanol (3 mL, 0.5 M) were stirred at 60 °C for 4 hours. The reaction mixture was then evaporated under vacuum. Conversion yields were determined by proton NMR. Crude mixture was then purified by column chromatography on C-18 reverse silica gel using appropriate eluent system (H₂O:methanol 80:20). 219.3 mg of a yellow/white powder was recovered (71%).

**¹H NMR** (300 MHz, 25°C, (CD₃)₂CO): δ (ppm) = 7.62 (1H, s, H-3), 7.58 (2H, d, J = 8.6 Hz, H-5 and H-5'), 6.91 (2H, d, J = 8.7 Hz, H-6 and H-6').

**¹³C NMR** (75 MHz, 25°C, (CD₃)₂CO): δ (ppm) = 168.5 and 165.9 (C-1 and C-1'), 160.8 (C-7), 141.7 (C-3), 132.9 (C-5 and C-5'), 125.4 (C-4), 124.4 (C-2), 116.7 (C-6 and C-6').

**Melting point:** 183-186 ± 0.1 °C.

**TOF MS ES⁺:** [M+H]+ for C₁₀H₁₀O₂: m/z 209.0450; found: m/z 209.0450.

**UV:** λ max = 314 nm.

**DPPH:** EC₅₀ = no EC₅₀ was obtained at studied concentrations.

Sinapic diacid

**Synthesis:** Syringaldehyde (270.5 mg, 1.5 mmol), malonic acid (153.9 mg, 1.5 mmol, 1 eq), proline (17.1 mg, 0.15 mmol, 0.1 eq) and ethanol (3 mL, 0.5 M) were stirred at 60 °C for 4 hours. The reaction mixture was then evaporated under vacuum. Conversion yields were determined by proton NMR. Crude mixture was then purified by column chromatography on C-18 reverse silica gel using appropriate eluent system (H₂O:methanol 80:20 to 50:50). 269.5 mg of a yellow/white powder was recovered (68%).

**¹H NMR** (300 MHz, 25°C, (CD₃)₂CO): δ (ppm) = 7.99 (1H, s, H-10), 7.61 (1H, s, H-3), 7.06 (2H, s, H-5 and H-5'), 3.84 (6H, s, H-8 and H-8').

**¹³C NMR** (75 MHz, 25°C, (CD₃)₂CO): δ (ppm) = 168.9 and 165.8 (C-1 and C-1'), 148.8 (C-6 and C-6'), 142.3 (C-3), 139.8 (C-7), 124.9 (C-4), 124.5 (C-2), 108.7 (C-5 and C-5'), 56.1 (C-8 and C-8').

**Melting point:** 172-175 ± 0.1 °C.

**TOF MS ES⁺:** [M+H]+ for C₁₂H₁₃O₃: m/z 269.0661; found: m/z 269.0661.

**UV:** λ max = 330 nm.

**DPPH:** EC₅₀ = 3.86 nmol.

Caffeic diacid

**Synthesis:** 3,4-dihydroxybenzaldehyde (270.5 mg, 1.5 mmol), malonic acid (153.9 mg, 1.5 mmol, 1 eq), proline (17.1 mg, 0.15 mmol, 0.1 eq) and ethanol (3 mL, 0.5 M) were stirred at 60 °C for 4 hours. The reaction mixture was then evaporated under vacuum. Conversion yields were determined by proton NMR. Crude mixture was then purified by column chromatography on C-18 reverse silica gel using appropriate eluent system (H₂O:methanol 90:10). 197.7 mg of a yellow/white powder was recovered (60%).

**¹H NMR** (300 MHz, 25°C, (CD₃)₂CO): δ (ppm) = 7.54 (1H, s, H-3), 7.24 (1H, d, J = 2.2 Hz, H-9), 7.08 (1H, dd, J = 2.2 and 8.3 Hz, H-5), 6.89 (1H, d, J = 8.3 Hz, H-6).

**¹³C NMR** (75 MHz, 25°C, (CD₃)₂CO): δ (ppm) = 168.4 and 165.9 (C-1 and C-1'), 149.0 (C-7), 146.1 (C-8), 141.8 (C-3), 125.9 (C-4), 124.7 (C-2), 124.4 (C-5), 116.9 (C-9), 116.3 (C-6).

**Melting point:** 162-164 ± 0.1 °C.

**TOF MS ES⁺:** [M+H]+ for C₁₀H₁₀O₂: m/z 225.0399; found: m/z 225.0399.

**UV:** λ max = 340 nm.

**DPPH:** EC₅₀ = 2.96 nmol.
| N° | Eq Proline | Eq Malonic Acid | Temperature (°C) | Time (h) | m Proline (mg) | m Malonic Acid (mg) | m Vanillin (mg) | V Ethanol (mL) | Vanillin (%) [a] | Diacid (%) [a] | Acid (%) [a] | Other (%) [a] | Total (%) |
|----|------------|----------------|-----------------|---------|---------------|-------------------|----------------|----------|----------------|---------------|-------------|-------------|-------------|-----------|
| 1  | 0.1        | 1              | 20              | 4       | 5.7           | 51.3              | 75             | 1        | 59             | 34            | 2           | 5           | 100         |
| 2  | 0.1        | 4              | 20              | 4       | 5.7           | 205.2             | 75             | 1        | 55             | 40            | 3           | 2           | 100         |
| 3  | 1          | 4              | 20              | 4       | 56.8          | 205.2             | 75             | 1        | 6              | 83            | 3           | 8           | 100         |
| 4  | 0.1        | 1              | 60              | 4       | 5.7           | 51.3              | 75             | 1        | 19             | 78            | 1           | 2           | 100         |
| 5  | 0.1        | 4              | 60              | 4       | 5.7           | 205.2             | 75             | 1        | 20             | 71            | 1           | 9           | 100         |
| 6  | 1          | 1              | 33.3            | 4       | 56.8          | 5103             | 75             | 1        | 11             | 78            | 2           | 10          | 100         |
| 7  | 1          | 2              | 60              | 4       | 56.8          | 102.6             | 75             | 1        | 5              | 63            | 13          | 18          | 100         |
| 8  | 0.7        | 4              | 60              | 4       | 39.7          | 205.2             | 75             | 1        | 4              | 90            | 3           | 2           | 100         |
| 9  | 0.6        | 1              | 40              | 4       | 31.2          | 51.3              | 75             | 1        | 16             | 66            | 6           | 12          | 100         |
| 10 | 1          | 1              | 20              | 8       | 56.8          | 51.3              | 75             | 1        | 11             | 61            | 14          | 14          | 100         |
| 11 | 1          | 4              | 46.7            | 8       | 56.8          | 205.2             | 75             | 1        | 3              | 75            | 12          | 10          | 100         |
| 12 | 0.7        | 1              | 60              | 8       | 39.7          | 51.3              | 75             | 1        | 13             | 57            | 19          | 12          | 100         |
| 13 | 0.4        | 4              | 20              | 8       | 22.7          | 205.2             | 75             | 1        | 6              | 82            | 0           | 11          | 100         |
| 14 | 0.1        | 2.5            | 40              | 8       | 5.7           | 128.2             | 75             | 1        | 11             | 79            | 0           | 10          | 100         |
| 15 | 0.1        | 1              | 20              | 16      | 5.7           | 51.3              | 75             | 1        | 39             | 39            | 5           | 17          | 100         |
| 16 | 0.1        | 4              | 20              | 16      | 5.7           | 205.2             | 75             | 1        | 53             | 40            | 2           | 5           | 100         |
| 17 | 0.1        | 1              | 60              | 16      | 5.7           | 51.3              | 75             | 1        | 28             | 60            | 2           | 9           | 100         |
| 18 | 1          | 1              | 60              | 16      | 56.8          | 51.3              | 75             | 1        | 15             | 30            | 38          | 17          | 100         |
| 19 | 0.1        | 4              | 60              | 16      | 5.7           | 205.2             | 75             | 1        | 17             | 68            | 4           | 11          | 100         |
| 20 | 1          | 4              | 60              | 16      | 56.8          | 205.2             | 75             | 1        | 11             | 20            | 25          | 45          | 100         |
| 21 | 1          | 4              | 33.3            | 16      | 56.8          | 205.2             | 75             | 1        | 2              | 89            | 2           | 7           | 100         |
| 22 | 1          | 2              | 20              | 16      | 56.8          | 102.6             | 75             | 1        | 4              | 80            | 2           | 14          | 100         |
| 23 | 1          | 3              | 20              | 16      | 56.8          | 153.9             | 75             | 1        | 4              | 81            | 3           | 13          | 100         |
| 24 | 0.6        | 1              | 40              | 16      | 31.2          | 51.3              | 75             | 1        | 19             | 60            | 4           | 17          | 100         |
| 25 | 0.6        | 2.5            | 60              | 16      | 31.2          | 128.2             | 75             | 1        | 10             | 41            | 17          | 32          | 100         |
| 26 | 0.6        | 2.5            | 40              | 8       | 31.2          | 128.2             | 75             | 1        | 1              | 91            | 3           | 5           | 100         |
| 27 | 0.6        | 2.5            | 40              | 8       | 31.2          | 128.2             | 75             | 1        | 2              | 83            | 3           | 11          | 100         |
| 28 | 0.6        | 2.5            | 40              | 8       | 31.2          | 128.2             | 75             | 1        | 2              | 83            | 4           | 11          | 100         |
| 29 | 0.1        | 1              | 60              | 4       | 5.7           | 51.3              | 75             | 1        | 15             | 77            | 1           | 7           | 100         |
| 30 | 0.1        | 1              | 60              | 5       | 5.7           | 51.3              | 75             | 1        | 20             | 73            | 1           | 6           | 100         |
| 31 | 0.1        | 1              | 60              | 6       | 5.7           | 51.3              | 75             | 1        | 18             | 72            | 1           | 9           | 100         |
| 32 | 0.1        | 1              | 60              | 4       | 17.1          | 153.9             | 225            | 3        | 19             | 75            | 1           | 4           | 100         |
| 33 | 0.1        | 1              | 60              | 4       | 17.1          | 153.9             | 225            | 3        | 17             | 80            | 1           | 2           | 100         |
| 34 | 1.1        | 3              | 40              | 16      | 187.3         | 461.7             | 225            | 3        | 25             | 68            | 7           | 0           | 100         |
| 35 | 0.1        | 1              | 60              | 2       | 17.1          | 153.9             | 225            | 3        | 41             | 55            | 3           | 1           | 100         |
| 36 | 0.1        | 1              | 60              | 3       | 17.1          | 153.9             | 225            | 3        | 36             | 60            | 3           | 1           | 100         |

[a] percentage of conversions were determined from the $^1$H NMR spectrum of the crude reaction mixture  
[b]: according to Peyrot et al.\textsuperscript{12}

Table S1. DOE experiments.
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<th>Benzaldehyde</th>
<th>Eq Proline</th>
<th>Eq Malonic</th>
<th>Temperature (°C)</th>
<th>Time (h)</th>
<th>m Proline (mg)</th>
<th>m Malonic Acid (mg)</th>
<th>m Aldehyde (mg)</th>
<th>V Ethanol (mL)</th>
<th>Aldehyde (%)</th>
<th>Diacid (%) [a]</th>
<th>Acid (%) [a]</th>
<th>Other (%) [a]</th>
<th>Total (%)</th>
<th>Isolated Yield (%)</th>
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<td>Vanilline</td>
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<td>153.9</td>
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<td>17</td>
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<td>71</td>
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<tr>
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<td>100</td>
<td>68</td>
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[a] percentage of conversions were determined from the ¹H NMR spectrum of the crude reaction mixture.

Table S2. Application of the optimal conditions designed by the DOE to the p-hydroxybenzaldehydes.

### Table S3. PMI score calculation between our procedure (Entry 33, Table S1) and Peyrot et al.¹² conditions (Entry 34, Table S1).
Figure S2: UV spectra of diacids.

UV Ferulic diacid

Ferulic Diacid $\lambda_{\text{max}} = 328 \text{ nm}$

UV Coumaric diacid

Coumaric Diacid $\lambda_{\text{max}} = 314 \text{ nm}$

UV Sinapic diacid

Sinapic Diacid $\lambda_{\text{max}} = 330 \text{ nm}$

UV Caffeic diacid

Caffeic Diacid $\lambda_{\text{max}} = 340 \text{ nm}$
Figure S3: Photostability of diacids.

**Photostability Ferulic diacid**

- $\lambda_{\text{max}}$ (nm): 328
- $A_{\text{max}}$: 0.170
- Absorbance loss (%): 7.9

**Photostability Coumaric diacid**

- $\lambda_{\text{max}}$ (nm): 314
- $A_{\text{max}}$: 0.224
- Absorbance loss (%): 3.0

**Photostability Sinapic diacid**

- $\lambda_{\text{max}}$ (nm): 330
- $A_{\text{max}}$: 0.174
- Absorbance loss (%): 8.9

**Photostability Caffeic diacid**

- $\lambda_{\text{max}}$ (nm): 340
- $A_{\text{max}}$: 0.150
- Absorbance loss (%): 4.9
Figure S4: Photostability of Octinoxate.

<table>
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<th>t0</th>
<th>t60</th>
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<td>$\lambda_{\text{max}}$ (nm)</td>
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<tr>
<td>$A_{\text{max}}$</td>
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<td>0.177</td>
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<td>Absorbance loss (%)</td>
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<td>26.5</td>
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</table>
Figure S5: Determination of the antiradical activity of diacids.

Ferulic Diacid
$EC_{50} = 20.72$ nmol

Coumaric Diacid
$EC_{50} = $ Non Determined

Sinapic Diacid
$EC_{50} = 3.86$ nmol

Caffeic Diacid
$EC_{50} = 2.96$ nmol
NMR analysis

Figure S6: $^1$H spectrum of aromatic zone for ferulic diacid conversion

Total diacid aromatic protons: vanillin 3, diacid 4, monoacid 5.
Total spectra aromatic protons = 5.13
Vanillin still presents: (0.32x3)/5.13 = 19%
Ferulic diacid conversion: (1x4)/5.13 = 78%
Ferulic acid conversion: (0.01x5)/5.13 = 1%
Figure S7: $^1$H spectrum of ferulic diacid

Figure S8: $^{13}$C spectrum of ferulic diacid
Figure S9: $^1$H spectrum of coumaric diacid

Figure S10: $^{13}$C spectrum of coumaric diacid
Figure S11: $^1$H spectrum of sinapic diacid

Figure S12: $^{13}$C spectrum of sinapic diacid
Figure S13: $^1$H spectrum of caffeic diacid

Figure S14: $^{13}$C spectrum of caffeic diacid
HRMS analysis

Figure S15: HRMS spectrum of ferulic diacid

Figure S16: HRMS spectrum of coumaric diacid
Figure S17: HRMS spectrum of sinapic diacid

Figure S18: HRMS spectrum of caffeic diacid