Table S1. List of oligonucleotide primers used in this study. For *BM3_fwd_BamHI* and *BM3_rev_XhoI*, the introduced restriction site sequences are shown in red. For all other primers, red text represents mutated bases relative to the wild-type $P450_{BM3}$ sequence (Genbank accession CP009920.1).

Name	Sequence (5' -> 3')
BM3_fwd_BamHI	TAAGCAGGATCCACAATTAAAGAAATGCCTCAGC
BM3_rev_XhoI	TACATACTCGAGTTACCCAGCCCACACGTC
F87A_fwd	AGACGGGTTAGCGACAAGCTGGAC
F87A_rev	CCTGCAAAATCACGTACAAATTTAAG
E267V_fwd	TGCGGGACACGTGACAACAAGTG
R47L_fwd	GGCGCCTGGTCTGGTAACGCGCT
F81I_fwd	TGTACGTGATATTGCAGGAGACG
E64G_fwd	AGCATGCGATGGCTCACGCTTTG
G74A_fwd	CTTAAGTCAAGCGCTTAAATTTG
L86I_87V_fwd	AGGAGACGGGATTGCGACAAGCTG
L86I_87A_fwd	AGGAGACGGGATTGTTACAAGCTG
S72D_fwd	TAAAAACTTAGATCAAGGGCTTAAATTTGTACG
L437S_fwd	TAAAGAAACTAGCACGTTAAAACCTGAAG

Table S2. Identification of the noscapine metabolites detected in LC-MS/MS screening experiments of $P450_{BM3}$ metabolism of noscapine. The chromatographic retention time is given, together with the observed mass and the name and formula of the identified compound. Metabolite numbers used throughout this work are also provided where appropriate.

Retention time (min)	Targeted precursor m/z	Formula	Observed m/z	Mass error (ppm)	Identification	Metabolite number
4.24	402.1547	$[C_{21}H_{24}NO_7]^+$	402.1543	-1.18	Cleavage of methylenedioxy bridge	3
4.40	388.1391	$[C_{20}H_{22}NO_7]^+$	388.1377	3.56	Cleavage of methylenedioxy bridge and demethylated	-
5.05	220.0968	$[C_{12}H_{14}NO_3]^+$	220.0959	4.20	Cotarnine	4
5.75	195.0652	$[C_{10}H_{11}O_4]^+$	195.0652	-0.08	Meconine	5
6.60	416.1340	$[C_{21}H_{22}NO_8]^+$	416.1347	-1.70	Demethylated and hydroxylated	-
7.00	416.1340	$[C_{21}H_{22}NO_8]^+$	416.1349	-2.18	Demethylated and hydroxylated	-
7.12	430.1496	$[C_{22}H_{24}NO_8]^+$	430.1494	0.57	Hydroxylated	2a
7.32	400.1391	$[C_{21}H_{22}NO_7]^+$	400.1389	0.45	Demethylated	1
7.70	414.1547	$[C_{22}H_{24}NO_7]^+$	414.1558	-2.59	Noscapine	-
8.10	430.1496	$[C_{22}H_{24}NO_8]^+$	430.1504	-1.76	Hydroxylated	2b

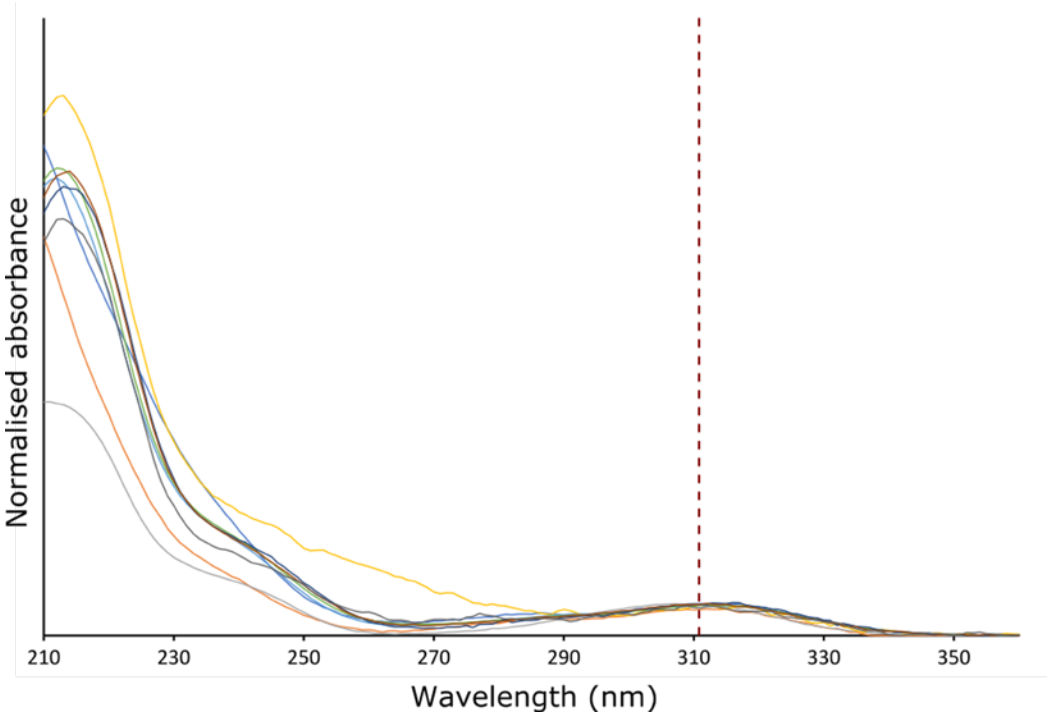

Figure S1. Normalised UV absorption spectra for the nine metabolites quantified by UV peak integration. Quantification was performed at 311 nm (indicated by the dashed line), as all metabolites share a similar broad peak in this region. Spectra were normalised by dividing the absorption spectrum at the peak apex by the peak height.

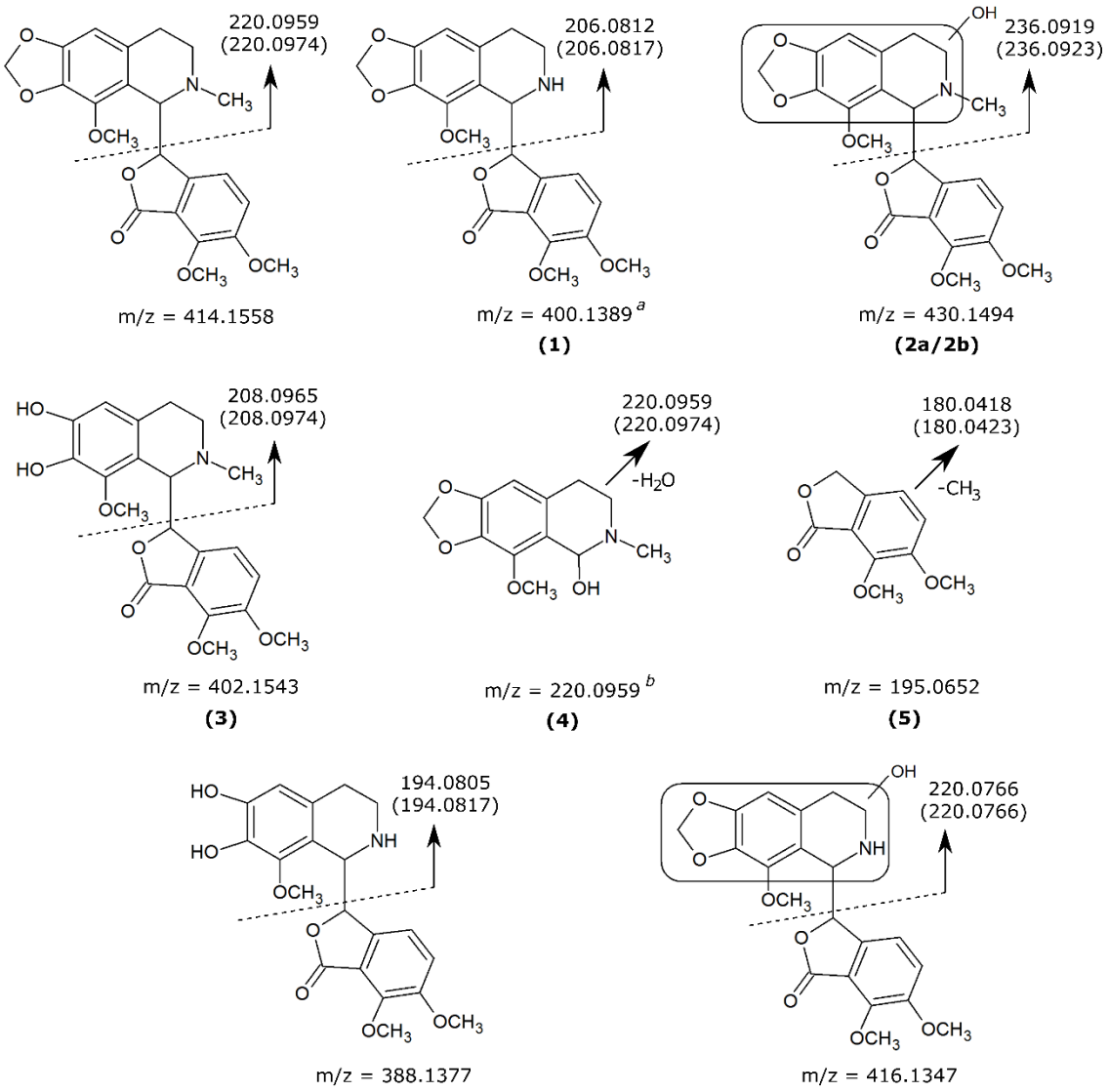

Figure S2. Metabolite identification based on LC-MS/MS data for the compounds listed in **Table S2**. The m/z of the parent compound is given below each structure. A unique MS/MS transition is shown for each metabolite, with the detected fragment indicated by a dashed line (where appropriate) and solid arrow. The observed *m/z* for each fragment is indicated; the calculated *m/z* is shown in parentheses. Metabolite numbers used throughout this work are provided in bold where appropriate. Fragments for metabolites **4** and **5** were produced by loss of a water molecule and methyl group, respectively.

[a] The demethylation site was confirmed by comparison to a *N*-demethylated noscapine standard (**Figure S3**).

[b] The expected *m/z* for cotarnine (**4**) is 238.1, however, no ion of this *m/z* was detected. It was shown by Tsunoda & Yashimura that cotarnine does not produce a detectable ion at *m/z* 238 under conditions of chemical ionisation and this metabolite instead produces a major fragment at *m/z* 220[1] from loss of a water molecule. This has also been observed with electrospray ionisation for noscapine[2] and noscapine analogues[3, 4].

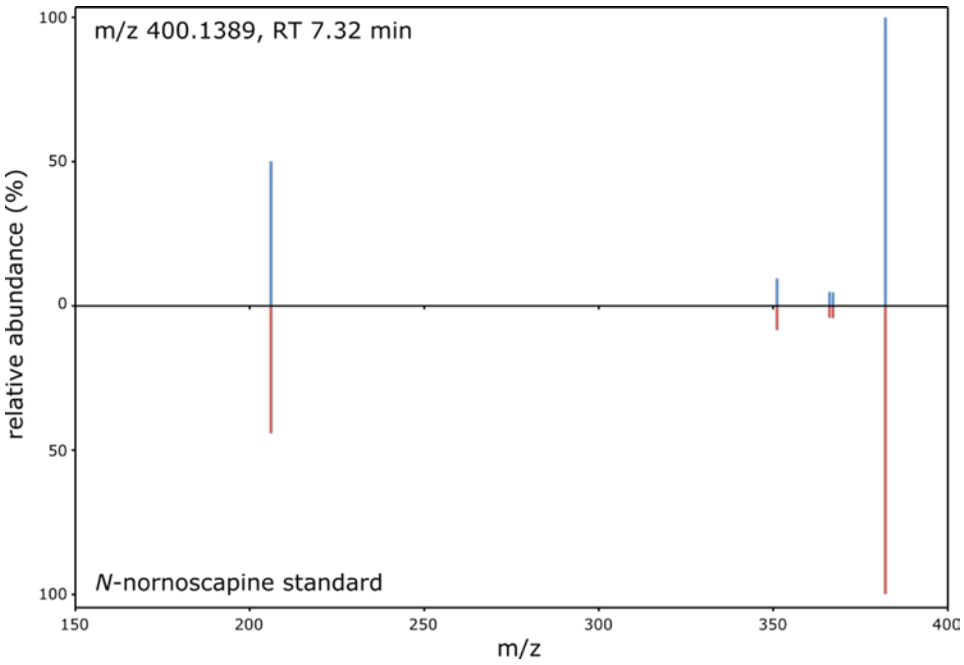

Figure S3. MS/MS fragment mirror comparison of the metabolite detected at RT 7.32 min (metabolite **1**) with a *N*-demethylated noscapine standard (*N*-nornoscapine). The five most abundant fragments are shown; two appear close together at m/z values of 366.1 and 367.1.

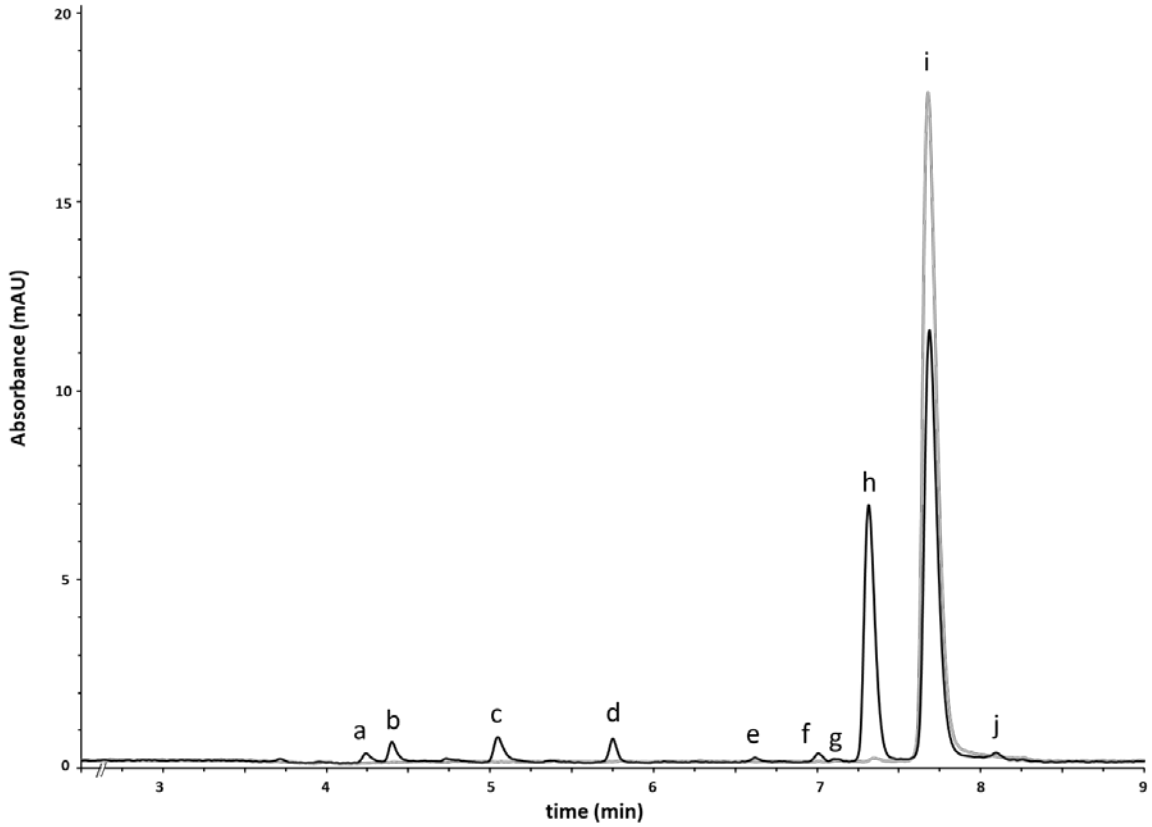

Figure S4. UHPLC chromatogram showing noscapine turnover by mutant A3 (black). Control without NADPH addition is shown in grey. Metabolite peaks: a, cleavage of methylenedioxy bridge (**3**); b, cleavage of methylenedioxy bridge and demethylated; c, cotarnine (**4**); d, meconine (**5**); e, demethylated and hydroxylated; f, demethylated and hydroxylated; g, hydroxylated (**2a**); h, *N*-demethylated (**1**); i, noscapine; b, hydroxylated (**2b**).

Table S3. Numerical data from screening experiments for the 12 active $P450_{BM3}$ mutants. Three replicates were used for each mutant; errors are in SE.

			% product distribution				
	Noscapine metabolised (%)	**Coupling efficiency (%)**	***N*-nornoscapine (1)**	**Hydroxylated (2a)**	**Hydroxylated (2b)**	**Cleaved methylenedioxy (3)**	**Meconine (5)**
V1	22.3 ± 0.1	3.3 ± 0.2	57.8 ± 2.1	3.1 ± 0.2	-	6.3 ± 0.2	32.8 ± 1.0
V2	27.0 ± 0.7	5.8 ± 0.3	68.8 ± 1.4	3.5 ± 0.2	-	4.7 ± 0.2	23.0 ± 2.0
V3	38.3 ± 0.6	8.8 ± 0.5	73.7 ± 1.4	3.0 ± 0.0	-	7.1 ± 0.2	16.2 ± 0.6
V4	36.4 ± 0.8	8.8 ± 0.5	70.2 ± 1.7	2.9 ± 0.1	-	10.0 ± 0.5	17.0 ± 1.8
V4A	21.2 ± 1.1	4.9 ± 0.6	62.4 ± 7.0	4.5 ± 0.5	-	6.3 ± 0.7	26.7 ± 2.3
A1	37.5 ± 1.8	9.5 ± 0.5	85.3 ± 2.4	2.7 ± 0.0	1.3 ± 0.0	3.7 ± 0.2	7.1 ± 0.2
A2	50.4 ± 1.6	11.2 ± 0.5	88.0 ± 3.4	1.8 ± 0.1	1.2 ± 0.1	2.8 ± 0.1	6.2 ± 0.2
A3	39.3 ± 0.6	15.6 ± 0.8	88.1 ± 3.4	1.1 ± 0.1	1.8 ± 0.2	2.8 ± 0.1	6.3 ± 0.2
A4	44.6 ± 0.7	17.2 ± 0.9	84.8 ± 1.8	1.2 ± 0.1	2.0 ± 0.1	3.4 ± 0.7	8.6 ± 0.3
A4A	31.4 ± 1.1	10.7 ± 0.7	64.8 ± 1.0	1.3 ± 0.0	5.5 ± 0.3	6.5 ± 0.4	21.9 ± 1.0
A3I	29.4 ± 0.8	13.9 ± 0.6	82.5 ± 1.7	1.4 ± 0.0	0.8 ± 0.1	8.4 ± 0.3	7.0 ± 0.1
A3S	59.1 ± 1.9	22.0 ± 1.4	59.3 ± 1.3	1.6 ± 0.1	11.7 ± 0.3	10.0 ± 0.4	17.3 ± 0.3

[1] N. Tsunoda, H. Yoshimura, Metabolic fate of noscapine. II. Isolation and identification of novel metabolites produced by C-C bond cleavage, Xenobiotica 9(3) (1979) 181-7.
[2] Z.Z. Fang, K.W. Krausz, F. Li, J. Cheng, N. Tanaka, F.J. Gonzalez, Metabolic map and bioactivation of the anti-tumour drug noscapine, Br J Pharmacol 167(6) (2012) 1271-86.
[3] H.J. Qu, Y. Qian, Metabolism profiling of amino-noscapine, Eur J Drug Metab Pharmacokinet 41(2) (2016) 171-7.
[4] Y. Yao, Y. Xiong, Metabolic pathway profiling of the derivative of important herbal component noscapine, Eur J Drug Metab Pharmacokinet 41(1) (2016) 27-32.