Supplementary Materials

Molecules investigated in this study:

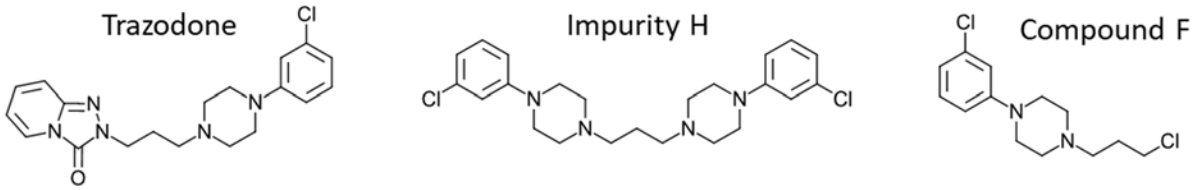

Figure S1. Chemical structures of trazodone and the synthesis side products impurity H (trazodone EP impurity H) and compound F (trazodone USP related compound F).

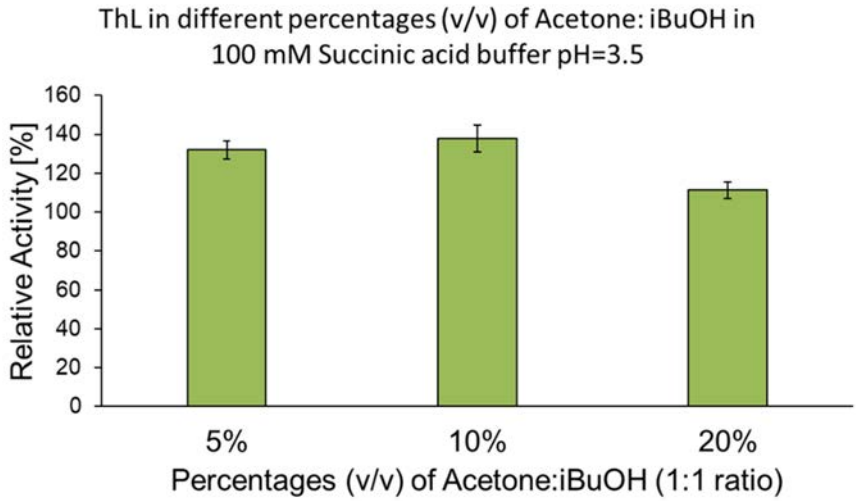

Figure S2. Relative activity of *Trametes hirsuta* laccase (ThL) in different mixtures of 100 mM succinic acid at pH 3.5 and 5, 10 and 20% (v/v) of acetone and isobutyl alcohol in a 1:1 ratio. The figure shows the mean ± SD.

Table S1. Amounts of mediator and solvents used for laccase-catalysed oxidation of trazodone, impurity H and compound F in 50 mM succinic acid buffer pH = 3.5. HBT was dissolved in the same buffer, as described in section 2.5. The reactions were carried out in duplicate for each compound.

N°	Sample name	HBT mediator [mM]	Each organic solvent [%]	Isobutyl alcohol [uL]	Acetone [uL]	HBT in succinic acid buffer [uL]	Laccase *ThL* [uL]	Aqueous buffer [uL]
1	**Blanks**	0	0	0	0	0	no enzyme	1000
2		5	0	0	0	200		800
3		10	0	0	0	400		600
4		15	0	0	0	600		400
5		0	2.5	25	25	0		950
6		5	2.5	25	25	200		750
7		10	2.5	25	25	400		550
8		15	2.5	25	25	600		350
9		0	5	50	50	0		900
10		5	5	50	50	200		700
11		10	5	50	50	400		500
12		15	5	50	50	600		300
13		0	10	100	100	0		800
14		5	10	100	100	200		600
15		10	10	100	100	400		400
16		15	10	100	100	600		200
17	**ThL_0%**	0	0	0	0	0	140	860
18		0	0	0	0	0	140	860
19		5	0	0	0	200	140	660
20		5	0	0	0	200	140	660
21		10	0	0	0	400	140	460
22		10	0	0	0	400	140	460
23		15	0	0	0	600	140	260
24		15	0	0	0	600	140	260
25	**ThL_5%**	0	2.5	25	25	0	140	810
26		0	2.5	25	25	0	140	810
27		5	2.5	25	25	200	140	610
28		5	2.5	25	25	200	140	610
29		10	2.5	25	25	400	140	410
30		10	2.5	25	25	400	140	410
31		15	2.5	25	25	600	140	210
32		15	2.5	25	25	600	140	210
33	**ThL_10%**	0	5	50	50	0	140	760
34		0	5	50	50	0	140	760
35		5	5	50	50	200	140	560
36		5	5	50	50	200	140	560
37		10	5	50	50	400	140	360
38		10	5	50	50	400	140	360
39		15	5	50	50	600	140	160
40		15	5	50	50	600	140	160
41	**ThL_20%**	0	10	100	100	0	140	660
42		0	10	100	100	0	140	660
43		5	10	100	100	200	140	460
44		5	10	100	100	200	140	460
45		10	10	100	100	400	140	260
46		10	10	100	100	400	140	260
47		15	10	100	100	600	140	60
48		15	10	100	100	600	140	60

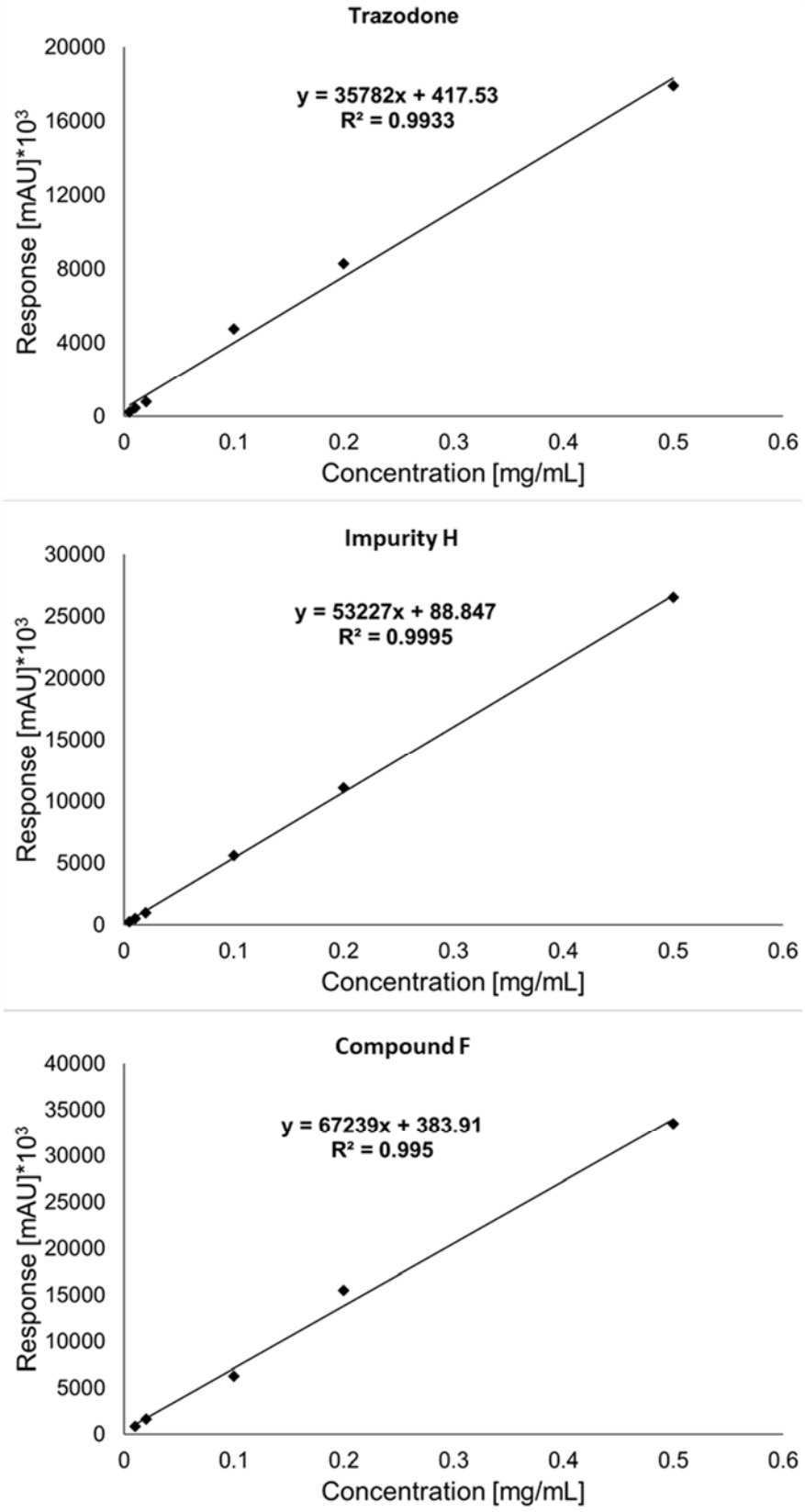

Figure S3. Standard curves for quantification of trazodone, impurity H and compound F by using HPLC-UV.

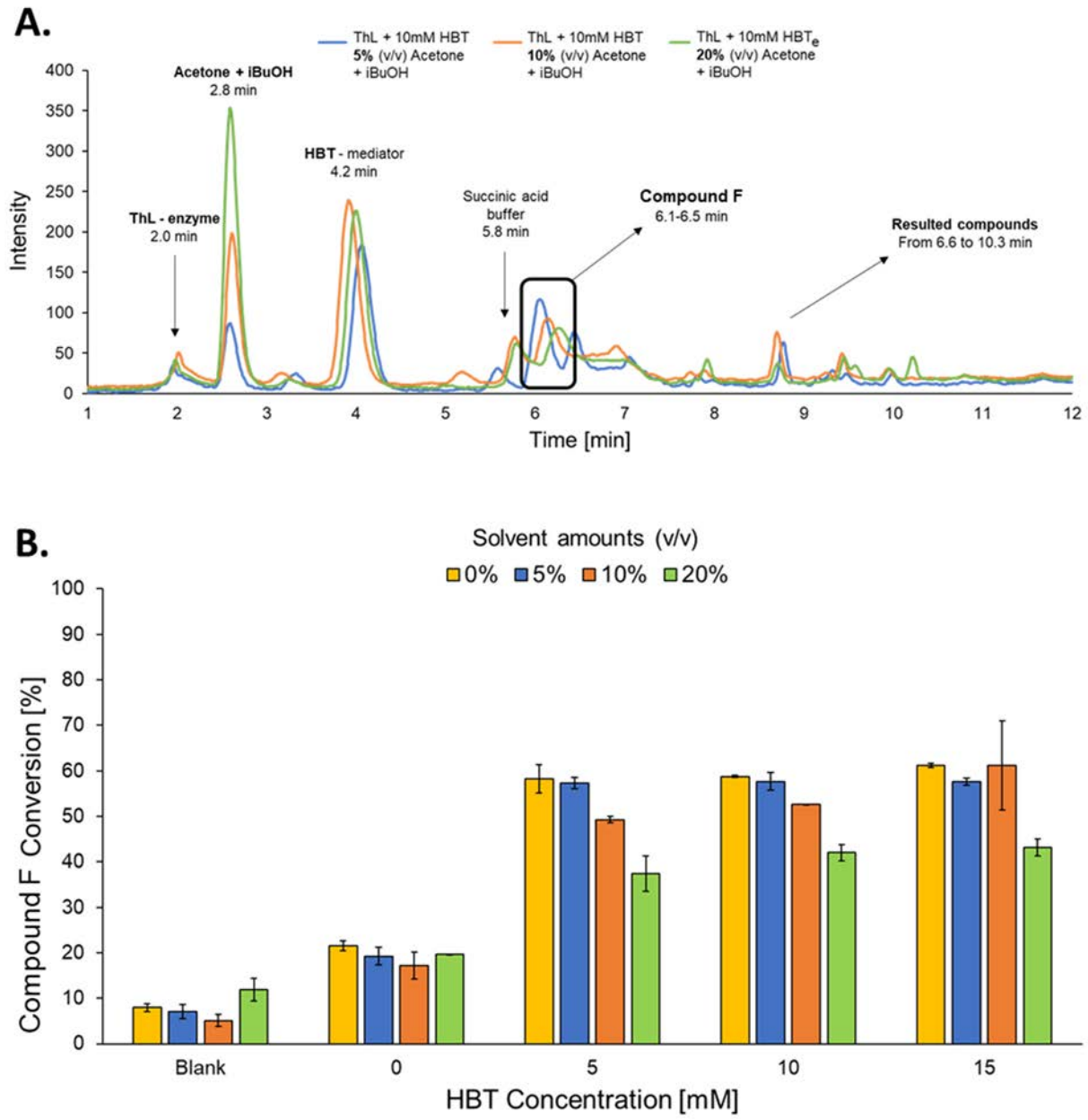

Figure S4. Oxidation of the trazodone synthesis side products compound F by a laccase from *Trametes hirsuta* in the presence of various concentrations of the mediator HBT and the solvents acetone and isobutyl alcohol (in a 1:1 ratio). A) HPLC chromatogram and B) Conversion. The figure shows the mean ± SD.

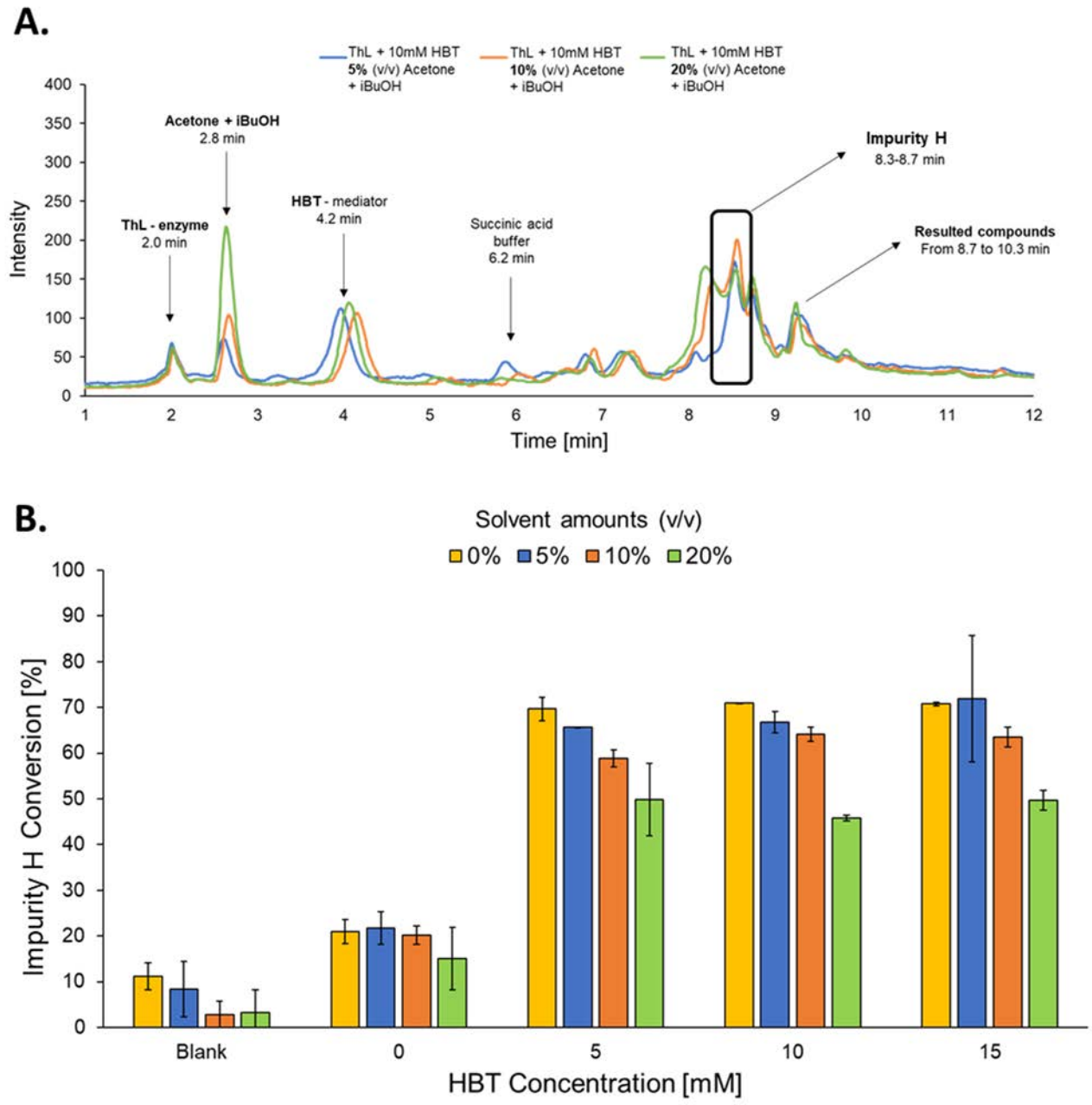

Figure S5. Oxidation of the trazodone synthesis side products impurity H by a laccase from *Trametes hirsuta* in the presence of various concentrations of the mediator HBT and the solvents acetone and isobutyl alcohol (in a 1:1 ratio). A) HPLC chromatogram and B) Conversion. The figure shows the mean ± SD.

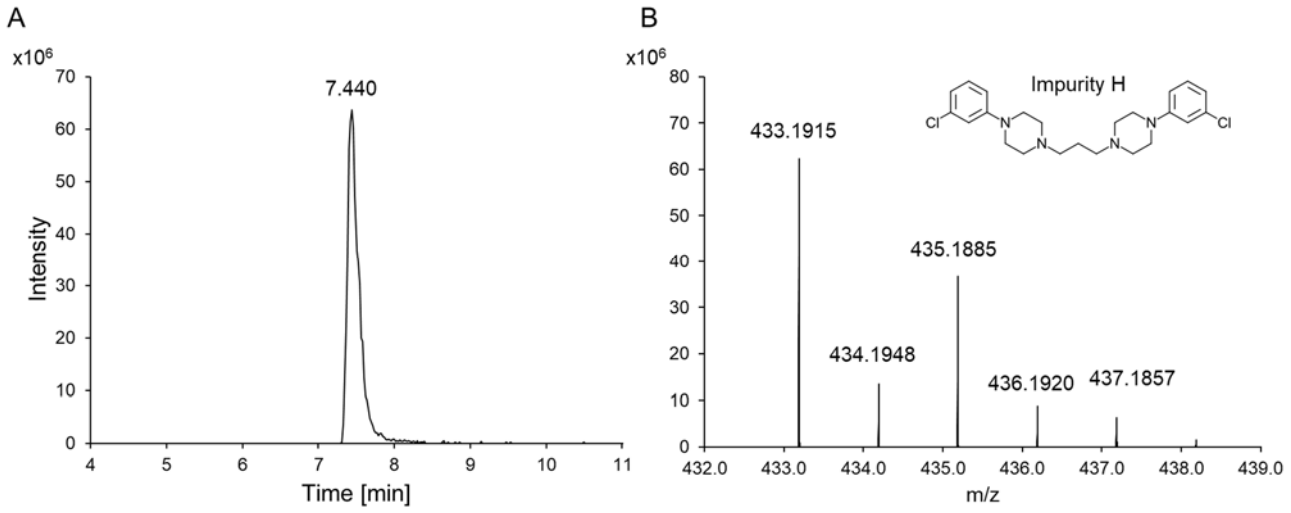

Figure S6. LC-HRMS of impurity H. The chromatogram (A) and the MS spectrum (B) are presented as intensity plotted against time [min] or m/z. The dominant signal for impurity H was present at $[M+H]^+$ at 433.1915 m/z and the calculated $[M+H]^+$ was 433.1920 m/z, with an error of -1.15 ppm.

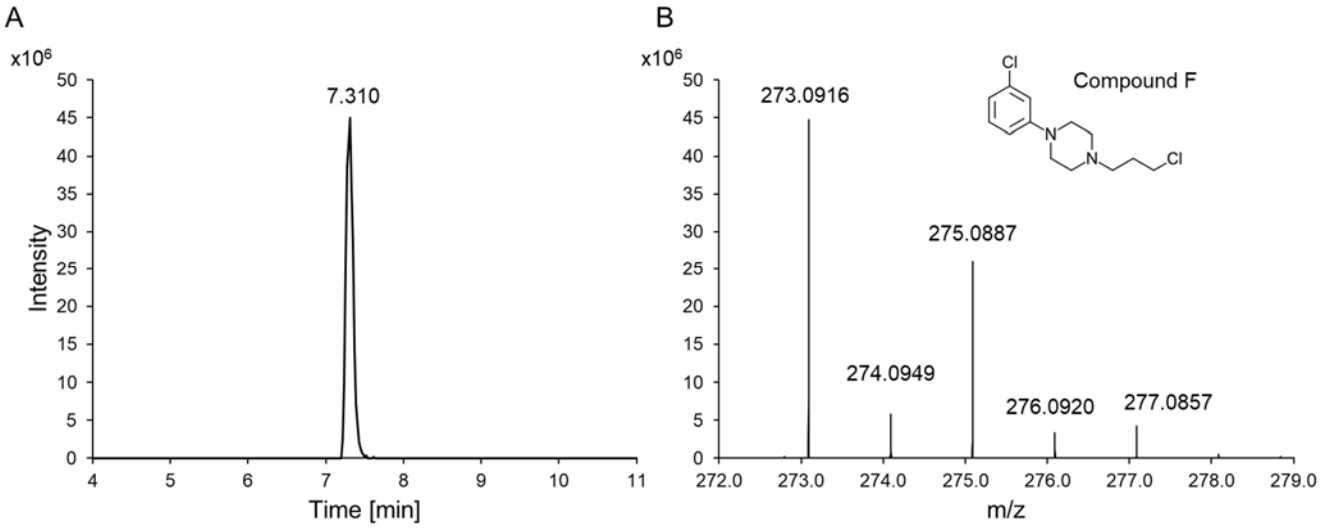

Figure S7. LC-HRMS of compound F. The chromatogram (A) and the MS spectrum (B) are presented as intensity plotted against time [min] or m/z. The dominant signal for impurity H was present at $[M+H]^+$ at 273.0916 m/z and the calculated $[M+H]^+$ was 273.0920 m/z, with an error of 1.46 ppm

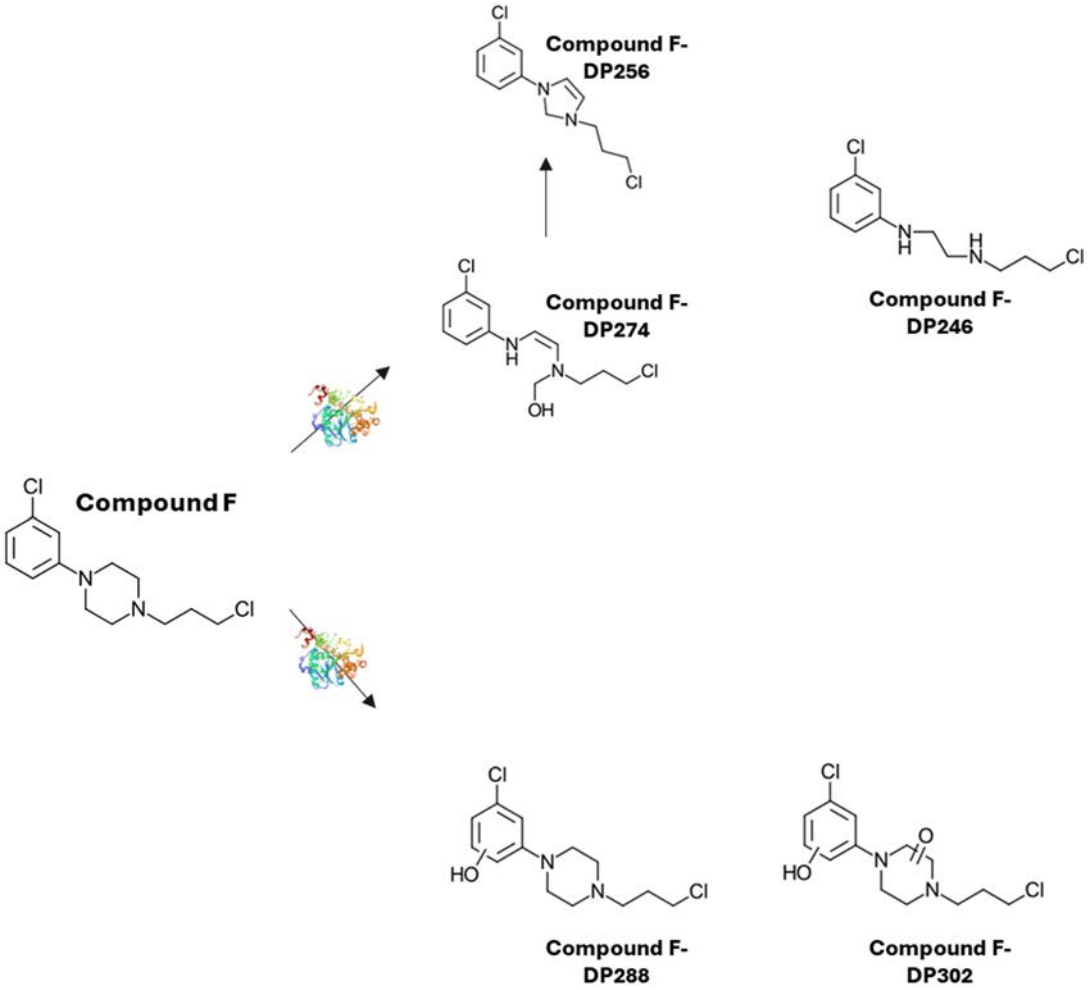

Figure S8. Oxidation pathway of the Trazodone synthesis side product compound F by a laccase from *Trametes hirsuta* in the presence of the mediator HBT as analysed by using LC-HRMS.

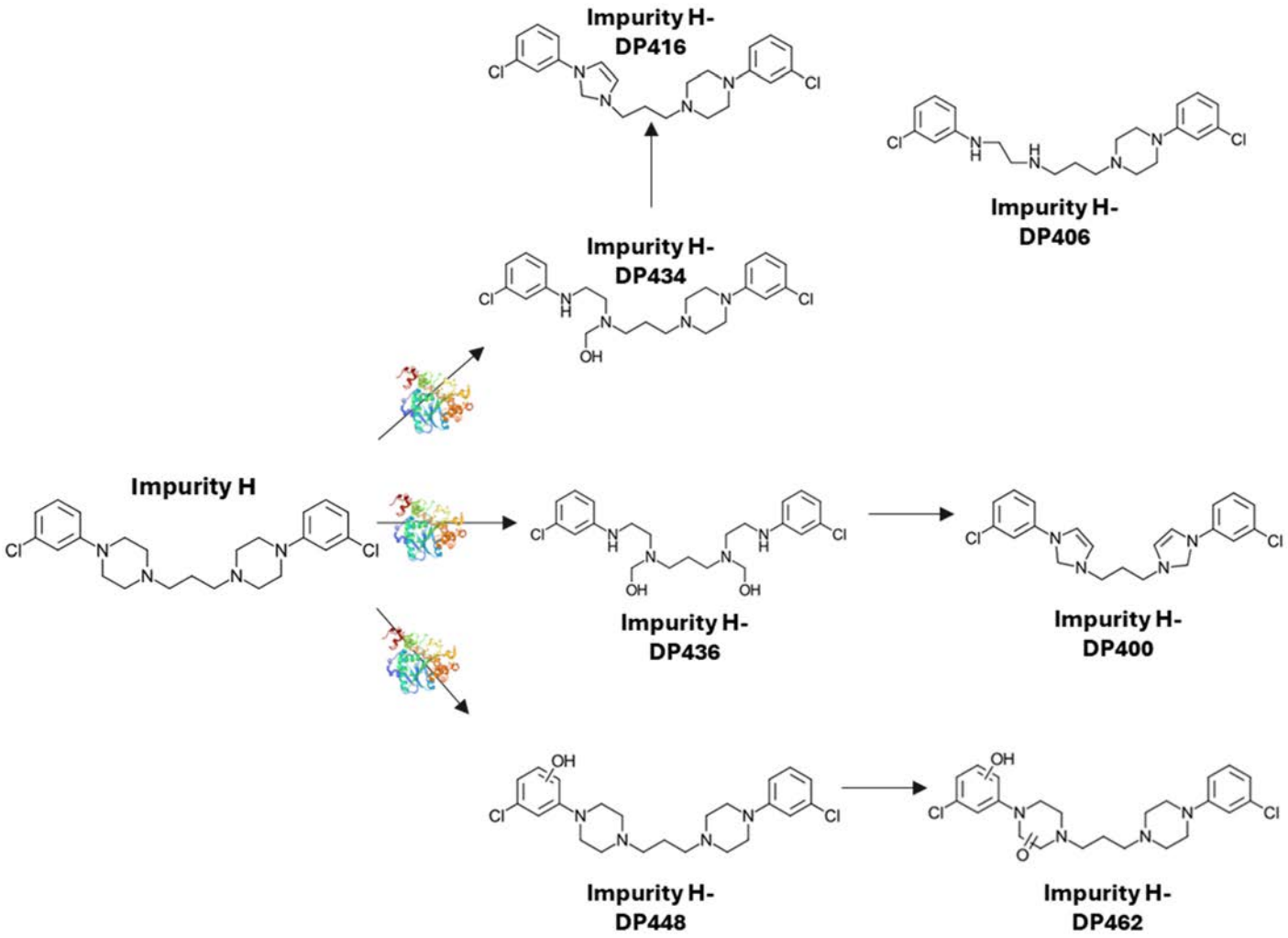

Figure S9. Oxidation pathway of the Trazodone synthesis side product impurity H by a laccase from *Trametes hirsuta* in the presence of the mediator HBT as analysed by using LC-HRMS.

Table S2. High-resolution mass data corresponding to the elemental composition of impurity H and its degraded products (DP) after the laccase-mediated remediation. All the compounds were detected as [M+H]+.

Description	Molecular formula	Observed m/z	Calculated m/z	Error [ppm]	Retention time [min]
Impurity H	$C_{19}H_{22}ClN_5O$	433.1920	433.1915	1.15	7.44
Impurity H-DP406	$C_{21}H_{28}N_4Cl_2$	407.1768	407.1764	0.98	8.07
Impurity H-DP416	$C_{22}H_{26}N_4Cl_2$	417.1610	417.1607	0.72	7.07
Impurity H-DP434	$C_{22}H_{28}N_4OCl_2$	435.1717	435.1713	0.92	9.3
Impurity H-DP448	$C_{23}H_{30}N_4OCl_2$	449.1873	449.1869	0.89	9.91
Impurity H-DP462	$C_{23}H_{28}N_4Cl_2O_2$	463.1663	463.1662	0.21	8.62
Impurity H-DP400	$C_{21}H_{22}N_4Cl_2$	401.1298	401.1294	0.99	9.51
Impurity H-DP436	$C_{21}H_{26}N_4Cl_2O_2$	437.1511	437.1506	1.14	9.10, 10.75*

* Multiple peaks due to isomers

Table S3. High-resolution mass data corresponding to the elemental composition of compound F and its degraded products (DP) after the laccase-mediated remediation. All the compounds were detected as [M+H]+.

Description	Molecular formula	Observed m/z	Calculated m/z	Error [ppm]	Retention time [min]
Compound F	$C_{13}H_{18}Cl_2N_2$	273.0916	273.0920	1.46	7.31
Compound F-DP246	$C_{11}H_{16}Cl_2N_2$	247.0766	247.0763	1.21	7.98
Compound F-DP256	$C_{12}H_{14}N_2Cl_2$	257.0609	257.0607	0.78	6.55
Compound F-DP274	$C_{12}H_{16}N_2Cl_2O$	275.0714	275.0707	2.54	10.16

Compound F-DP288	$C_{13}H_{18}Cl_2N_2O$	289.0869	289.0869	0.00	5.64, 10.23*
Compound F-DP302	$C_{13}H_{16}O_2Cl_2N_2$	303.0665	303.0662	0.99	9.23

* Multiple peaks due to isomers