

Supporting information for

The efficient and selective catalytic oxidation of *para*-substituted cinnamic acid derivatives by the cytochrome P450 monooxygenase, CYP199A4

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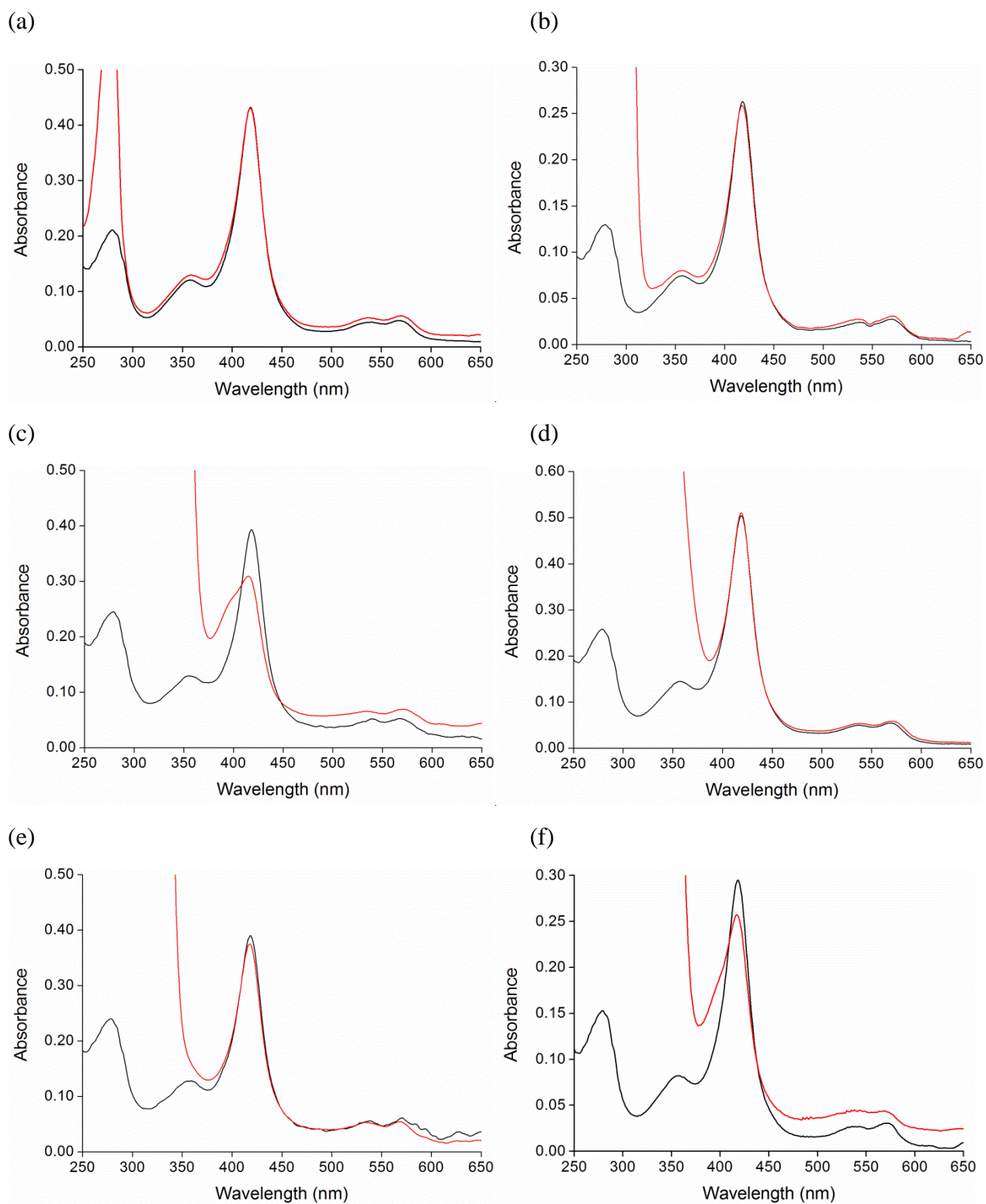
Table S1 HPLC retention times of cinnamic acid derivatives. Where two times are given this refers to the *cis* and *trans* isomers of the molecule.

	RT (min)
4-methoxycinnamic acid	18.5 (<i>cis</i>) and 19.3 (<i>trans</i>)
cinnamic acid	19.1
4-methylcinnamic acid	22.6
3-hydroxycinnamic acid	12.5
4-hydroxycinnamic acid	11.0
3,4-(methylenedioxy)cinnamic acid	18.7
3-hydroxy-4-methoxycinnamic acid	12.8
3-methoxy-4-hydroxycinnamic acid	11.8
3,5-dimethoxy-4-hydroxycinnamic acid	11.1
4-isopropylcinnamic acid	27.9
3,5-dimethoxycinnamic acid	21.5
3,4-dimethoxycinnamic acid	16.0 (<i>cis</i>) and 16.7 (<i>trans</i>)
2,4-dimethoxycinnamic acid	20.3 (<i>cis</i>) and 22.1 (<i>trans</i>)
4-methoxyphenylacetic acid	15.0
3-(4'-methoxyphenyl)propionic acid	18.1
4-hydroxymethylcinnamic acid	9.3
3,4-dihydroxycinnamic acid	7.5
4-(2'-hydroxyisopropyl)cinnamic acid	15.0
4-(1'-hydroxyisopropyl)cinnamic acid	14.5
4-(1',2'-epoxyisopropyl)cinnamic acid	7.6
4-(prop-1'-en-2'-yl)cinnamic acid	27.5
3-hydroxy-5-methoxycinnamic acid	13.9
3-methoxy-4-hydroxycinnamic acid	12.3
2-methoxy-4-hydroxycinnamic acid	13.4 (<i>cis</i>) and 14.0 (<i>trans</i>)
4-hydroxyphenylacetic acid	7.1
3-(4'-hydroxyphenyl)propionic acid	9.6

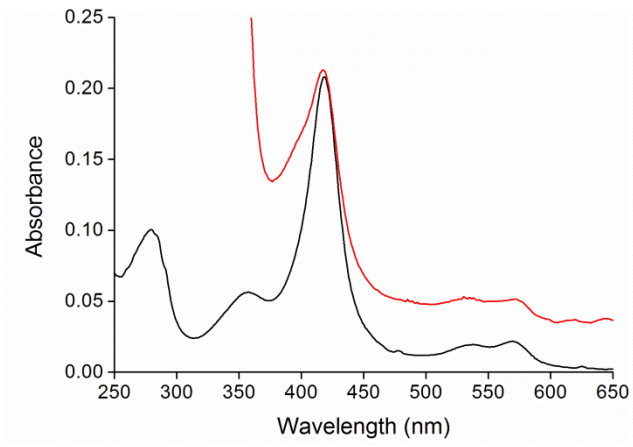
Table S2 GC-MS retention times and masses of TMS derivatised cinnamic acid derivatives. Where two times are given this refers to the *cis* and *trans* isomers of the molecule.

	R. T. (min)	Substrate Masses: Actual (Expected)
4-methoxycinnamic acid	12.7 (<i>cis</i>) and 15.3 (<i>trans</i>)	250.2 (250.4)
4-isopropylcinnamic acid	12.2 (<i>cis</i>) and 15.1 (<i>trans</i>)	262.3 (262.4)
3,5-dimethoxycinnamic acid	15.4 (<i>cis</i>) and 18.6 (<i>trans</i>)	280.2 (280.4)
3,4-dimethoxycinnamic acid	15.8 (<i>cis</i>) and 18.6 (<i>trans</i>)	280.3 (280.4)
2,4-dimethoxycinnamic acid	15.7 (<i>cis</i>) and 19.0 (<i>trans</i>)	280.0 (280.4)
4-hydroxycinnamic acid	14.4 (<i>cis</i>) and 17.1 (<i>trans</i>)	308.2 (308.5)
4-(2'-hydroxyisopropyl)cinnamic acid	20.4	350.3 (350.6)
4-(1'-hydroxyisopropyl)cinnamic acid	19.7	350.2 (350.6)
4-(1',2'-epoxyisopropyl)cinnamic acid	21.5	276.3 (276.4)
4-(prop-1'-en-2'-yl)cinnamic acid	16.1	260.3 (260.4)
3-hydroxy-5-methoxycinnamic acid	19.5	338.3 (338.6)
3-methoxy-4-hydroxycinnamic acid	16.7 (<i>cis</i>) and 19.7 (<i>trans</i>)	338.2 (338.6)
2-methoxy-4-hydroxycinnamic acid	17.0 (<i>cis</i>) and 20.4 (<i>trans</i>)	338.0 (338.6)

Figure S1 Spin state shifts of CYP199A4 with (a) 4-methoxyphenylacetic acid,¹ (b) cinnamic acid, (c) 3-(4-methylenedioxy)-cinnamic acid, (d) 4-hydroxycinnamic acid, (e) 3-hydroxycinnamic acid, (f) 2,4-dimethoxycinnamic acid, (g) 3,4-dimethoxycinnamic acid and (h) 3-hydroxy-4-methoxycinnamic acid.



(g)



(h)

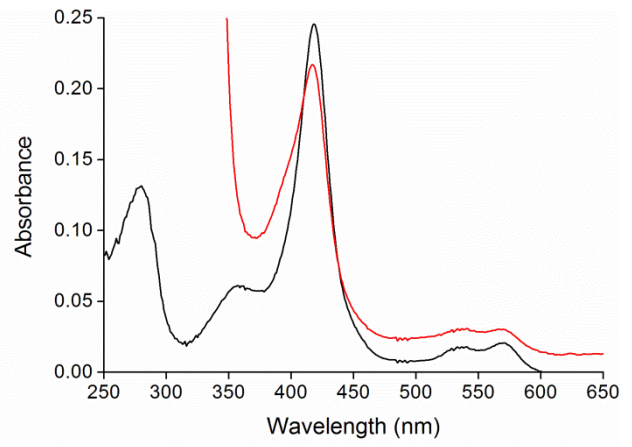
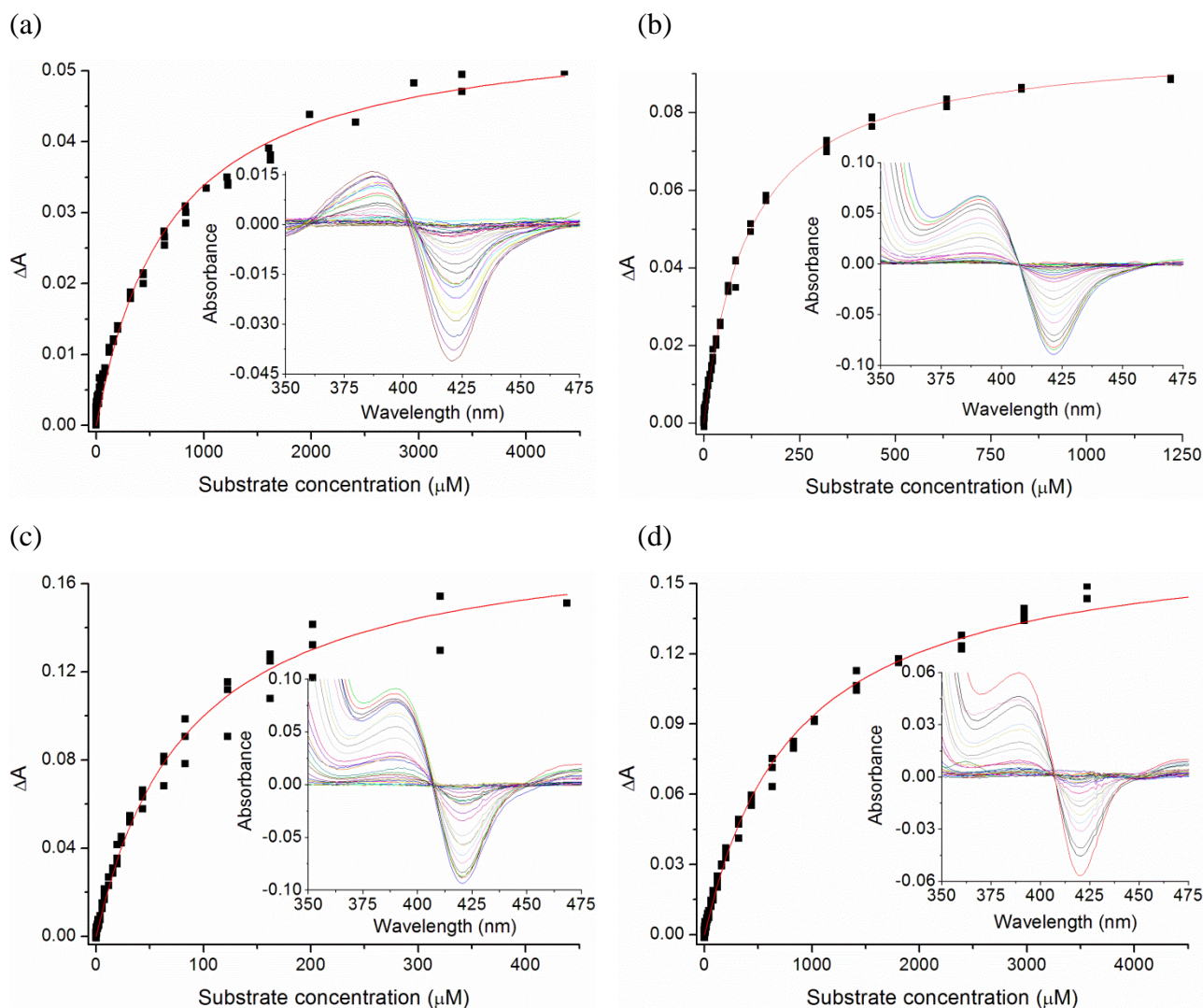
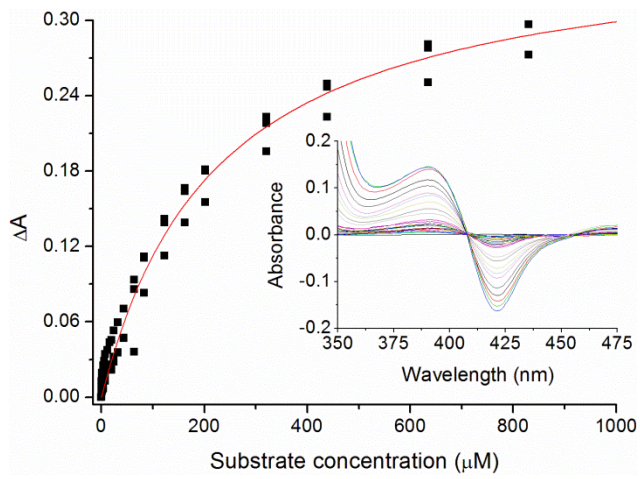


Figure S2 Dissociation constant analyses of CYP199A4 with different substrates. The protein concentration, the absorbance of the peak and trough used to calculate ΔA and the K_d are also provided. The data for 4-methoxyphenylacetic acid were reported previously and are included for comparison (a) 4-methoxyphenylacetic acid¹ (b) 3-(4-methylenedioxy)cinnamic acid (CYP199A4 concentration, 2.6 μM , A_{391} - A_{421} and K_d 120 μM), (c) 2,4-dimethoxycinnamic acid (CYP199A4 concentration, 5.5 μM , A_{390} - A_{421} and K_d 86 μM), (d) 3,4-dimethoxycinnamic acid (CYP199A4 concentration, 4.6 μM , A_{388} - A_{420} and K_d 840 μM), (e) 3-hydroxy-4-methoxycinnamic acid (CYP199A4 concentration, 5.1 μM , A_{390} - A_{421} and K_d 224 μM) and (f) 4-isopropylcinnamic acid (CYP199A4 concentration, 2.1 μM , A_{390} - A_{421} and K_d 3.4 μM).



(e)



(f)

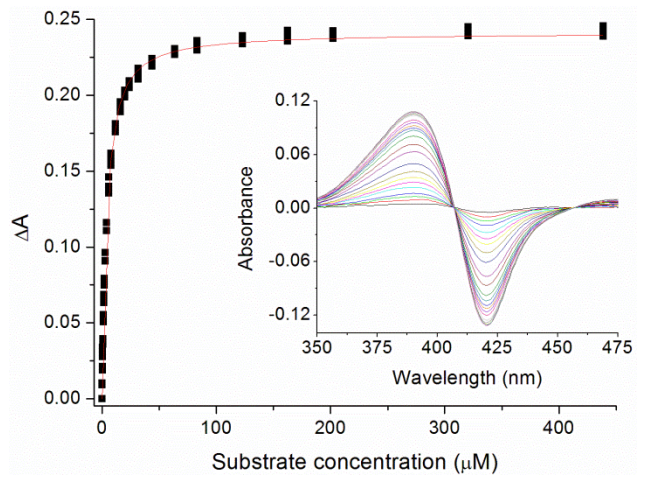
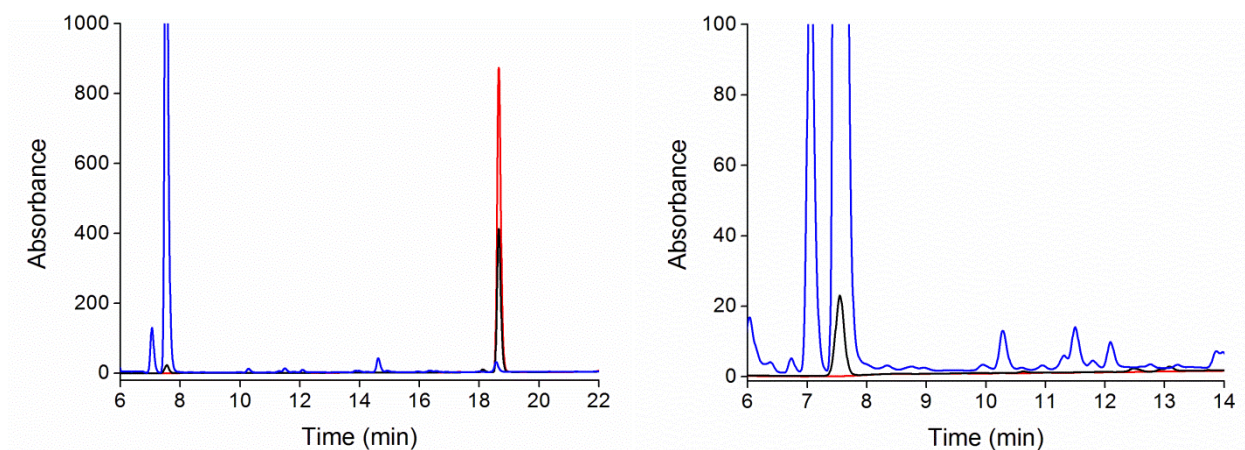
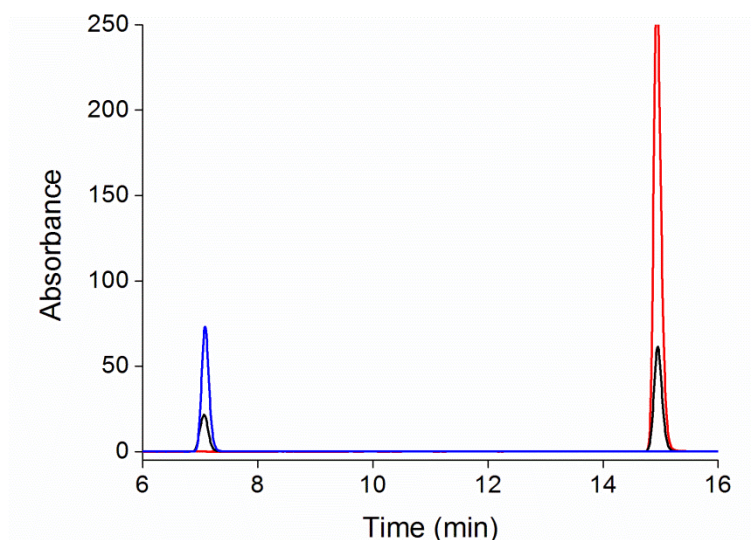


Figure S3 HPLC analysis of the *in vitro* turnovers of various cinnamic acid derivatives with CYP199A4; turnover is in black, substrate control in red and product control in blue.

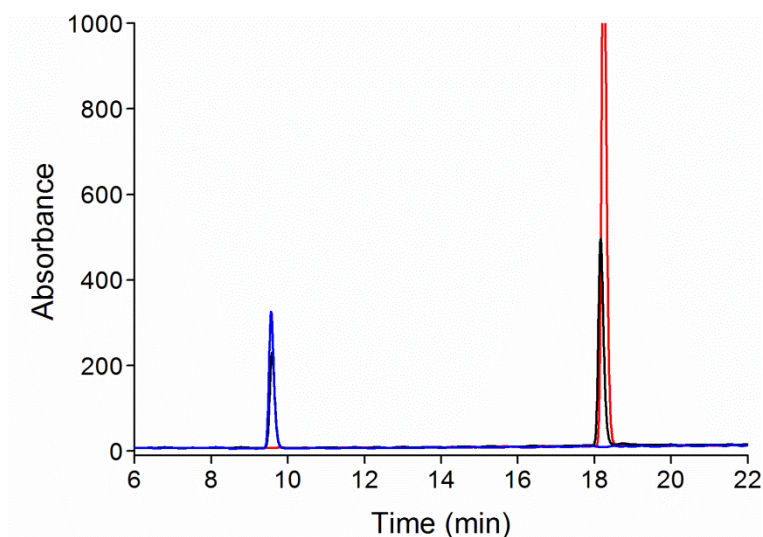
- (a) HPLC trace of the 3,4-(methylenedioxy)cinnamic acid turnover by CYP199A4 (black), 3,4-(methylenedioxy)cinnamic acid control (red) and 3,4-dihydroxycinnamic acid (3,4-dihydroxycinnamic acid) control (blue). 3,4-Dihydroxycinnamic acid, RT 7.5 min, and 3,4-(methylenedioxy)cinnamic acid, RT 18.7 min. A zoomed in version of the product region is shown on the right.



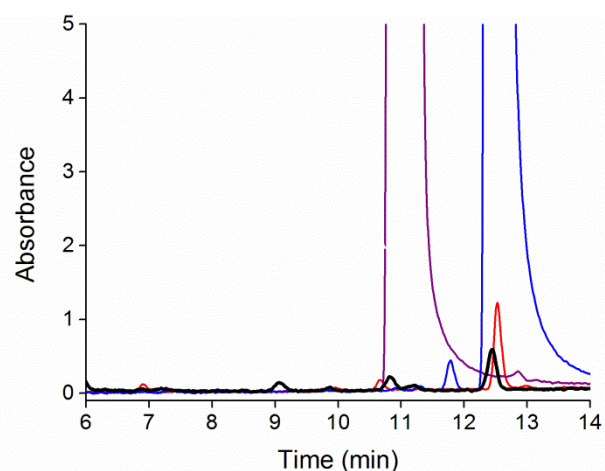
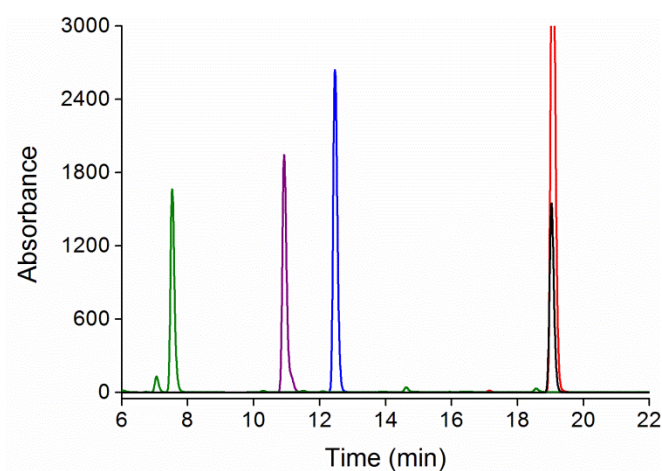
- (b) HPLC analysis of the 4-methoxyphenylacetic acid turnover by CYP199A4 (black), 4-methoxyphenylacetic acid control (red) and 4-hydroxyphenylacetic acid control (blue). 4-Hydroxyphenylacetic acid, RT 7.1 min, and 4-methoxyphenylacetic acid, RT 15.0 min.¹



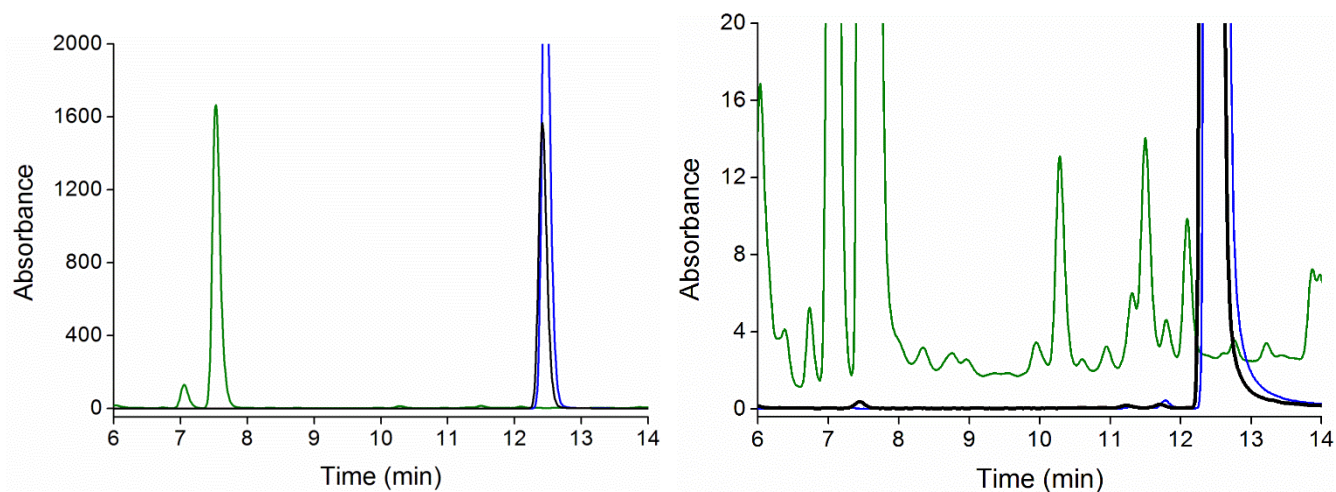
- (c) HPLC analysis of the 3-(4'-methoxyphenyl)propionic acid turnover by CYP199A4 (black), 3-(4'-methoxyphenyl)propionic acid control (red) and 3-(4'-hydroxyphenyl)propionic acid control (blue). 3-(4'-Hydroxyphenyl)propionic acid, RT 9.6 min, and 3-(4'-methoxyphenyl)propionic acid, RT 18.1 min.



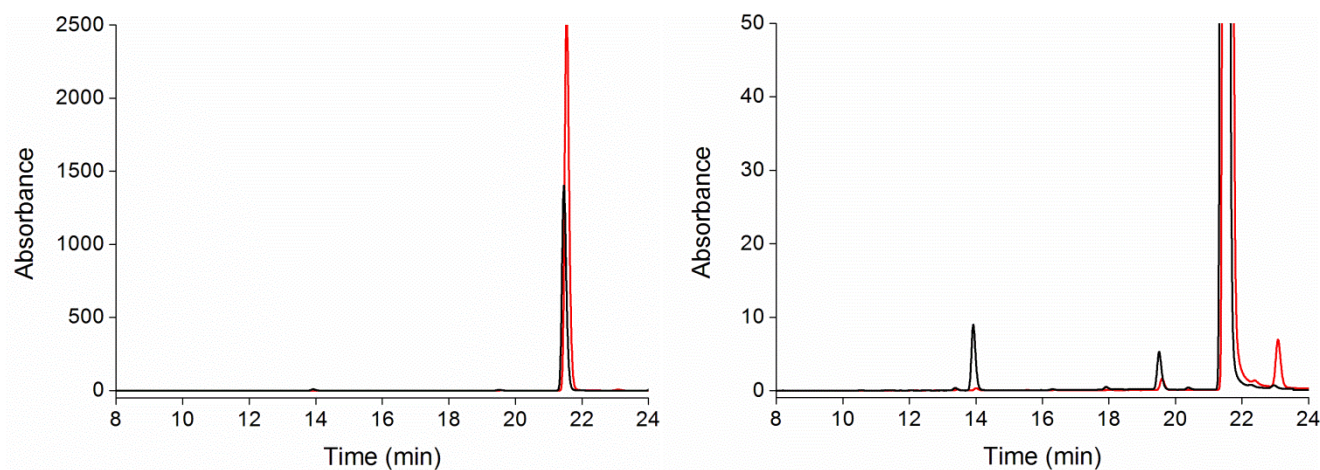
- (d) HPLC analysis of the cinnamic acid turnover by CYP199A4 (black), cinnamic acid control (red), 4-hydroxycinnamic acid (purple), 3,4-dihydroxycinnamic acid (3,4-dihydroxycinnamic acid, green) and 3-hydroxycinnamic acid control (blue). Cinnamic acid (RT 19.1 min), 4-hydroxycinnamic acid (RT 11.0 min), 3-hydroxycinnamic acid (RT 12.6 min) and 3,4-dihydroxycinnamic acid (7.5 min). A zoomed in version of the likely product region is included to show the presence of low levels of 3- and 4-hydroxycinnamic acids. It should be noted that the level of the 3-hydroxycinnamic acid impurity in the cinnamic acid sample could account for the majority of this product in the turnover.



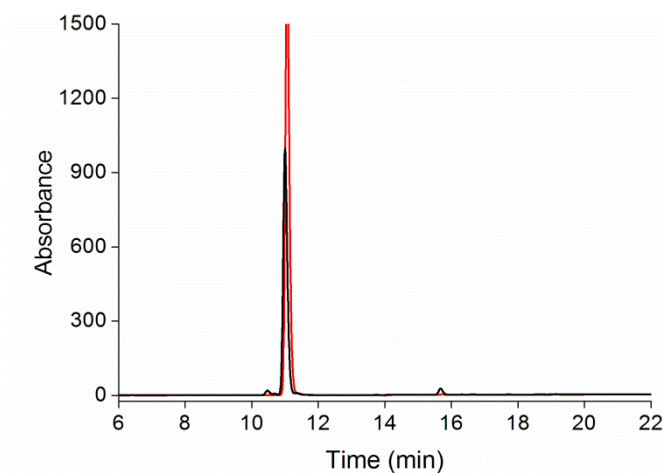
- (e) HPLC analysis of the 3-hydroxycinnamic acid turnover by CYP199A4 (black), 3,4-dihydroxycinnamic acid (3,4-dihydroxycinnamic acid, green) and 3-hydroxycinnamic acid control (blue). 3-Hydroxycinnamic acid, RT 12.6 min, and 3,4-dihydroxycinnamic acid, RT 7.5 min. A zoomed in version of the likely product region is included to show the presence of low levels of 3,4-dihydroxycinnamic acid.



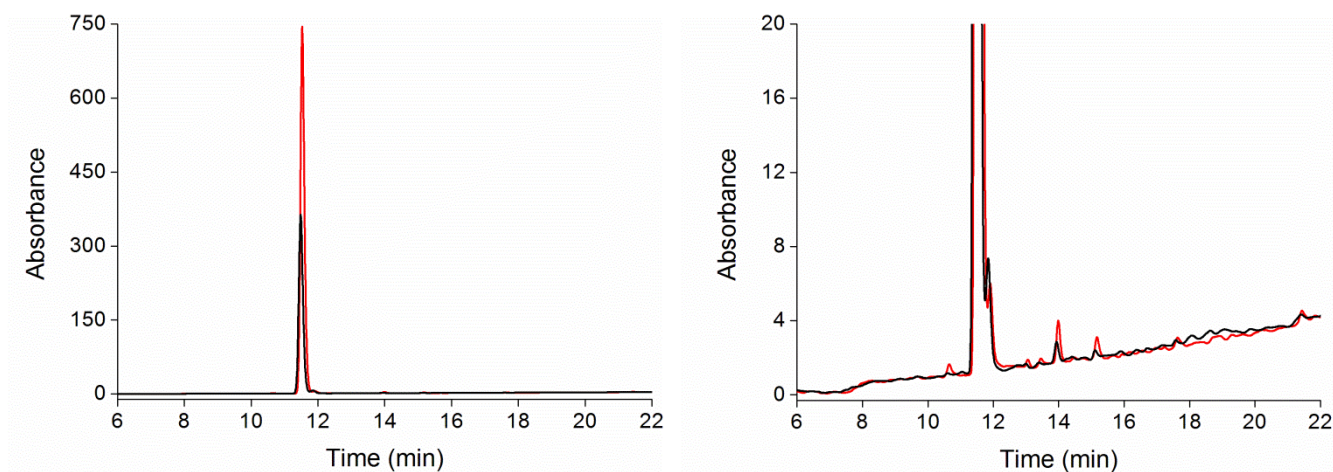
- (f) HPLC analysis of the 3,5-dimethoxycinnamic acid turnover by CYP199A4 (black) and 3,5-dimethoxycinnamic acid control (red). 3,5-Dimethoxycinnamic acid, RT 21.5 min. A zoomed in version of the likely product region is included to show the presence of low levels of a product at 13.9 min (3-hydroxy-5-methoxycinnamic acid).



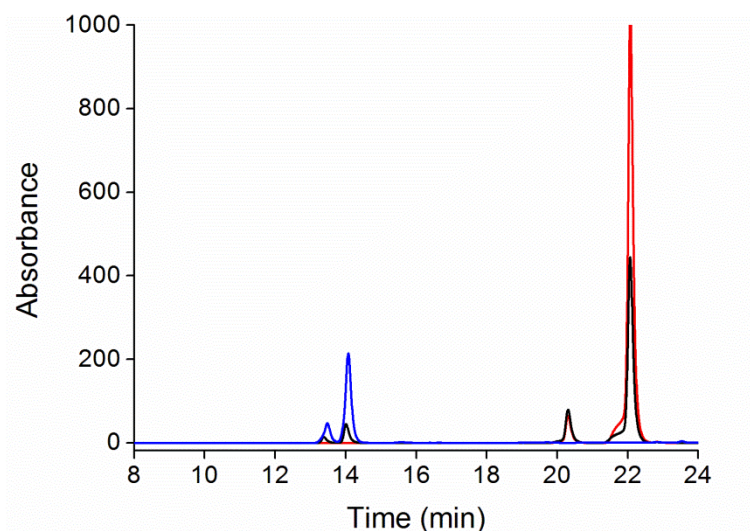
- (g) HPLC analysis of the 3,5-dimethoxy-4-hydroxycinnamic acid turnover by CYP199A4 (black) and 3,5-dimethoxy-4-hydroxycinnamic acid control (red). 3,5-Dimethoxy-4-hydroxycinnamic acid, RT 11.1 min. No potential product peaks are formed during the turnover.



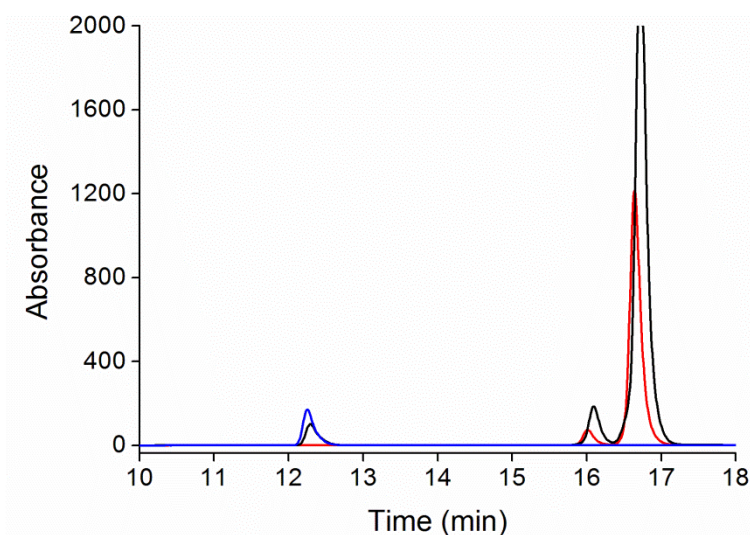
- (h) HPLC analysis of the 3-methoxy-4-hydroxycinnamic acid turnover by CYP199A4 (black) and 3-methoxy-4-hydroxycinnamic acid control (red). 3-Methoxy-4-hydroxycinnamic acid, RT 11.5 min. A zoomed in version of the turnover shows no potential products were observed during the turnover.



- (i) HPLC analysis of the turnover of 2,4-dimethoxycinnamic acid by CYP199A4 (black), 2,4-dimethoxycinnamic acid control (red) and 2-methoxy-4-hydroxycinnamic acid (blue). 2,4-Dimethoxycinnamic acid, RT 20.3 (*cis*) and 22.1 (*trans*) mins, and 2-methoxy-4-hydroxycinnamic acid, RT 13.4 (*cis*) and 14.0 (*trans*) mins.



- (j) HPLC analysis of the turnover of 3,4-dimethoxycinnamic acid turnover by CYP199A4 (black), 3,4-dimethoxycinnamic acid control (red) and 3-methoxy-4-hydroxycinnamic acid control (blue). 3,4-Dimethoxycinnamic acid, RT 16.0 (*cis*) and 16.7 (*trans*) mins, and 3-methoxy-4-hydroxycinnamic acid, RT 12.3 mins.



- (k) HPLC analysis of the turnover of 4-isopropylcinnamic acid by CYP199A4 (black) and 4-isopropylcinnamic acid control (red). 4-Isopropylcinnamic acid at 27.9 min. A zoomed in version of the product region is included to show the presence of the products, RT 7.6 min, 4-(1',2'-epoxyisopropyl)cinnamic acid; RT 14.5 min 4-(1'-hydroxyisopropyl)cinnamic acid; RT 15.0 min 4-(2'-hydroxyisopropyl)-cinnamic acid; and RT 27.5 min, 4-(prop-1'-en-2'-yl)cinnamic acid). *Impurity in the sample. Internal standard at 24.0 min.

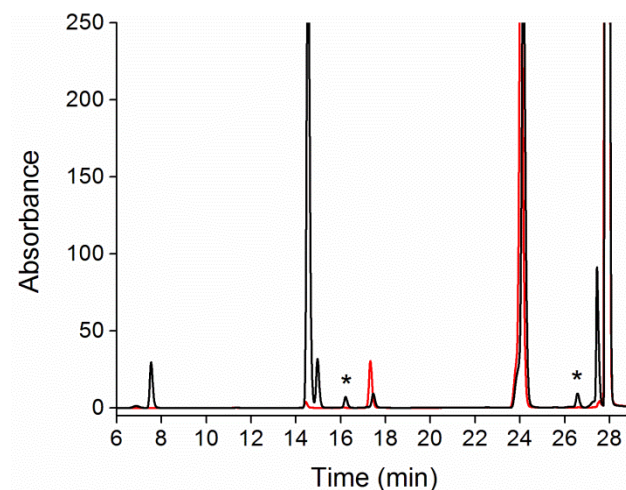
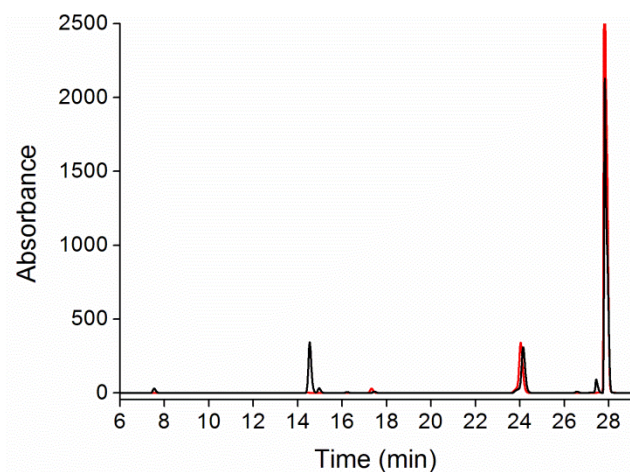
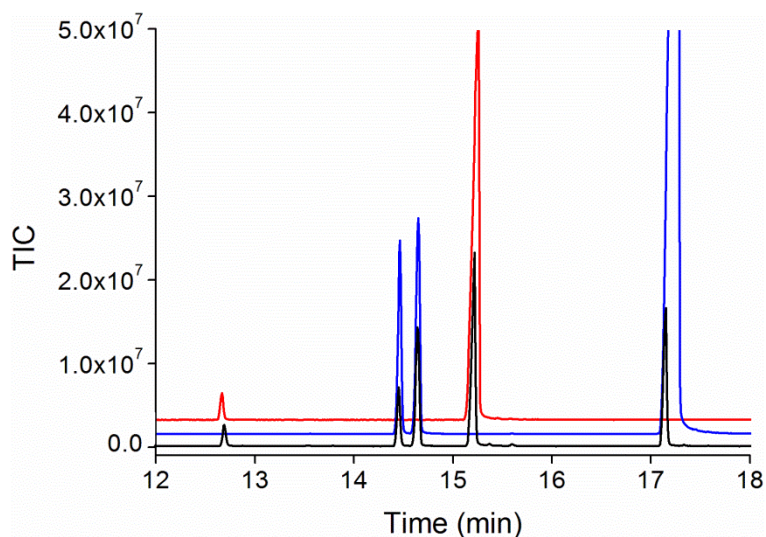
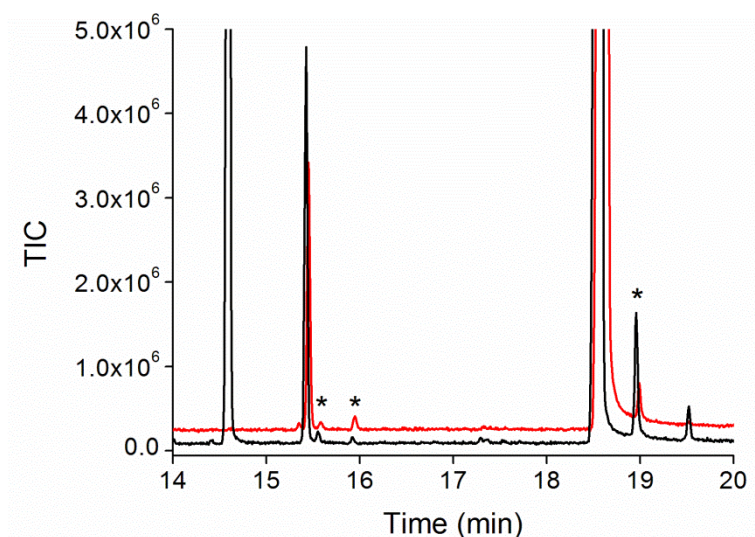


Figure S4 GC-MS analysis of the *in vitro* turnovers of various cinnamic acid derivatives.

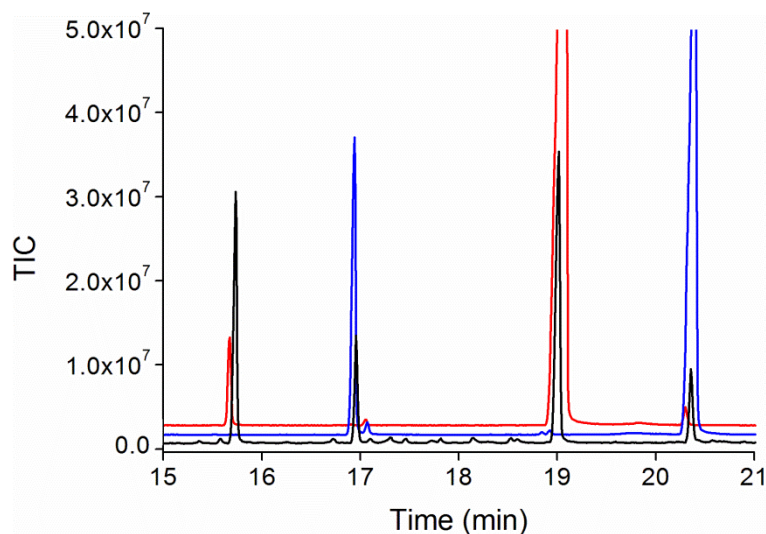
- (a) GC-MS analysis of the turnover of 4-methoxycinnamic acid by CYP199A4 (black), 4-methoxycinnamic acid substrate control (red) and 4-hydroxycinnamic acid product control (blue). 4-Methoxycinnamic acid, RT 12.7 (*cis*) and 15.3 (*trans*) mins, 4-hydroxycinnamic acid product, RT 14.4 (*cis*) and 17.1 (*trans*) mins, and internal standard, RT 14.6 min.



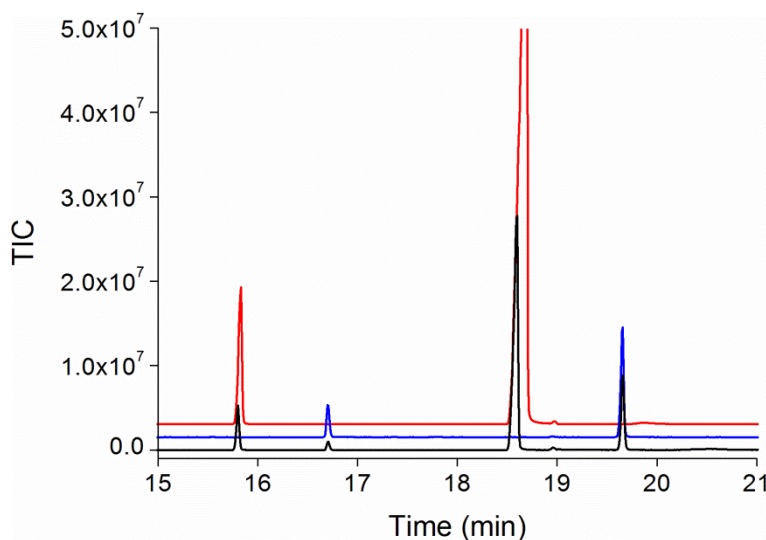
- (b) GC-MS analysis of the turnover of 3,5-dimethoxycinnamic acid by CYP199A4 (black), 3,5-dimethoxycinnamic acid control (red). 3,5-Dimethoxycinnamic acid, RT 15.4 (*cis*) and 18.6 (*trans*) mins, and internal standard, RT 14.6 min. Low levels of product (3-hydroxy-5-methoxycinnamic acid) are observed at 19.5 min. *Impurities in the sample.



- (c) GC-MS analysis of the turnover of 2,4-dimethoxycinnamic acid by CYP199A4 (black), 2,4-dimethoxycinnamic acid control (red) and 2-methoxy-4-hydroxycinnamic acid product control (blue). 2,4-Dimethoxycinnamic acid, RT 15.7 (*cis*) and 19.0 (*trans*) mins, and 2-methoxy-4-hydroxycinnamic acid, RT 17.0 (*cis*) and 20.4 (*trans*) mins.

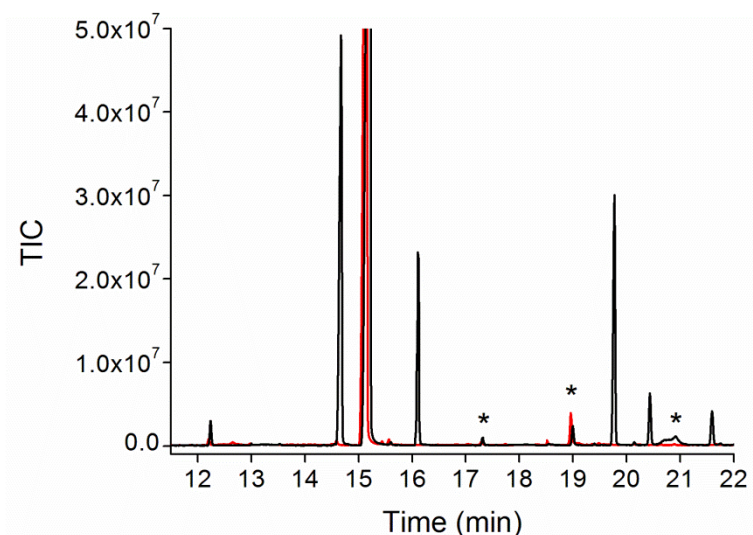


- (d) GC-MS analysis of the turnover of 3,4-dimethoxycinnamic acid by CYP199A4 (black), 2,4-dimethoxycinnamic acid control (red) and 3-methoxy-4-hydroxycinnamic acid (ferulic acid) product control (blue). 3,4-Dimethoxycinnamic acid, RT 15.8 (*cis*) and 18.6 (*trans*) mins, and 3-methoxy-4-hydroxycinnamic acid, RT 16.7 (*cis*) and 19.7 (*trans*) mins.



- (e) GC-MS analysis of the turnover of 4-isopropylcinnamic acid by CYP199A4 (black), 4-isopropylcinnamic acid control (red), RT 12.2 (*cis*) and 15.1 (*trans*) mins and internal standard at 14.6 mins.

Products are observed RT 16.1 min (4-(prop-1'-en-2'-yl)cinnamic acid), RT 19.7 min (4-(1'-hydroxyisopropyl)cinnamic acid), RT 20.4 min (4-(2'-hydroxyisopropyl)cinnamic acid) and RT 21.5 min (4-(1',2'-epoxyisopropyl)cinnamic acid). *Impurities in the sample.



- (f) GC-MS analysis of the turnover of 3,5-dimethoxy-4-hydroxycinnamic acid (sinapic acid) by CYP199A4 (black), 3,5-dimethoxy-4-hydroxycinnamic acid control (red). 3,5-Dimethoxy-4-hydroxycinnamic acid, RT 19.0 (*cis*) and 22.8 (*trans*) mins. No product is observed. *Impurity in the substrate control.

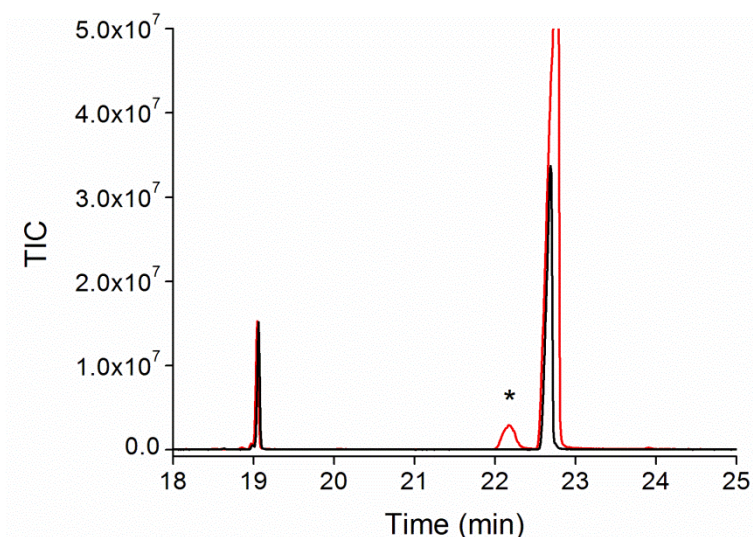
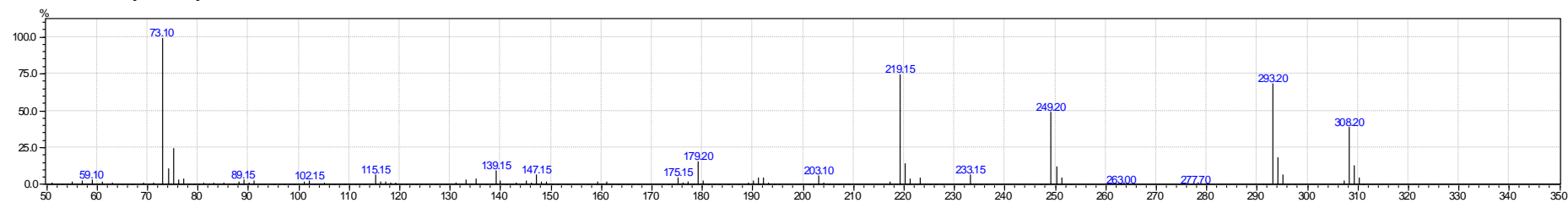
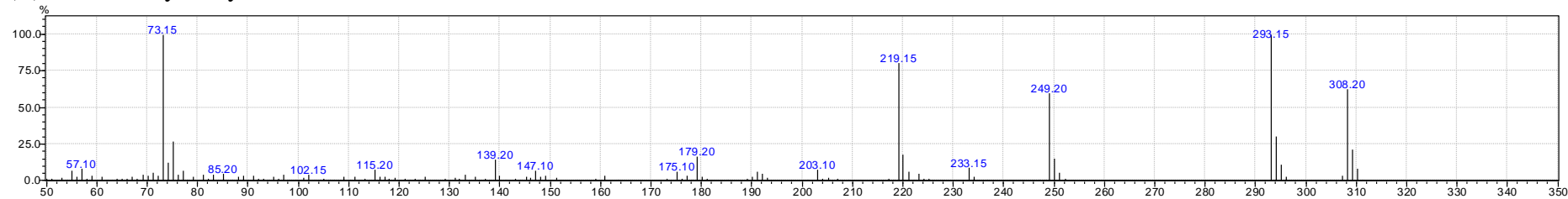


Figure S5 Mass spec analysis of the BSTFA/TMSCl derivatised products from the CYP199A4 turnover with (a) 4-methoxycinnamic acid (a)i *cis*-4-hydroxycinnamic acid product at 14.4 min (a)ii *trans*-product at 17.1 min (b) 3,5-dimethoxycinnamic acid (b)i 3-hydroxy-5-methoxycinnamic acid (c) 2,4-dimethoxycinnamic acid (c)i *cis*-2-methoxy-4-hydroxycinnamic acid product at 17.0 min (c)ii *trans*-product at 20.3 min (d) 3,4-dimethoxycinnamic acid (d)i *cis*-3-methoxy-4-hydroxycinnamic acid product at 16.7 min (d)ii *trans*-product at 19.7 min and (e) 4-isopropylcinnamic acid (e)i 4-(prop-1'-en-2'-yl)-cinnamic acid product at 16.1 min (e)ii 4-(1'-hydroxyisopropyl)-cinnamic acid product at 19.7 min (e)iii 4-(2'-hydroxyisopropyl)-cinnamic acid product at 20.4 min (e)iv 4-(1',2'-epoxyisopropyl)-cinnamic acid product at 21.5 min.

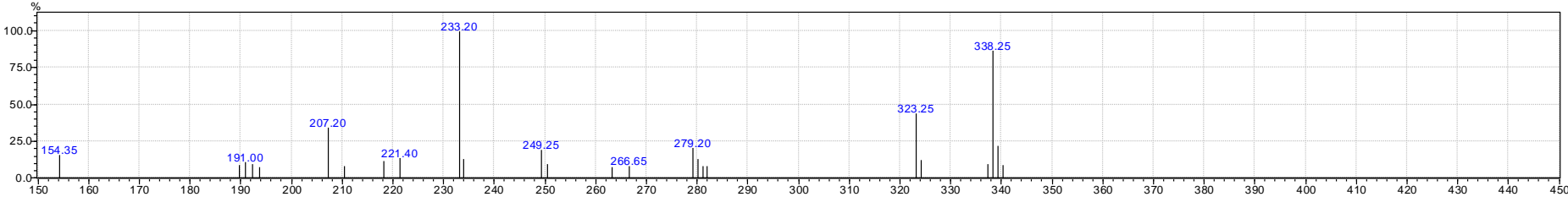
(a)i *cis*-4-hydroxycinnamic acid



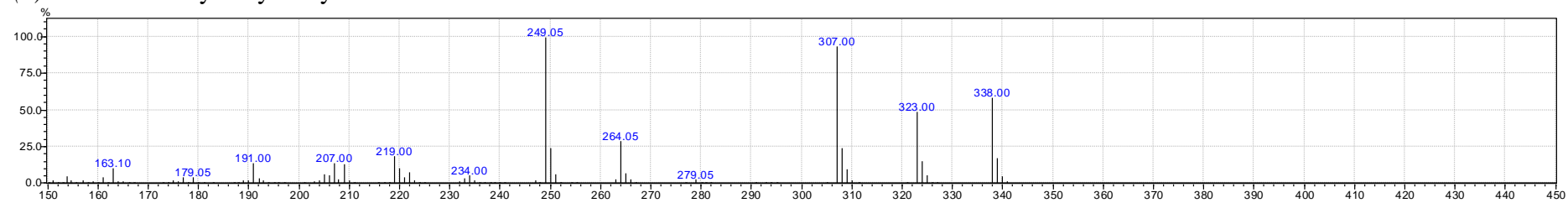
(a)ii *trans*-4-hydroxycinnamic acid



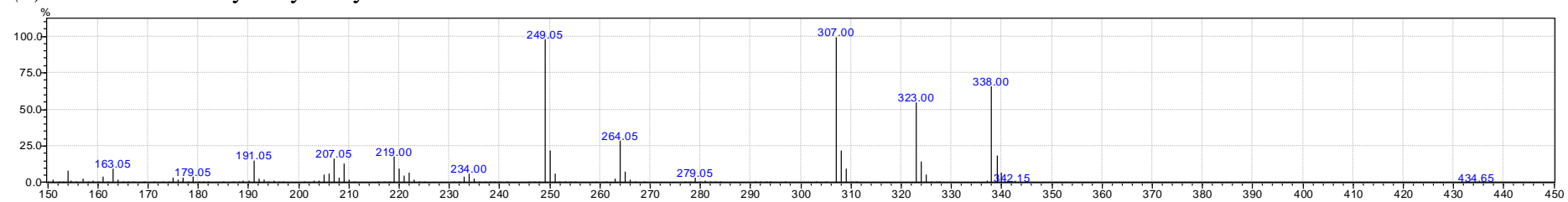
(b)i 3-hydroxy-5-methoxycinnamic acid



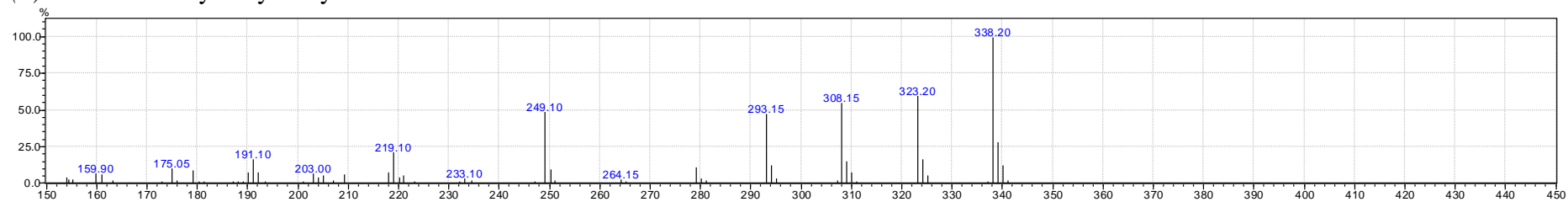
(c)i *cis*-2-methoxy-4-hydroxycinnamic acid



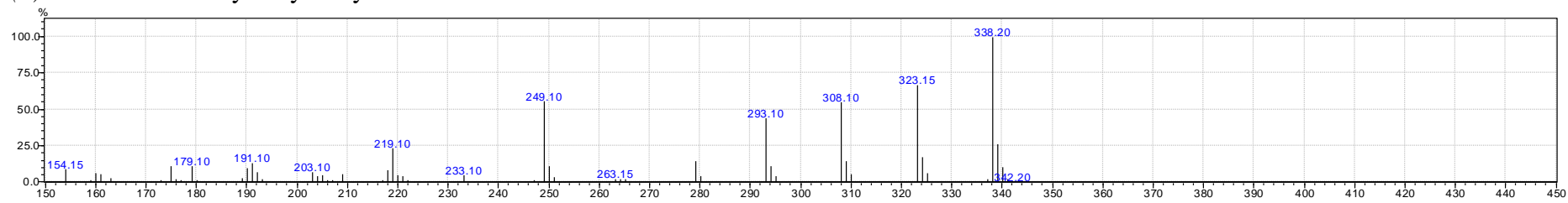
(c)ii *trans*-2-methoxy-4-hydroxycinnamic acid



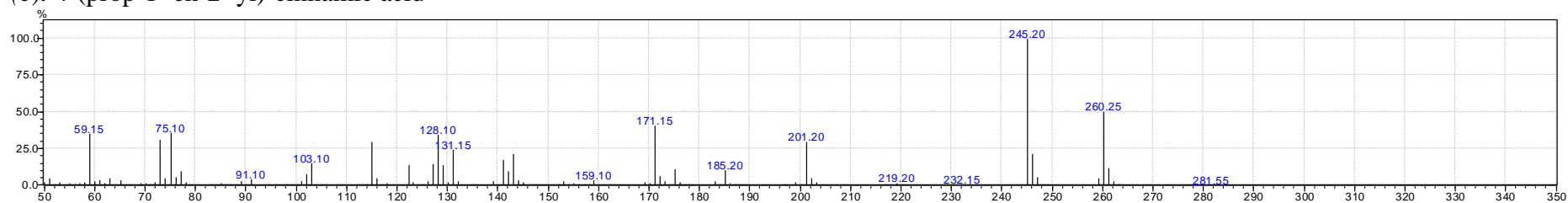
(d)i *cis*-3-methoxy-4-hydroxycinnamic acid



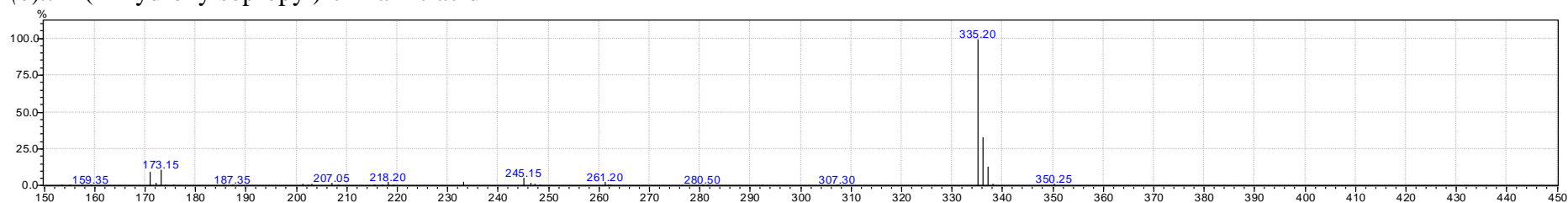
(d)ii *trans*-3-methoxy-4-hydroxycinnamic acid



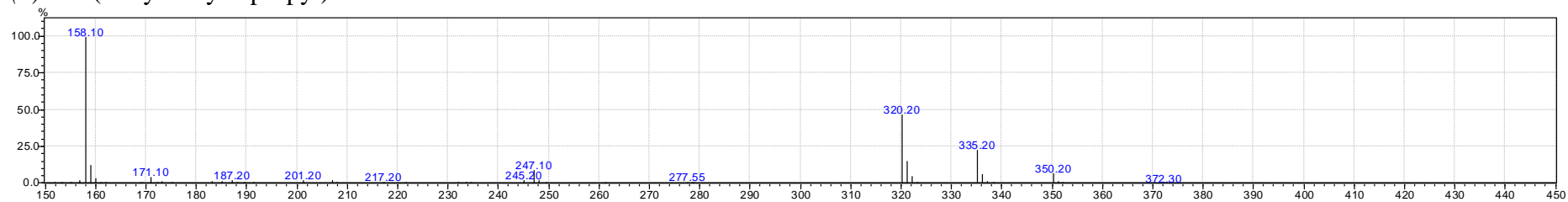
(e)i 4-(prop-1'-en-2'-yl)-cinnamic acid



(e)ii 4-(1'-hydroxyisopropyl)-cinnamic acid



(e)iii 4-(2'-hydroxyisopropyl)-cinnamic acid



(e)iv 4-(1',2'-epoxyisopropyl)-cinnamic acid

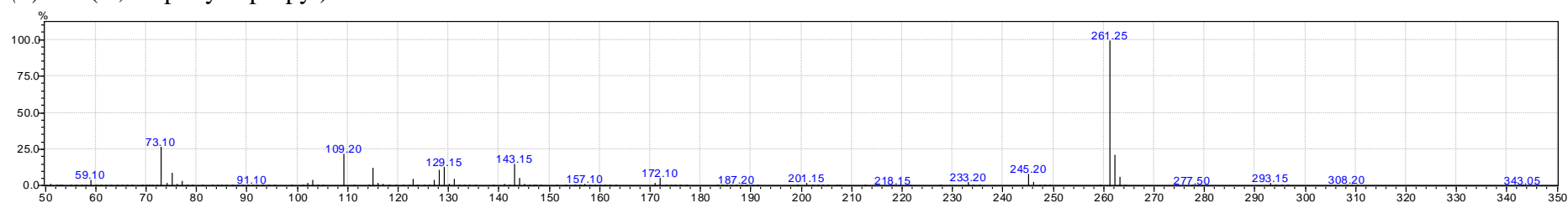
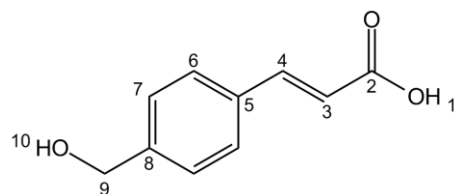


Figure S6 NMR analysis of the products

4-methylcinnamic acid turnover product

4-hydroxymethylcinnamic acid

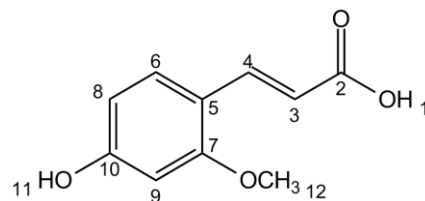
^1H NMR (500 MHz, DMSO) δ 7.63 (d, J = 7.9 Hz, 2H, H6), 7.57 (d, J = 16.0 Hz, 1H, H4), 7.35 (d, J = 7.9 Hz, 2H, H7), 6.49 (d, J = 16.0 Hz, 1H, H3), 5.25 (bs, 1H, H10), 4.52 (s, 2H, H9); ^{13}C NMR (125 MHz, DMSO) δ 167.64 (C2), 144.98 (C4), 143.82 (C8), 132.64 (C5), 128.02, (C7) 126.74 (C6), 118.55 (C3), 62.53 (C9).



2,4-dimethoxycinnamic acid turnover product

2-methoxy-4-hydroxycinnamic acid:

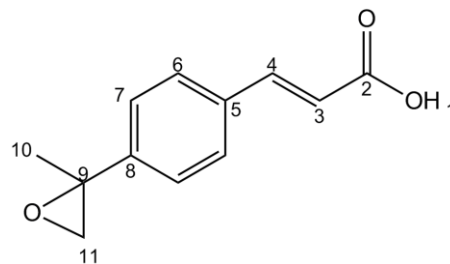
^1H NMR (500 MHz, DMSO) δ 10.05 (bs, 1H, H1), 7.73 (d, J = 16.1 Hz, 1H, H4), 7.48 (d, J = 8.5 Hz, 1H, H6), 6.44 (d, J = 2.2 Hz, 1H, H9), 6.40 (dd, J = 8.4, 2.2 Hz, 1H, H8), 6.29 (d, J = 16.0 Hz, 1H, H3), 3.81 (s, 3H, H12); ^{13}C NMR (125 MHz, DMSO) δ 168.32 (C2), 161.21 (C10), 159.50 (C7), 139.13 (C4), 130.03 (C6), 115.07 (C5), 113.81 (C3), 108.09 (C8), 99.04 (C9), 55.44 (C12).



4-isopropylcinnamic acid turnover products

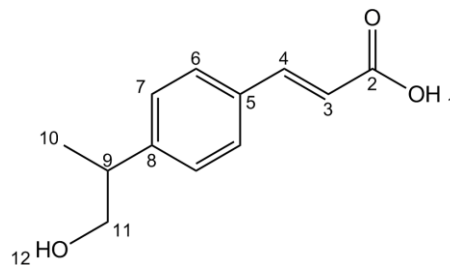
4-(1',2'-epoxyisopropyl)cinnamic acid:

^1H NMR (500 MHz, DMSO) δ 7.60 (d, J = 8.4 Hz, 2H, H6), 7.56 (d, J = 16.0 Hz, 1H, H4), 7.48 (d, J = 8.4 Hz, 2H, H7), 6.48 (d, J = 16.0 Hz, 1H, H3), 3.42 (d, J = 10.8 Hz, 1H, H11), 3.39 (d, J = 10.8 Hz, 1H, H11), 1.38 (s, 3H, H10); ^{13}C NMR (125 MHz, DMSO) δ 167.64 (C2), 149.94 (C4), 143.91 (C8), 132.09 (C5), 127.51 (C6), 126.08 (C7), 118.36 (C3), 73.71 (C9), 70.27 (C11), 25.96 (C10).



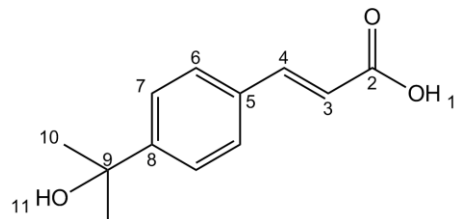
4-(2'-hydroxyisopropyl)cinnamic acid:

^1H NMR (500 MHz, DMSO) δ 7.59 (d, J = 8.2 Hz, 2H, H6), 7.54 (d, J = 16.0 Hz, 1H, H4), 7.26 (d, J = 8.2 Hz, 2H, H7), 6.46 (d, J = 16.0 Hz, 1H, H3), 3.50 (dd, J = 10.4, 6.5 Hz, 1H, H11), 3.44 (dd, J = 10.4, 6.5 Hz, 1H, H11), 2.87 - 2.77 (m, 1H, H9), 1.19 (d, J = 7.0 Hz, 3H, H10); ^{13}C NMR (125 MHz, DMSO) δ 167.64 (C2), 147.56 (C4), 143.92 (C8), 132.37 (C5), 128.07 (C6), 127.92 (C7), 118.21 (C3), 66.72 (C11), 41.91 (C9), 17.78 (C10).



4-(1'-hydroxyisopropyl)cinnamic acid:*

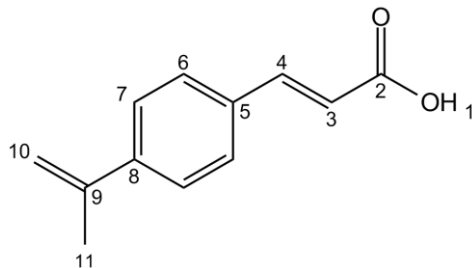
^1H NMR (500 MHz, DMSO) δ 7.66 (d, $J = 8.4$ Hz, 2H, H6), 7.60 (d, $J = 16.0$ Hz, 1H, H4), 7.49 (d, $J = 8.4$ Hz, 2H, H7), 6.67 (d, $J = 16.0$ Hz, 1H, H3), 1.42 (s, 6H, H10).

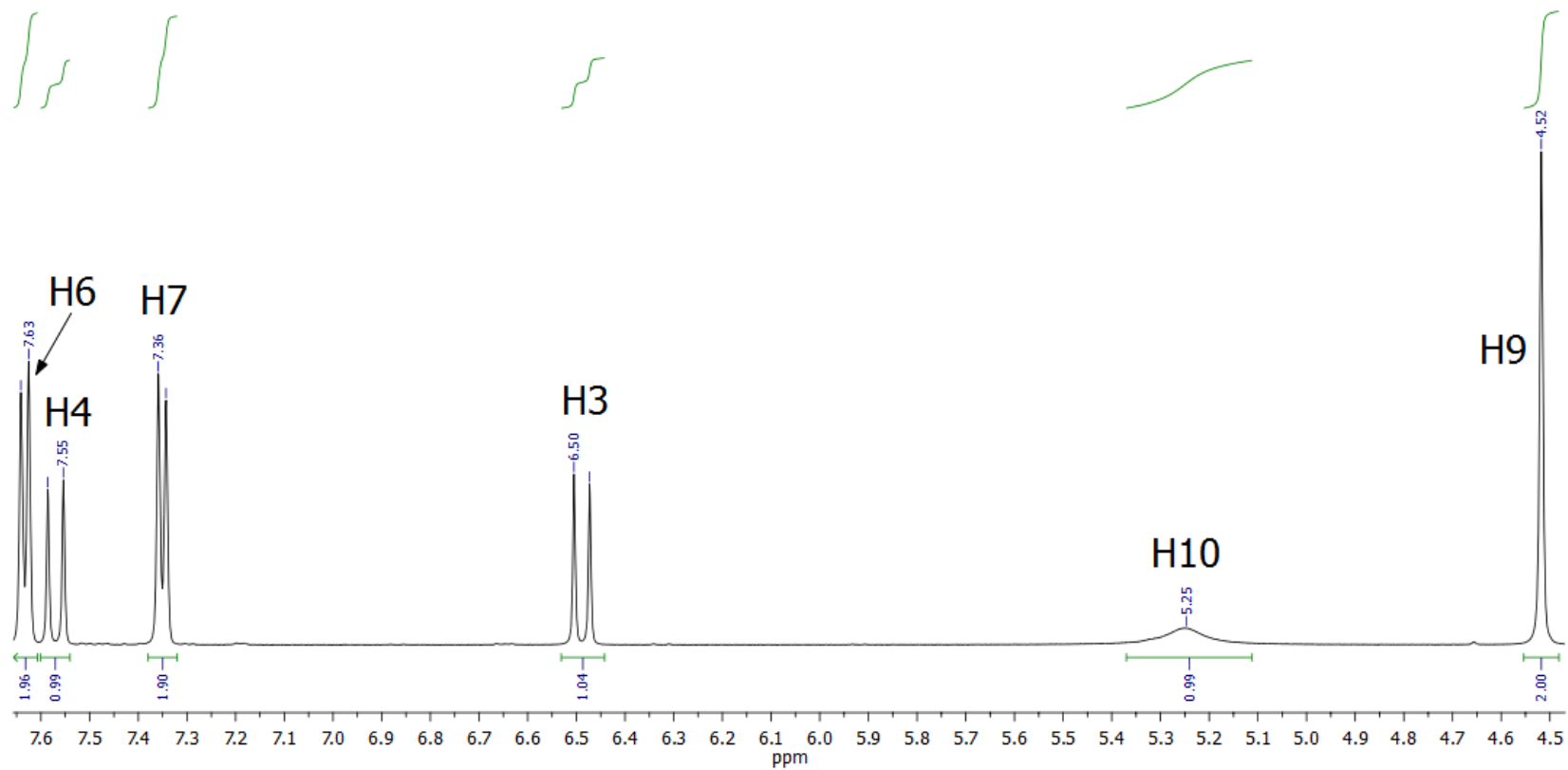


* After semi-prep HPLC purification, and NMR sample preparation the sample was found to contain significant levels of 4-(prop-1-en-2-yl)cinnamic acid suggesting that 4-(1-hydroxyisopropyl)cinnamic acid is unstable and can undergo a dehydration reaction to form the alkene.

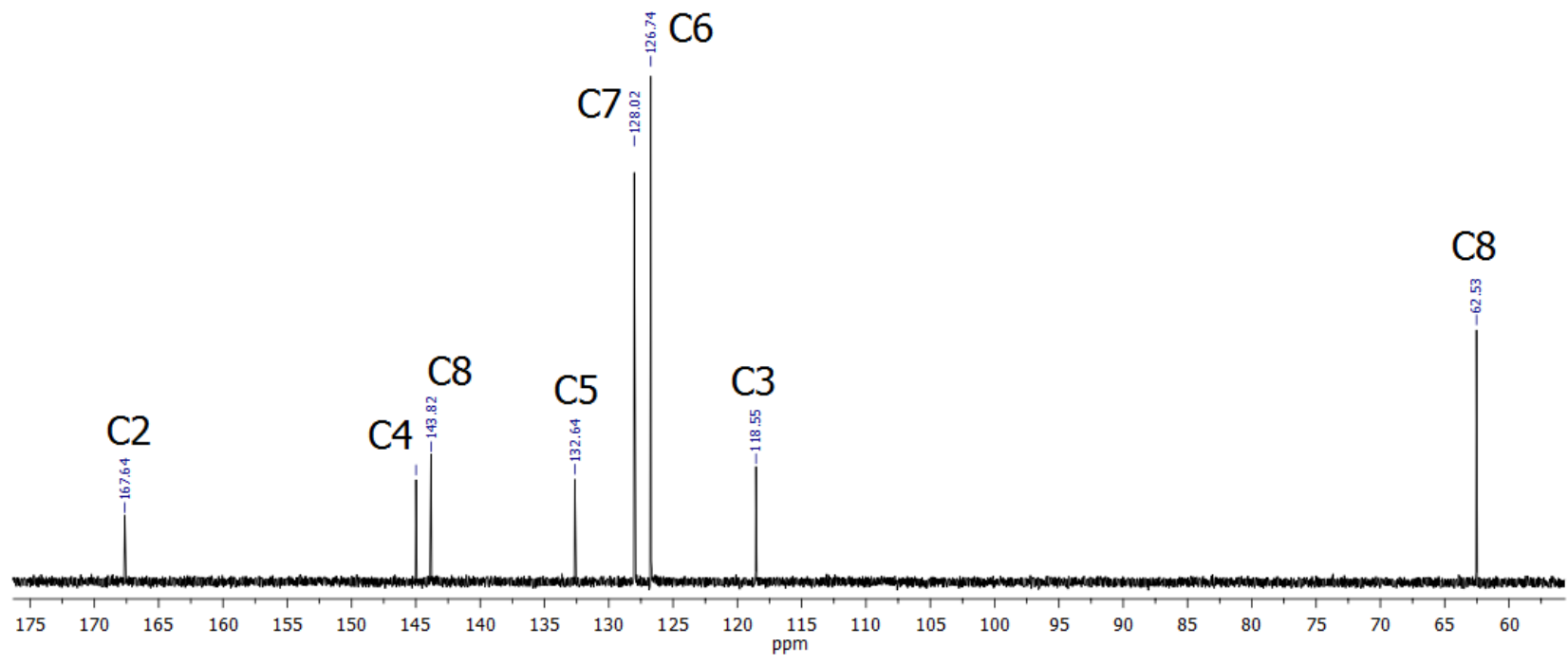
4-(prop-1'-en-2'-yl)cinnamic acid:

^1H NMR (500 MHz, DMSO) δ 7.62 (d, $J = 8.3$ Hz, 2H, H6), 7.57 (d, $J = 16.0$ Hz, 1H, H4), 7.34 (d, $J = 8.3$ Hz, 2H, H7), 6.52 (d, $J = 16.0$ Hz, 1H, H3), 5.52 (d, $J = 1.4$ Hz, 1H, H10), 5.16 (d, $J = 1.4$ Hz, 1H, H10), 2.12 (s, 3H, H11).

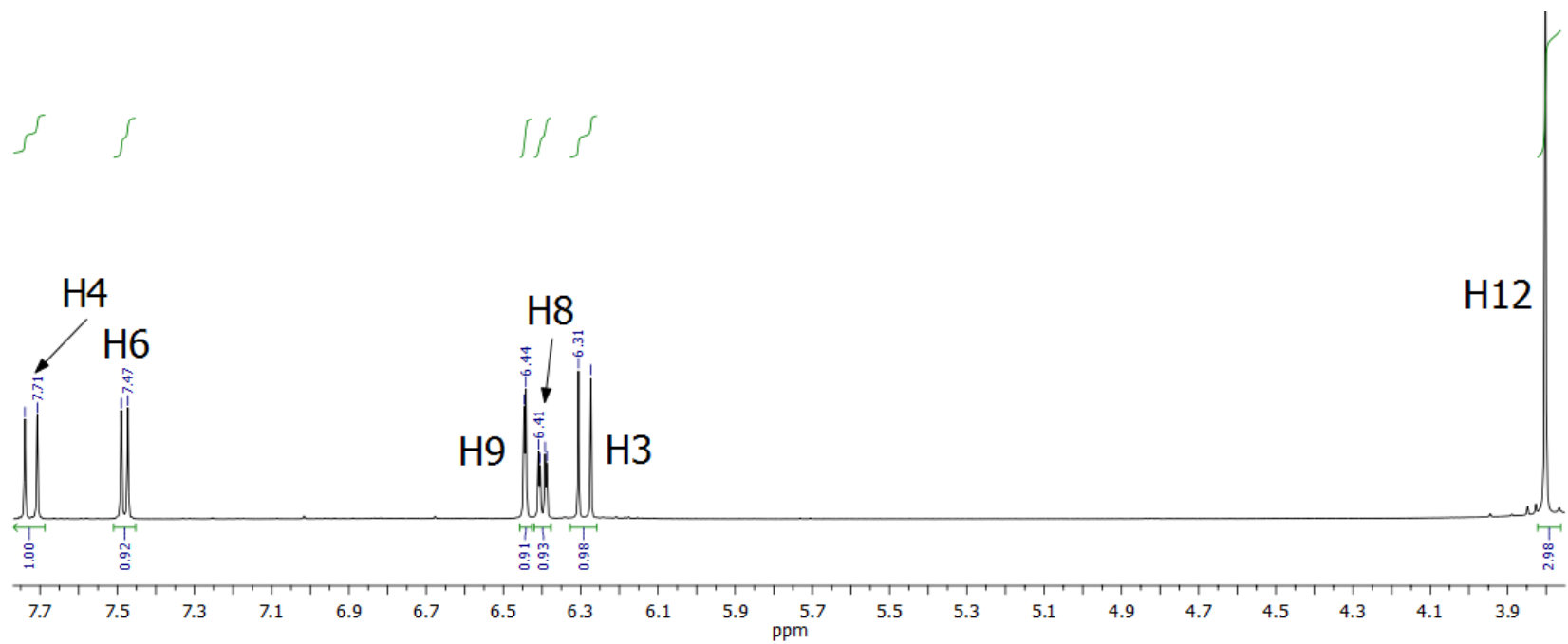




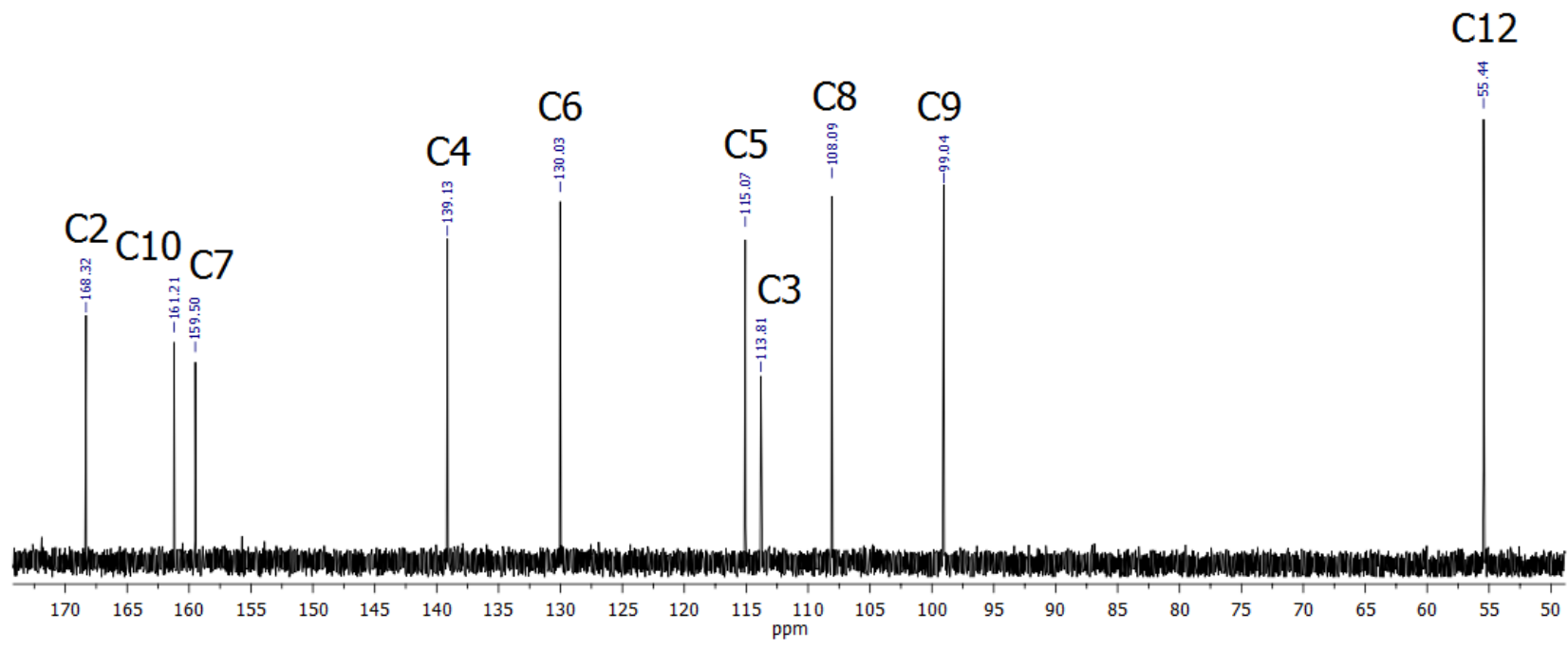
¹H NMR of 4-hydroxymethylcinnamic acid



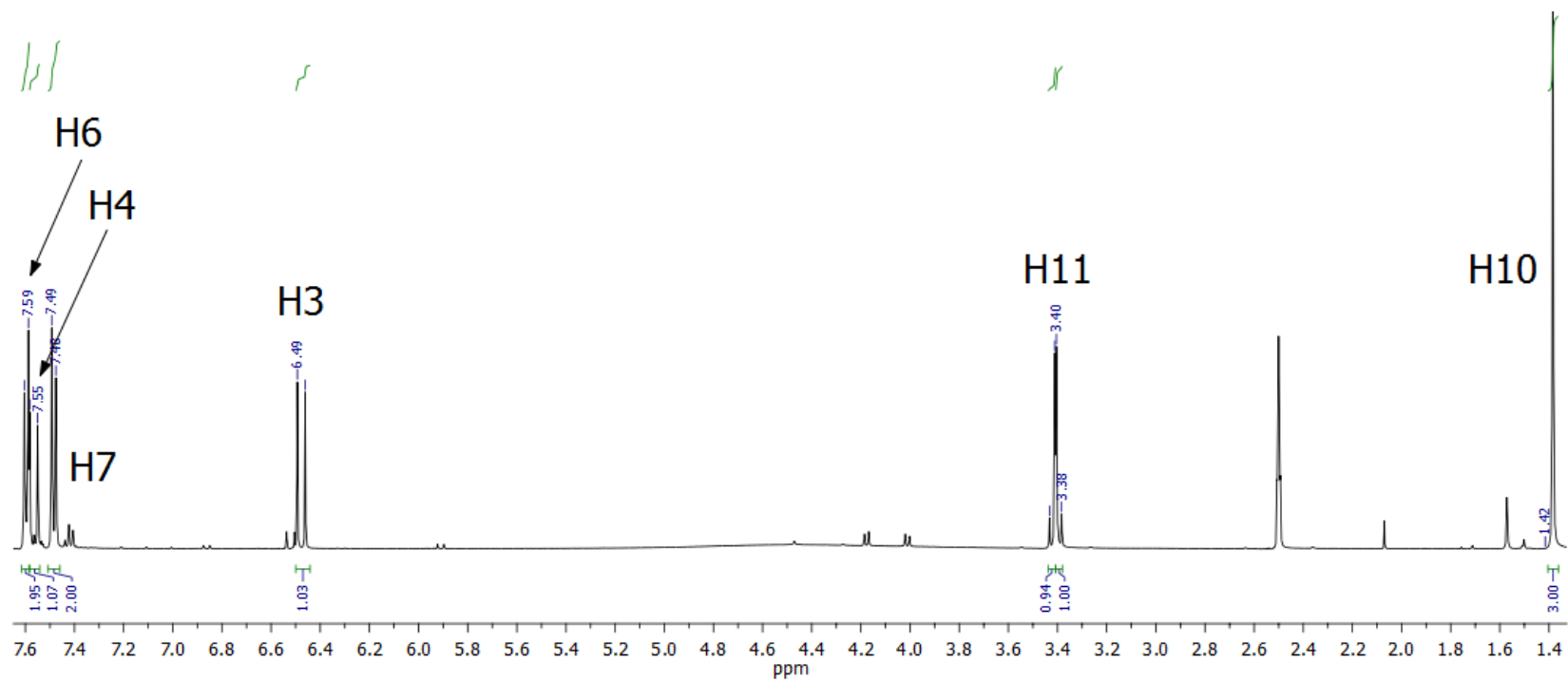
¹³C NMR of 4-hydroxymethylcinnamic acid



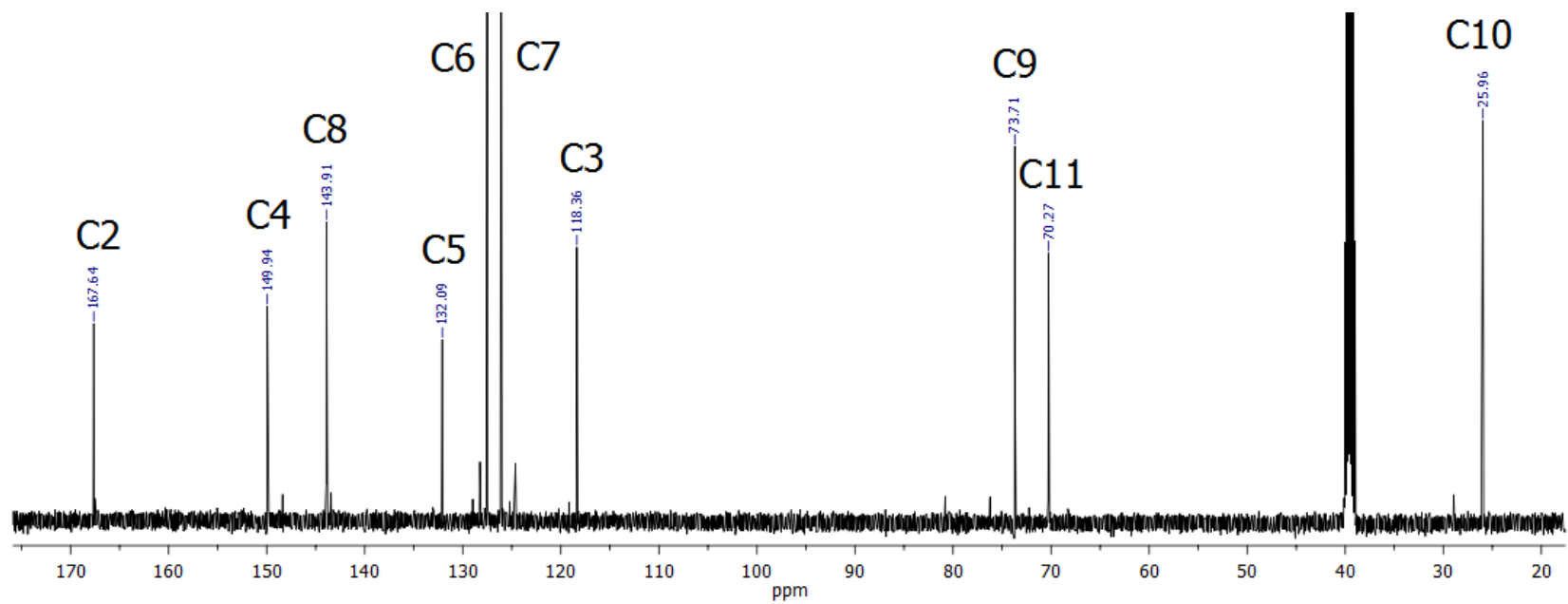
¹H NMR of 2-methoxy-4-hydroxycinnamic acid



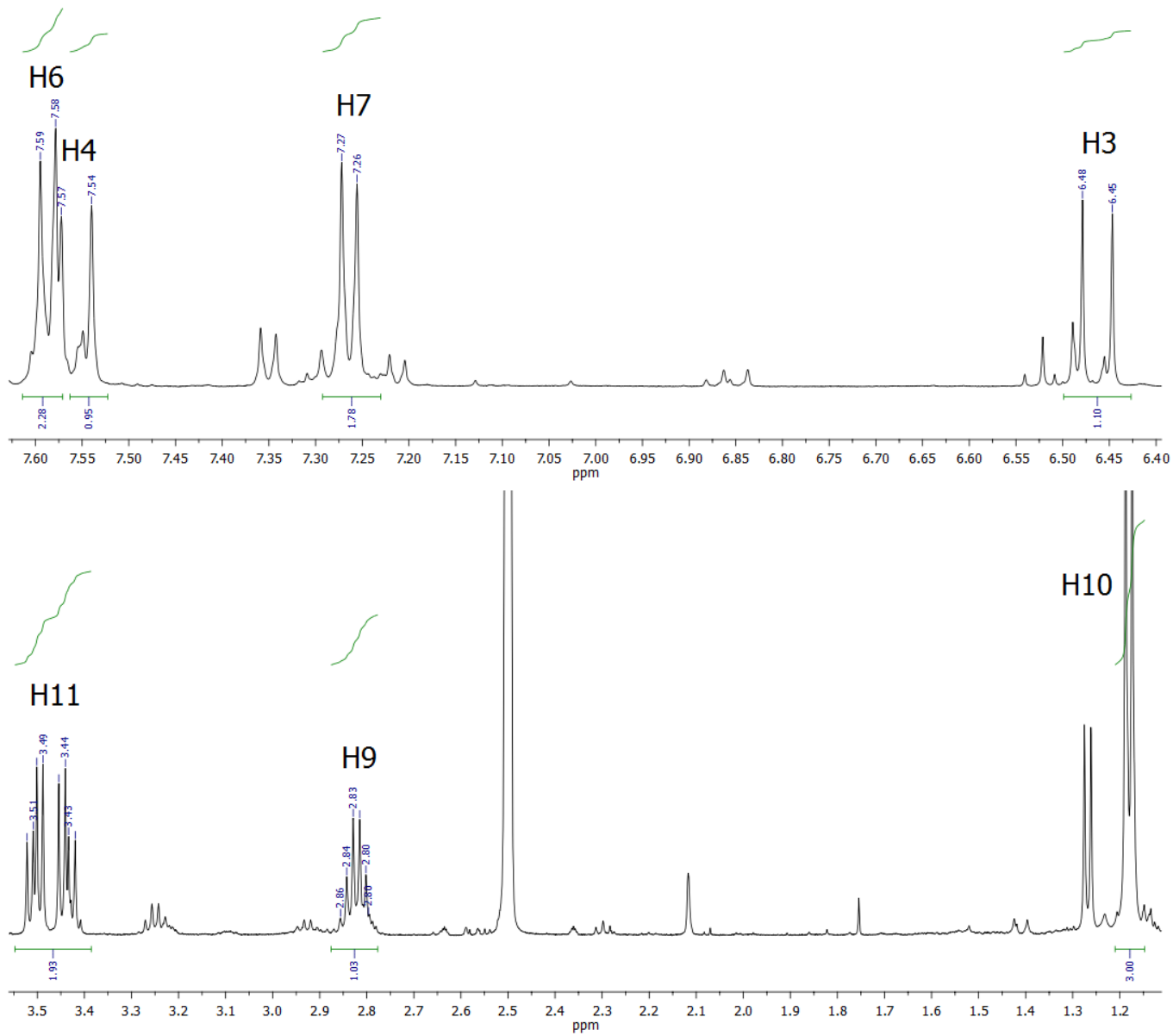
¹³C NMR of 2-methoxy-4-hydroxycinnamic acid



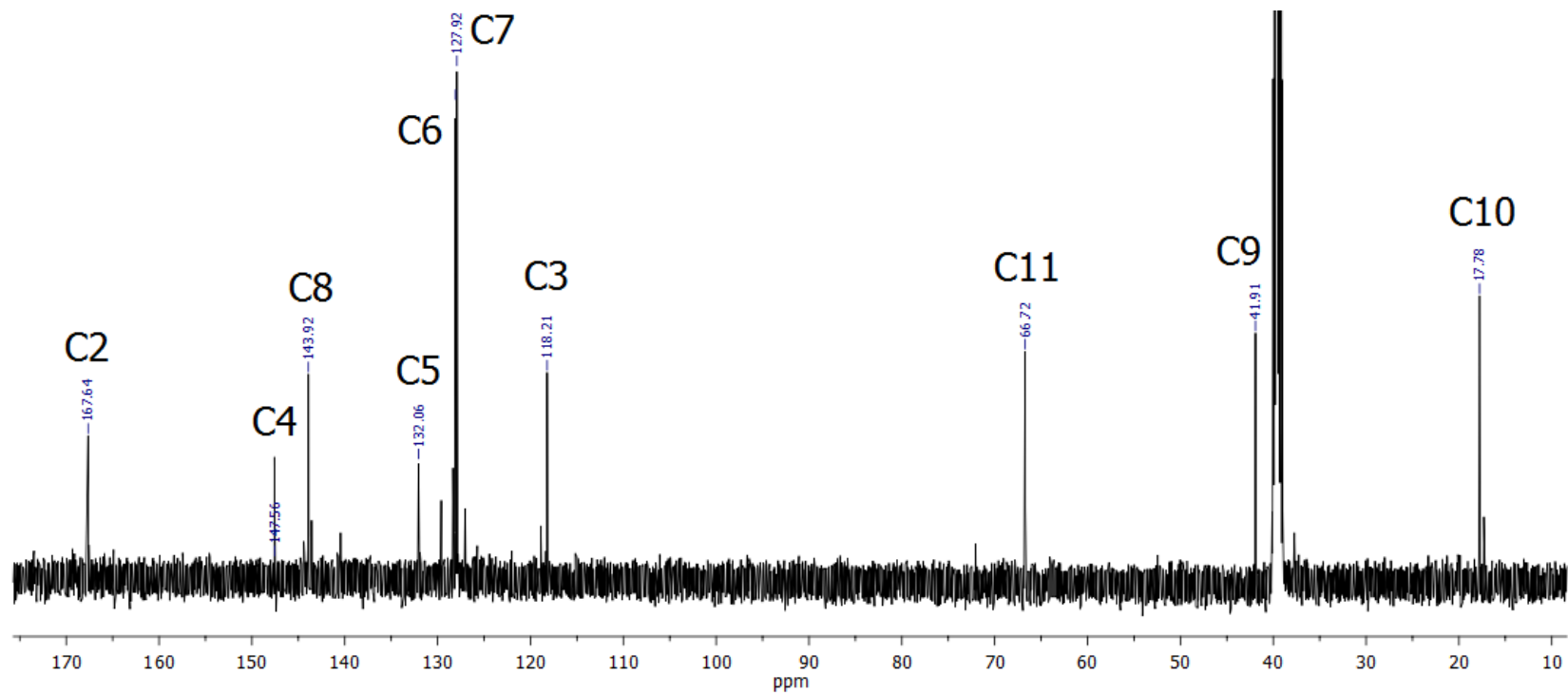
¹H NMR of 4-(1',2'-epoxyisopropyl)cinnamic acid



¹³C NMR of 4-(1',2'-epoxyisopropyl)cinnamic acid



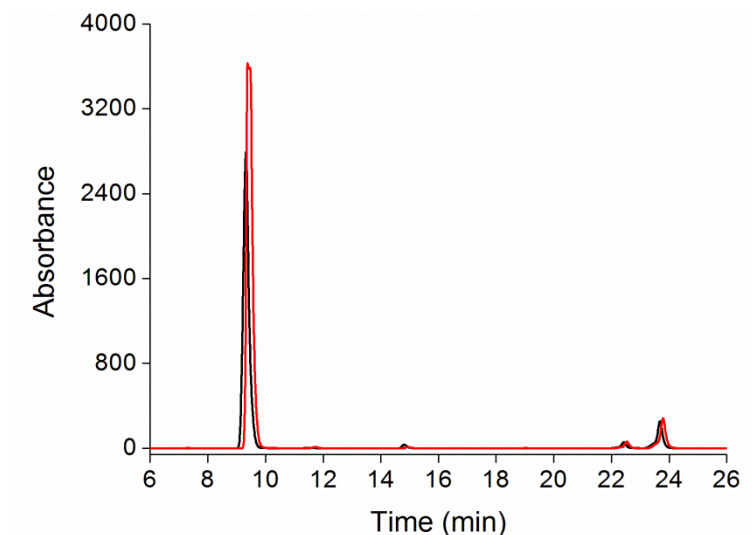
¹H NMR of 4-(2'-hydroxyisopropyl)cinnamic acid



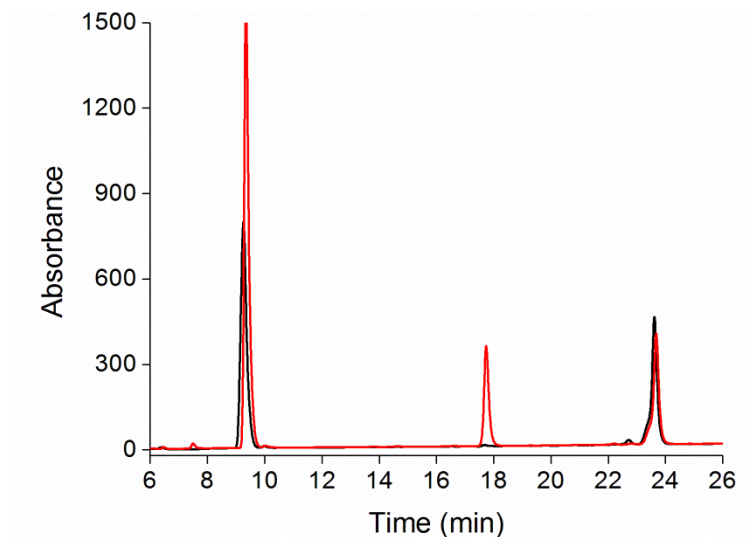
^{13}C NMR of 4-(2'-hydroxyisopropyl)cinnamic acid

Figure S7 HPLC analysis of the whole-cell oxidation of cinnamic acid derivatives; turnover at 4 hours (black), turnover overnight (red). 200 μ M 9-hydroxyfluorene standard, RT 23.8 min. Note there are minor changes in the retention times from the *in vitro* turnovers due to the use of a different C18 column.

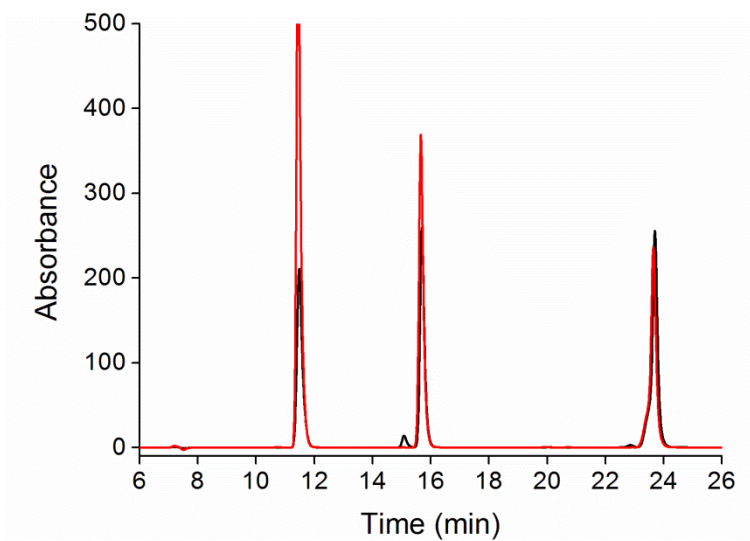
(a) 4-methylcinnamic acid – substrate, RT 22.8 min, product, RT 9.3 min.



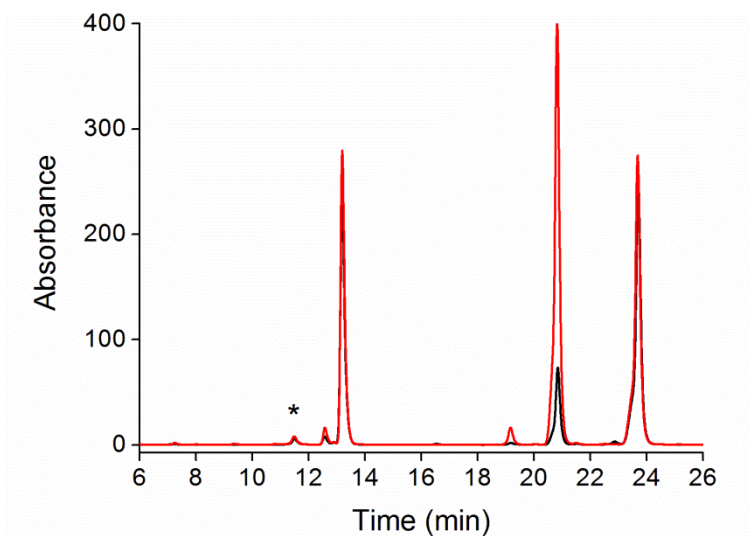
(b) 3-(4'-methoxyphenyl)propionic acid – substrate RT 18.1 min, product RT 9.6 min.



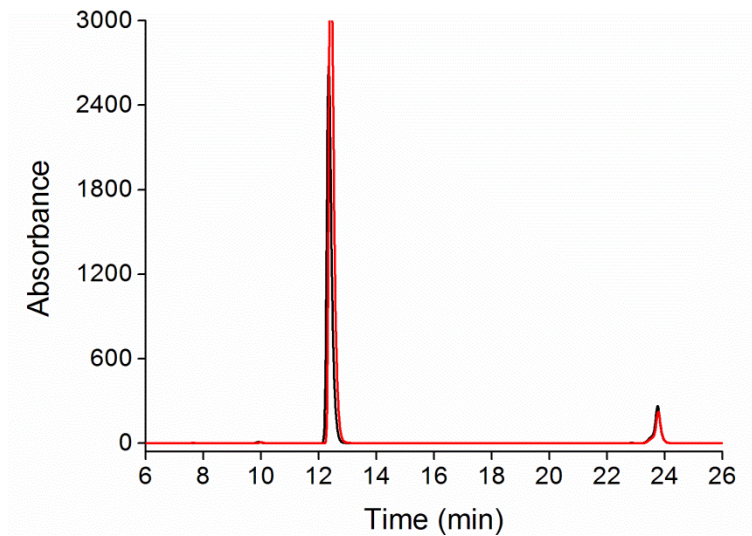
- (c) 3,4-dimethoxycinnamic acid – substrate RT 15.1 (*cis*) and 15.8 (*trans*) mins, product RT 11.5 min.



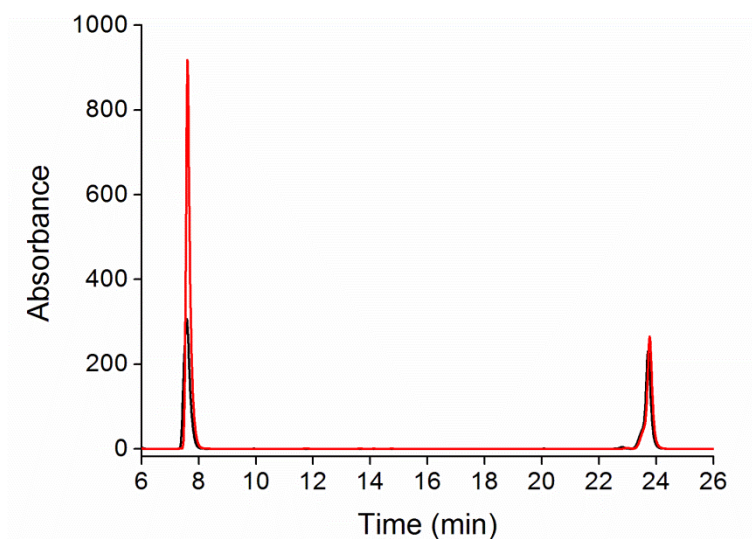
- (d) 2,4-dimethoxycinnamic acid – substrate at 19.2 (*cis*) and 20.8 mins (*trans*), product at 12.6 (*cis*) and 13.3 mins (*trans*). Impurity in the samples are labelled (*).



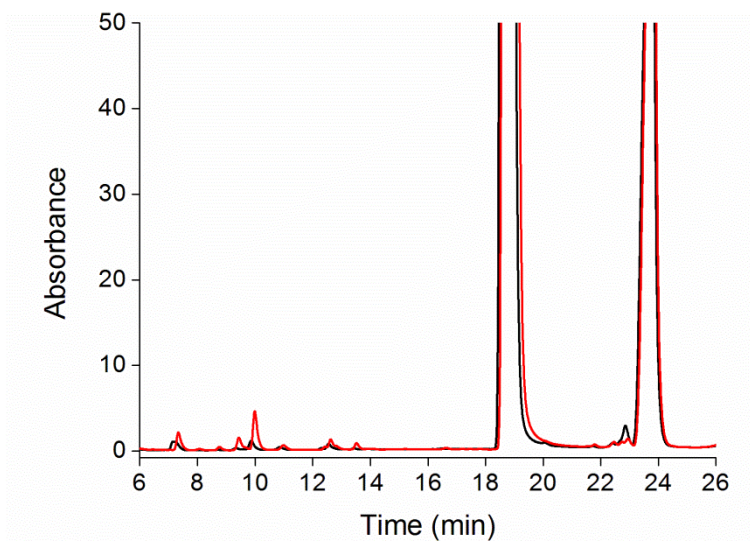
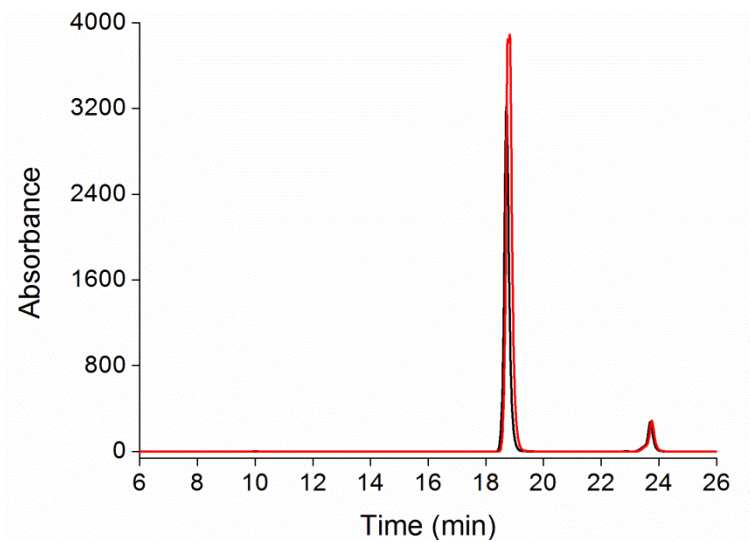
(e) 3-hydroxycinnamic acid – substrate RT 12.6 min (no product).



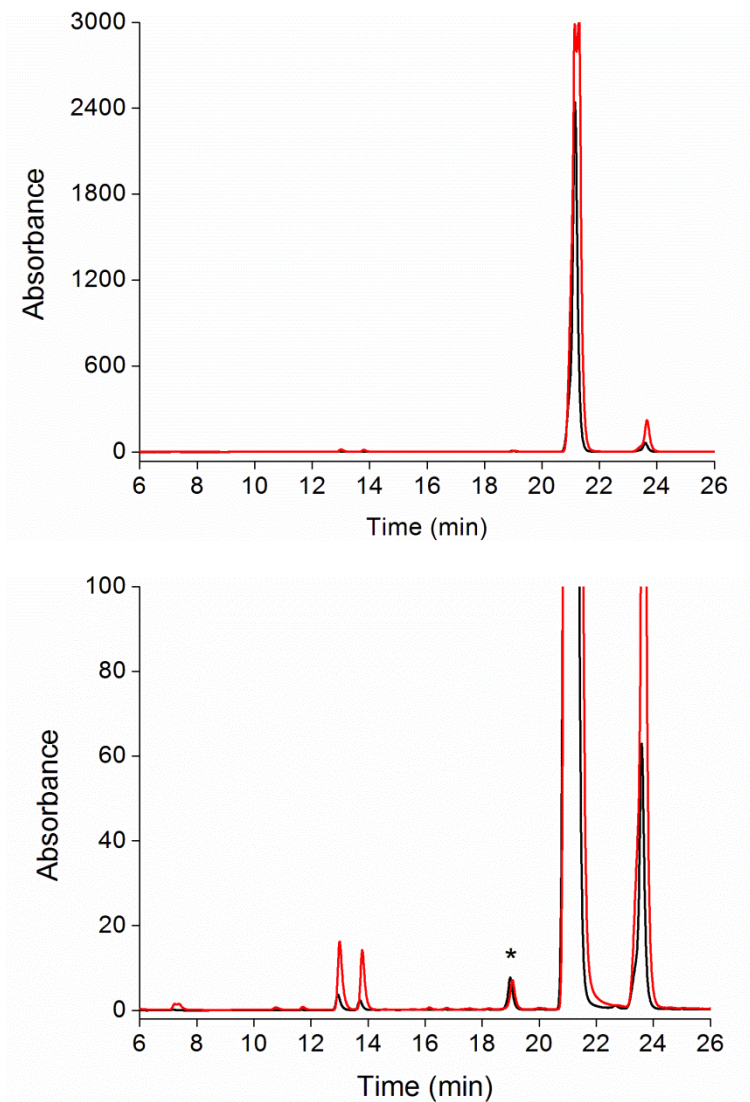
(g) 3-hydroxy-4-methoxycinnamic acid – no substrate remains, product at 7.5 min.



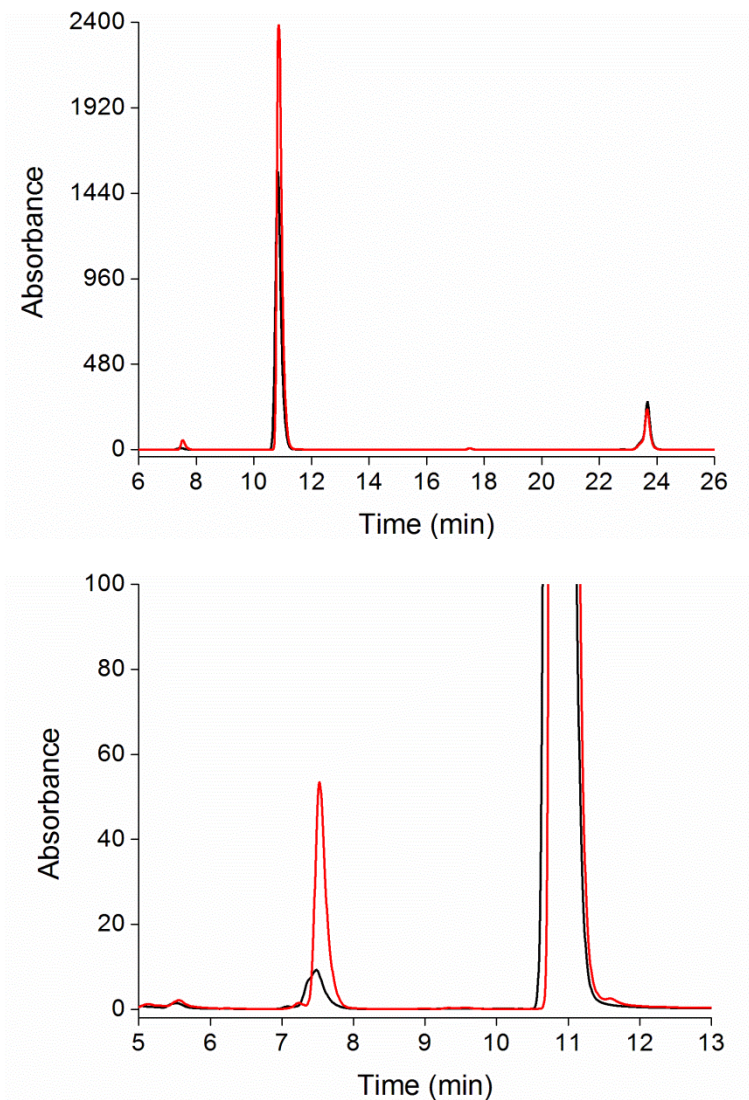
- (h) Cinnamic acid – substrate RT 18.9 min, very low levels of product at 7.5 min (3,4-dihydroxycinnamic acid), 11.0 min (4-hydroxycinnamic acid) and 12.5 min (3-hydroxycinnamic acid).



- (i) 3,5-dimethoxycinnamic acid – substrate RT 21.2 min, product 13.9 min (*trans*) a second peak RT 13.0 min was assigned as the *cis* isomer. Impurity in the samples are labelled (*).



(j) 4-hydroxycinnamic acid – substrate RT 11.0 min, product RT 7.5 min. A zoomed in version is shown below to highlight the product peak



(k) 4-methoxyphenylacetic acid – substrate RT 14.5 min, product RT 7.1 min.¹

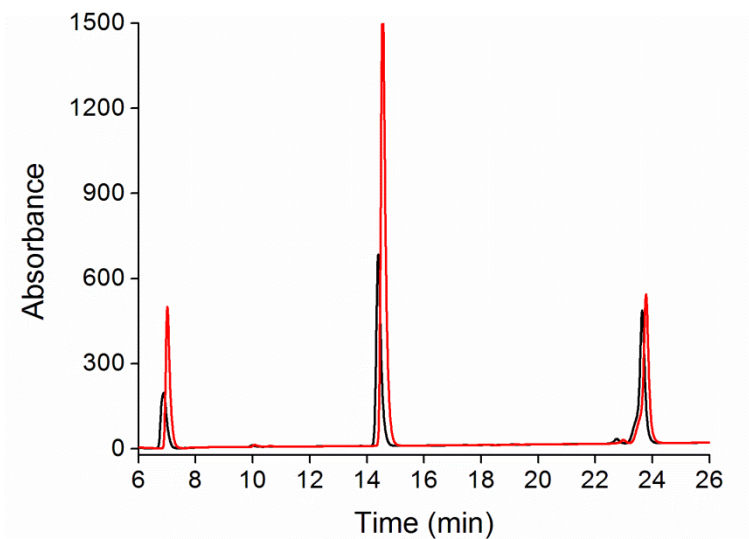
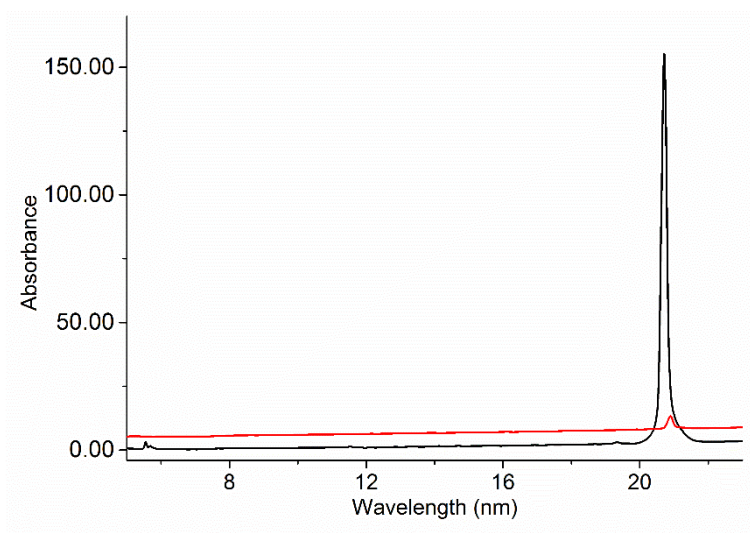
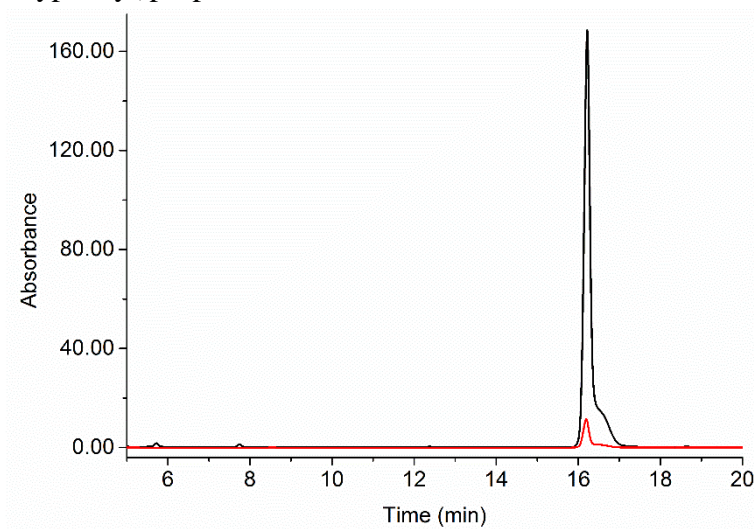


Figure S8 HPLC analysis of selected control whole-cell oxidation of cinnamic acid derivatives in systems which do not product the CYP199A4 enzyme; turnover (black), Note there are minor changes in the retention times due to the use of a different C18 column. There was no evidence of any product in any of the controls.

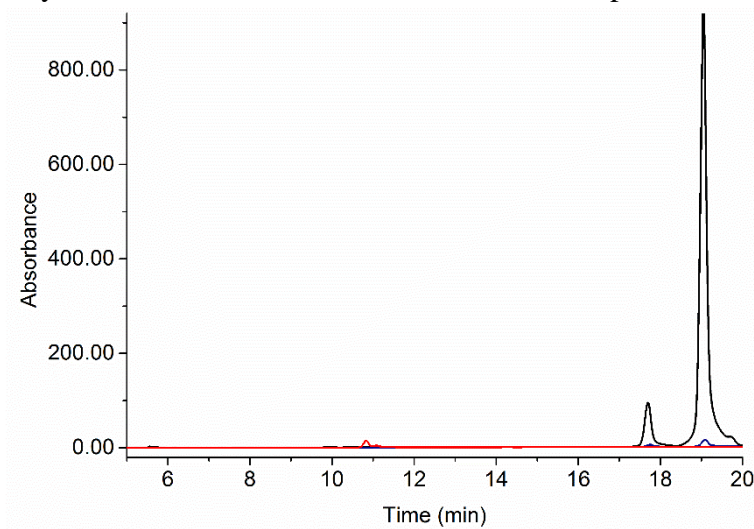
(a) 4-methylcinnamic acid. Substrate control is in red.



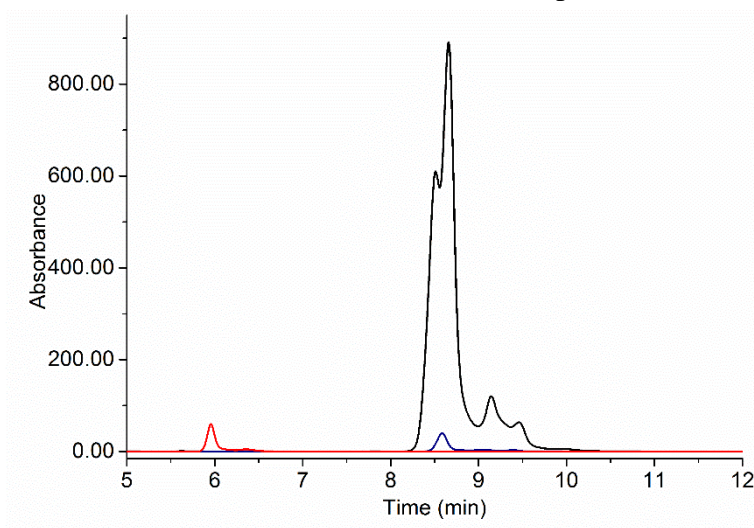
(b) 3-(4'-methoxyphenyl)propionic acid. Substrate control is in red.



(c) 2,4-dimethoxycinnamic acid. Substrate control is in blue, product control in red.



(d) 4-hydroxycinnamic acid. Substrate control is in blue, product control in red.



Reference

1. T. Coleman, R. R. Chao, J. De Voss and S. G. Bell, *Biochim. Biophys. Acta Proteins and Proteomics*, 2016, **1864**, 667-675.