Supporting information

Identification of novel pathways in idelalisib metabolism and bioactivation

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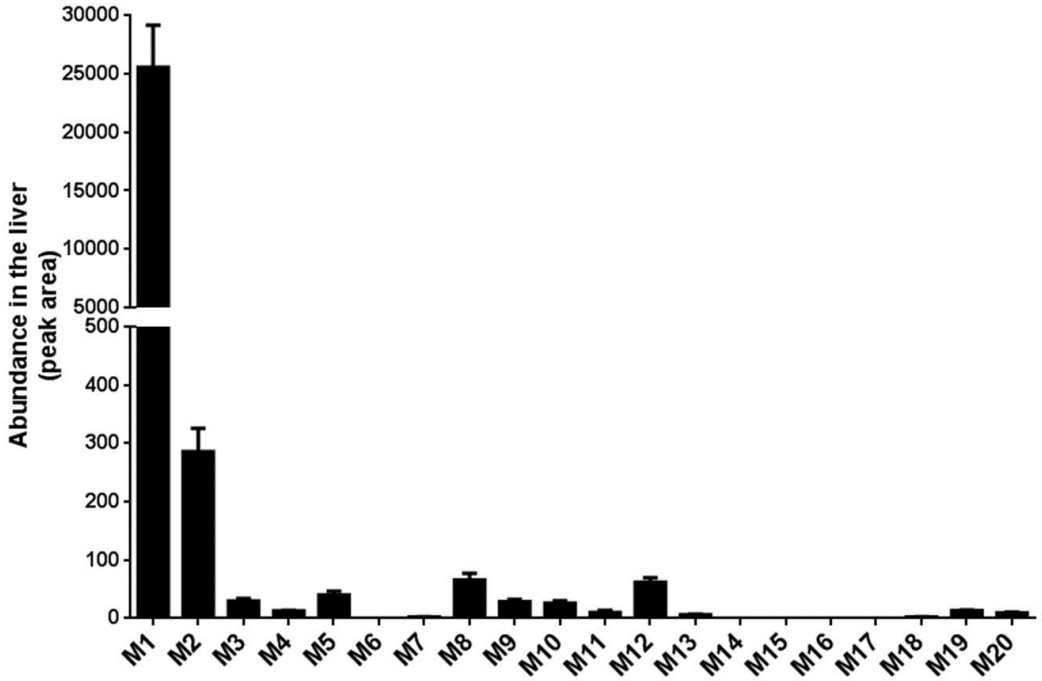


Figure S1. Relative abundance of ILB metabolites in the liver of mice treated with ILB (36 mg/kg, p.o.). The data are expressed as means ± SEM (n = 4).

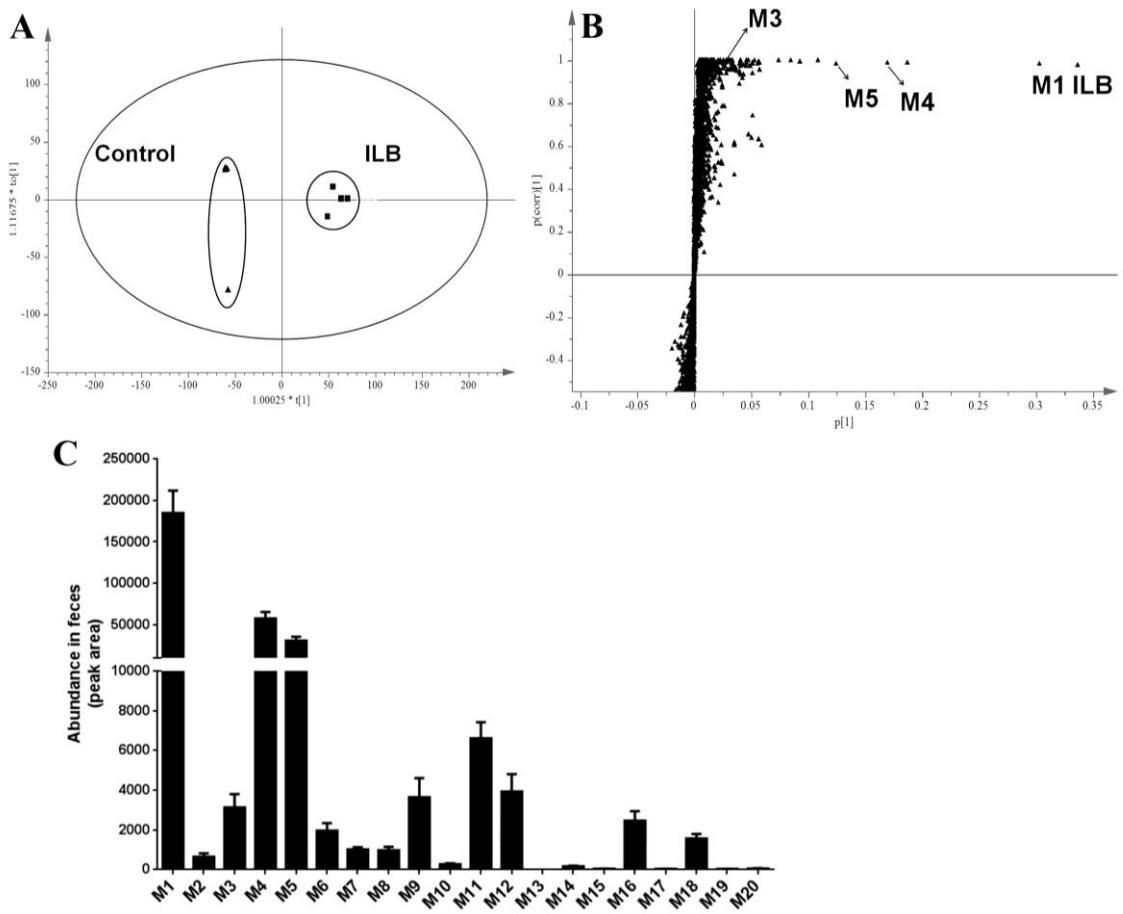


Figure S2. Metabolomic analysis of feces from mice treated with vehicle or ILB (100 mg/kg). All samples were analyzed by UPLC-QTOFMS. (A) Separation of fecal samples from control and ILB group in an OPLS-DA score plot. (B) Loading S-plot generated by OPLS-DA analysis. The top ranking ions were identified as ILB and its metabolites. (C) Relative abundance of ILB metabolites in feces. The data are expressed as means ± SEM (n = 4).

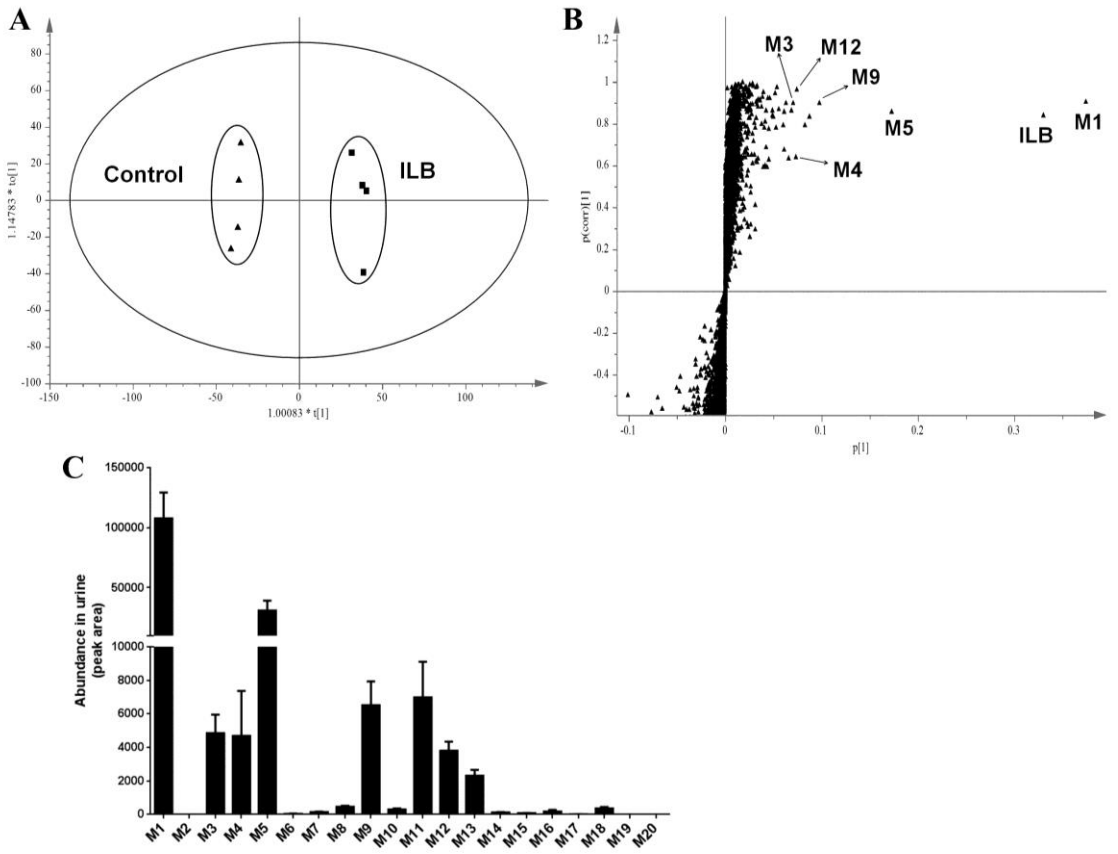


Figure S3. Metabolomic analysis of urine from mice treated with vehicle or ILB (100 mg/kg). All samples were analyzed by UPLC-QTOFMS. (A) Separation of urinary samples from control and ILB group in an OPLS-DA score plot. (B) Loading S-plot generated by OPLS-DA analysis. The top ranking ions were identified as ILB and its metabolites. (C) Relative abundance of metabolites of ILB in urine. The data are expressed as means ± SEM (n = 4).

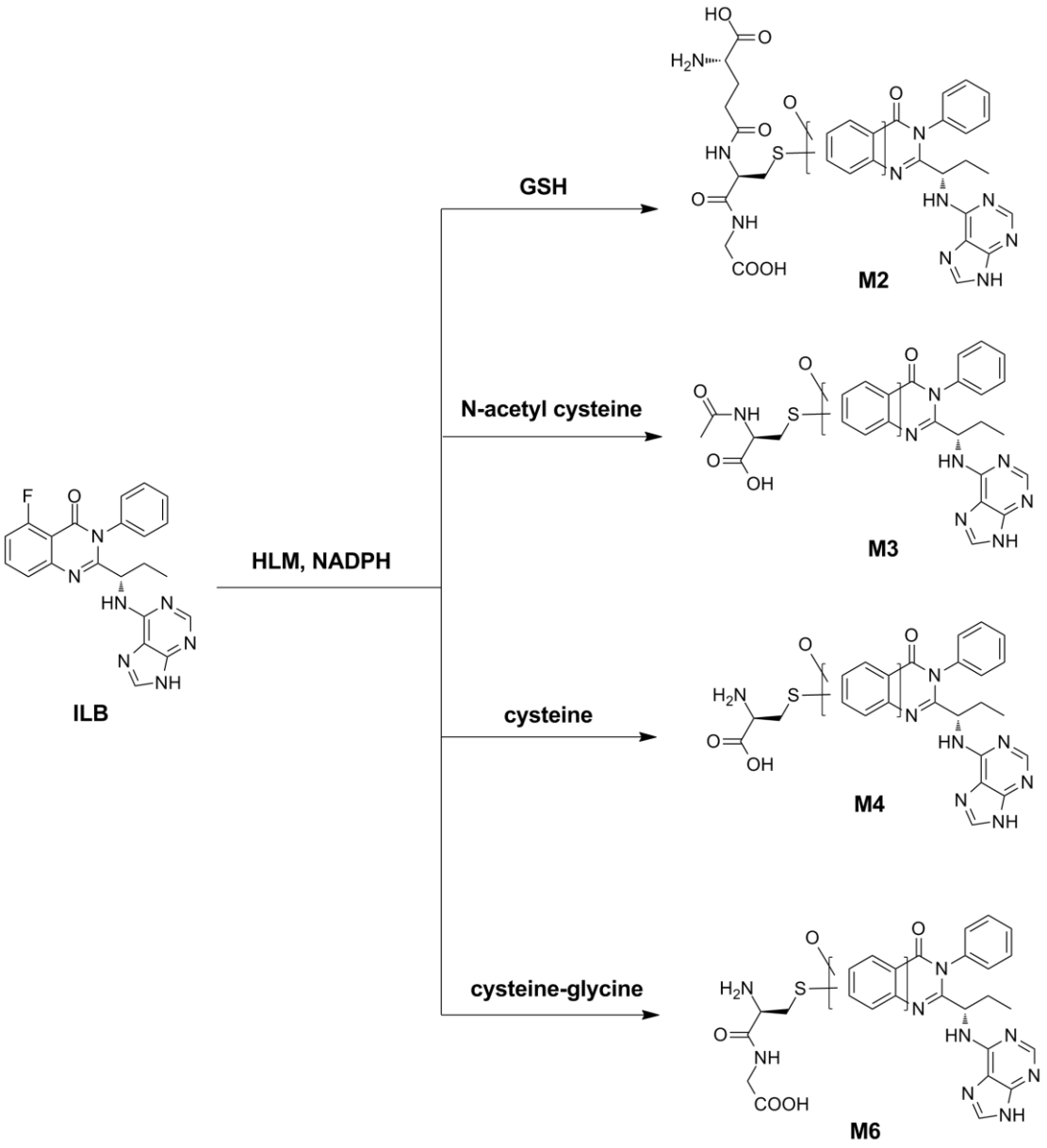
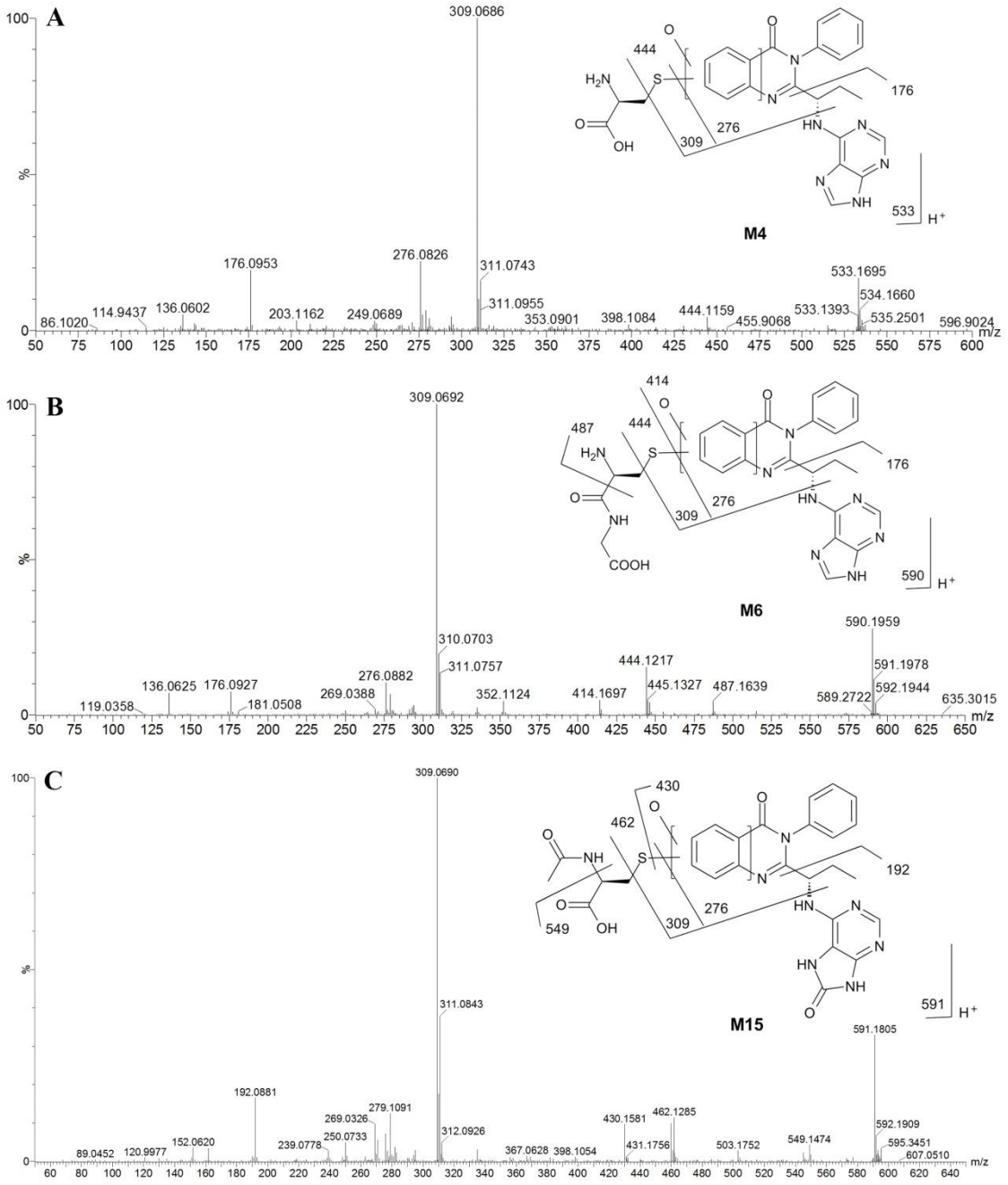


Figure S4. Trapping of M2, M3, M4 and M6 in HLM. M2, M3, M4 and M6 were recaptured by the incubation of ILB in HLM with trapping agents GSH, *N*-acetyl cysteine, cysteine and cysteine-glycine, respectively.


A
309.0686
444
176
276
309
533
H+
M4
176.0953
276.0826
311.0743
533.1695
534.1660
311.0955
86.1020
114.9437
136.0602
203.1162
249.0689
353.0901
398.1084
444.1159
455.9068
533.1393
535.2501
596.9024
m/z
B
309.0692
414
487
444
176
276
309
590
H+
M6
310.0703
590.1959
444.1217
311.0757
591.1978
136.0625
176.0927
276.0882
445.1327
487.1639
592.1944
269.0388
352.1124
414.1697
589.2722
635.3015
119.0358
181.0508
m/z
C
309.0690
430
462
192
276
309
549
591
H+
M15
311.0843
591.1805
192.0881
279.1091
269.0326
430.1581
462.1285
592.1909
312.0926
595.3451
152.0620
250.0733
549.1474
89.0452
120.9977
239.0778
367.0628
398.1054
431.1756
503.1752
607.0510
m/z

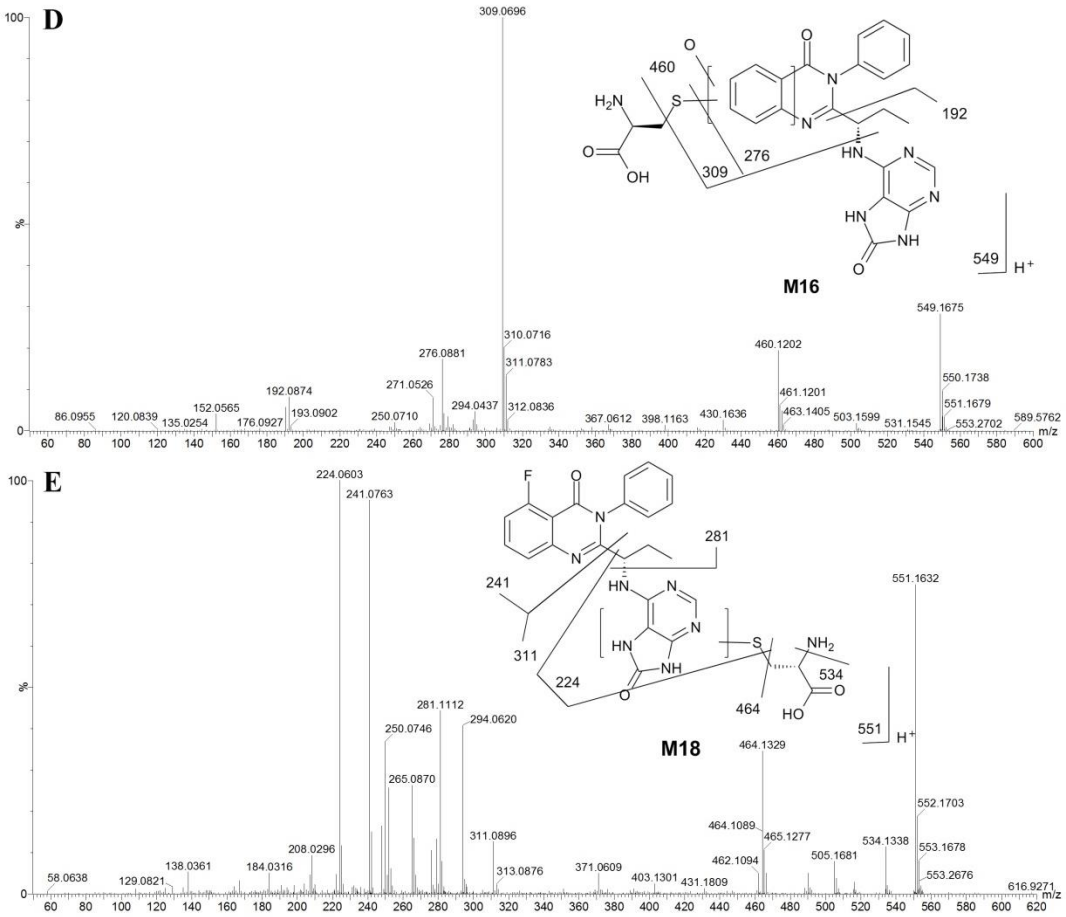


Figure S5. Identification of M4, M6, M15, M16 and M18. (A) MS/MS spectrum of M4. (B) MS/MS spectrum of M6. (C) MS/MS spectrum of M15. (D) MS/MS spectrum of M16. (E) MS/MS spectrum of M18.

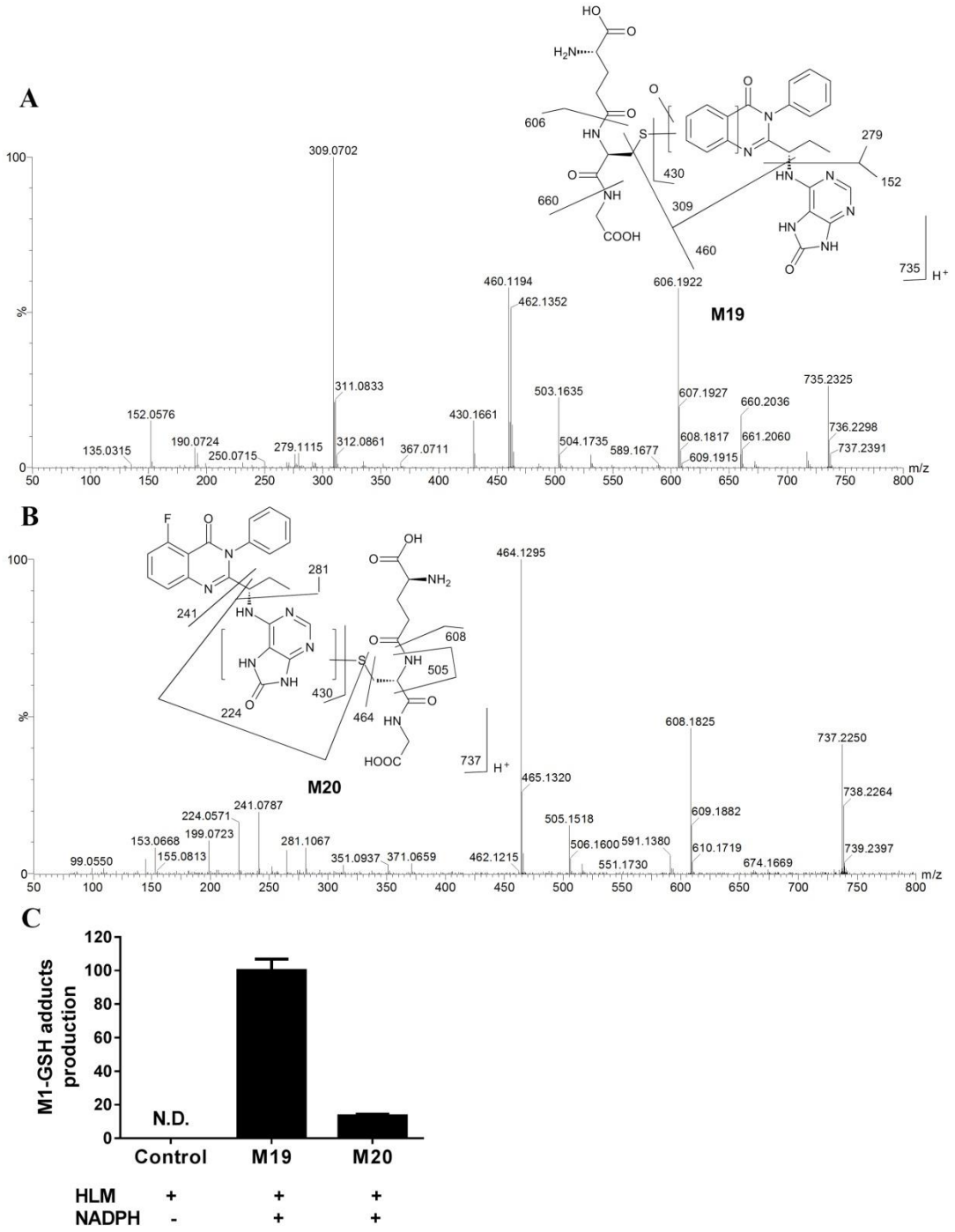


Figure S6. Identification of M19 and M20. (A) MS/MS spectrum of M19. (B) MS/MS spectrum of M20. (C) The abundance of M19 and M20 in the incubation of M1 with HLM. N.D., not detected. The abundance of M19 was set as 100%. All the data are expressed as means ± SEM (n = 3).

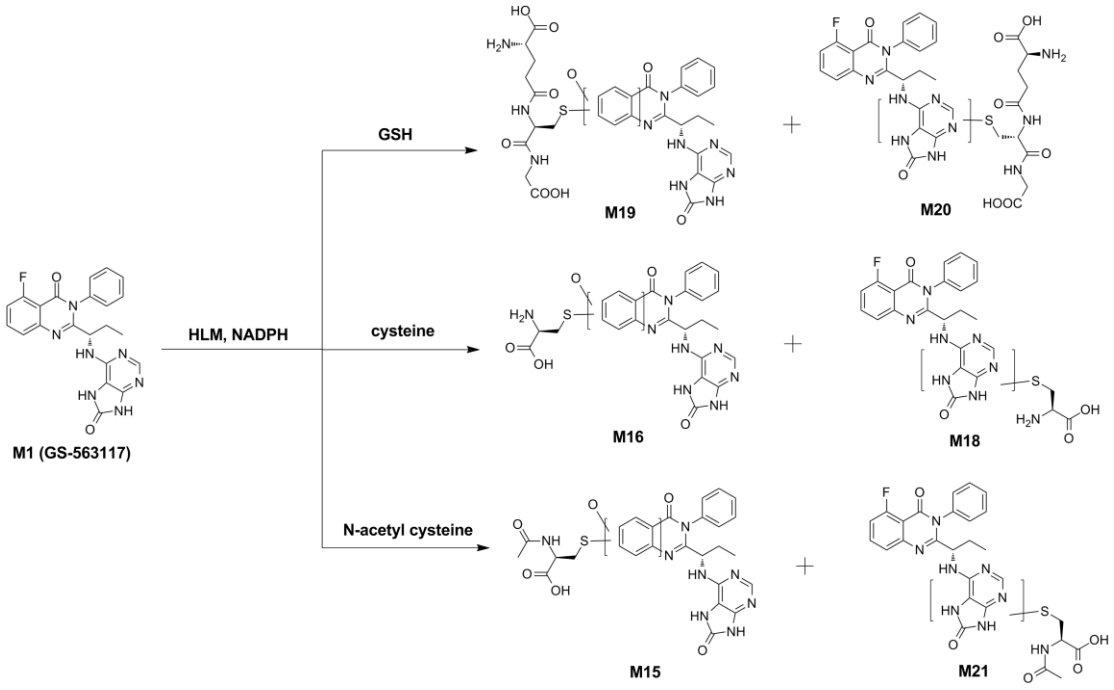


Figure S7. Trapping of M15, M16, M18, M19, M20 and M21 in HLM. M15, M16, M18, M19, M20 and M21 were recaptured by the incubation of M1 in HLM with trapping agents GSH, cysteine or *N*-acetyl cysteine.

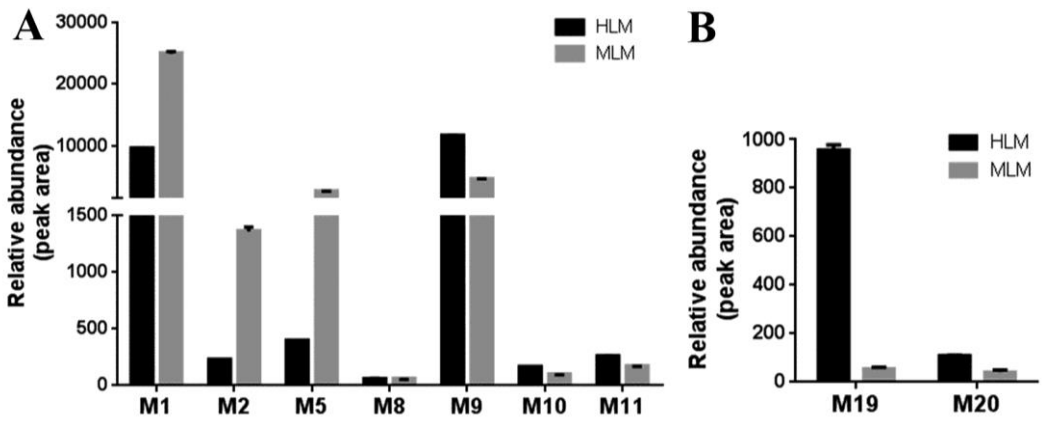


Figure S8. Comparison of the metabolism of ILB and M1 between HLM and MLM. (A) Metabolites in the incubations of ILB with HLM or MLM. (B) Metabolites in the incubations of M1 with HLM or MLM. All the metabolites were analyzed by UPLC-QTOFMS. The data are expressed as means ± SEM (n = 3).

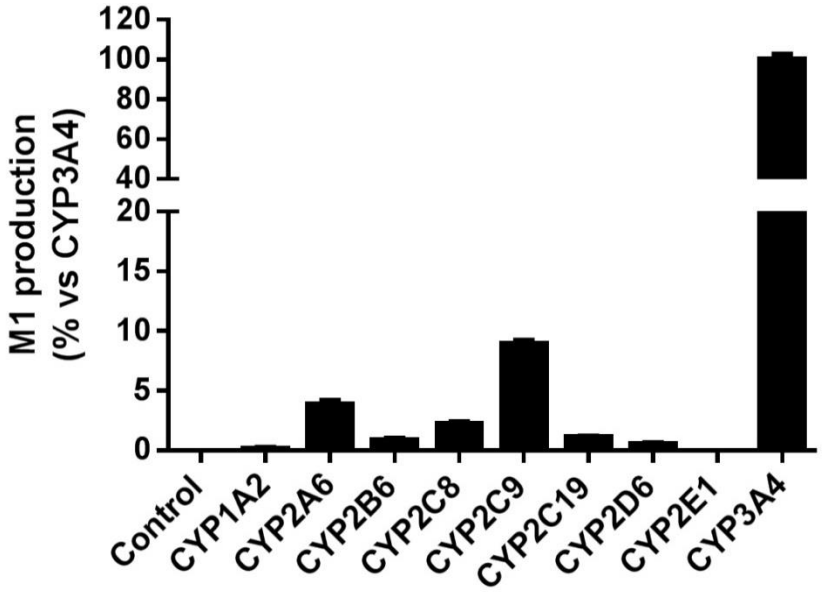
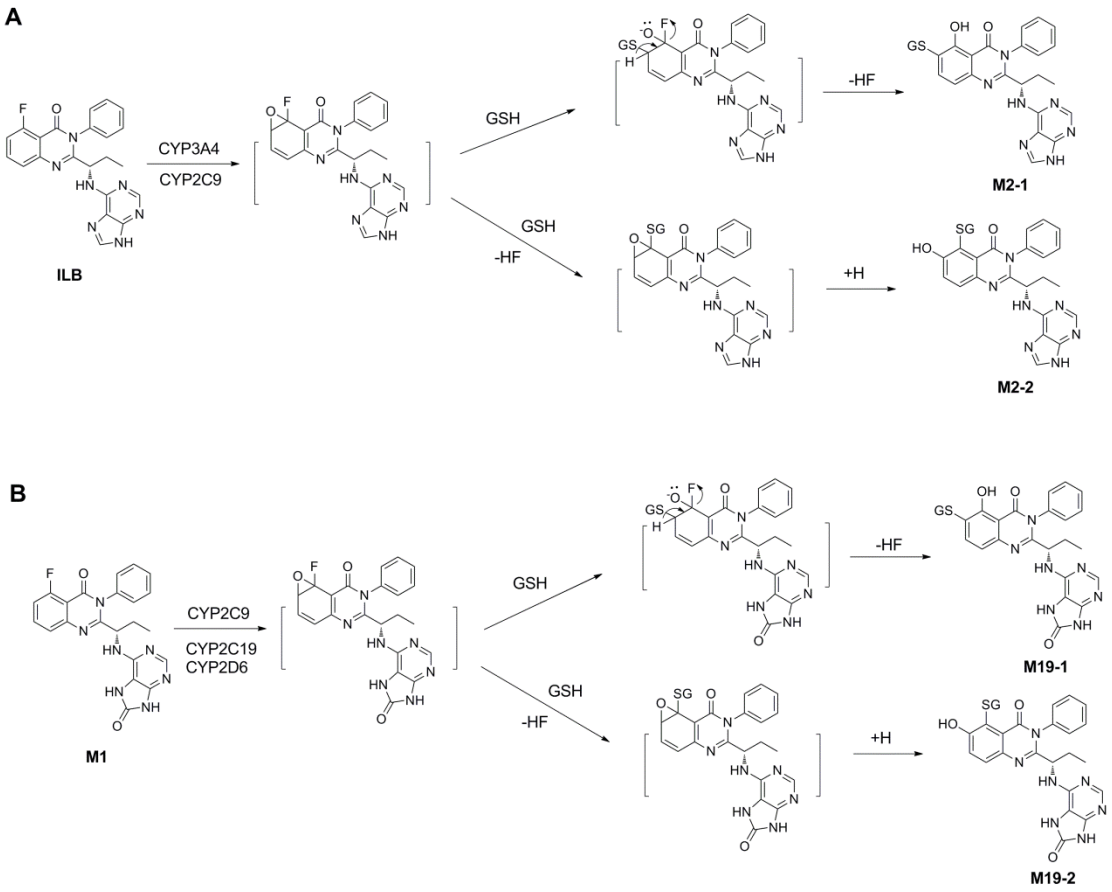


Figure S9. Role of CYPs in M1 formation. The relative abundance of M1 from the incubation with CYP3A4 was set as 100%. All the data are expressed as means ± SEM (n = 3).


A
CYP3A4
CYP2C9
ILB
GSH
GSH
-HF
-HF
M2-1
+H
M2-2
B
CYP2C9
CYP2C19
CYP2D6
M1
GSH
GSH
-HF
-HF
M19-1
+H
M19-2

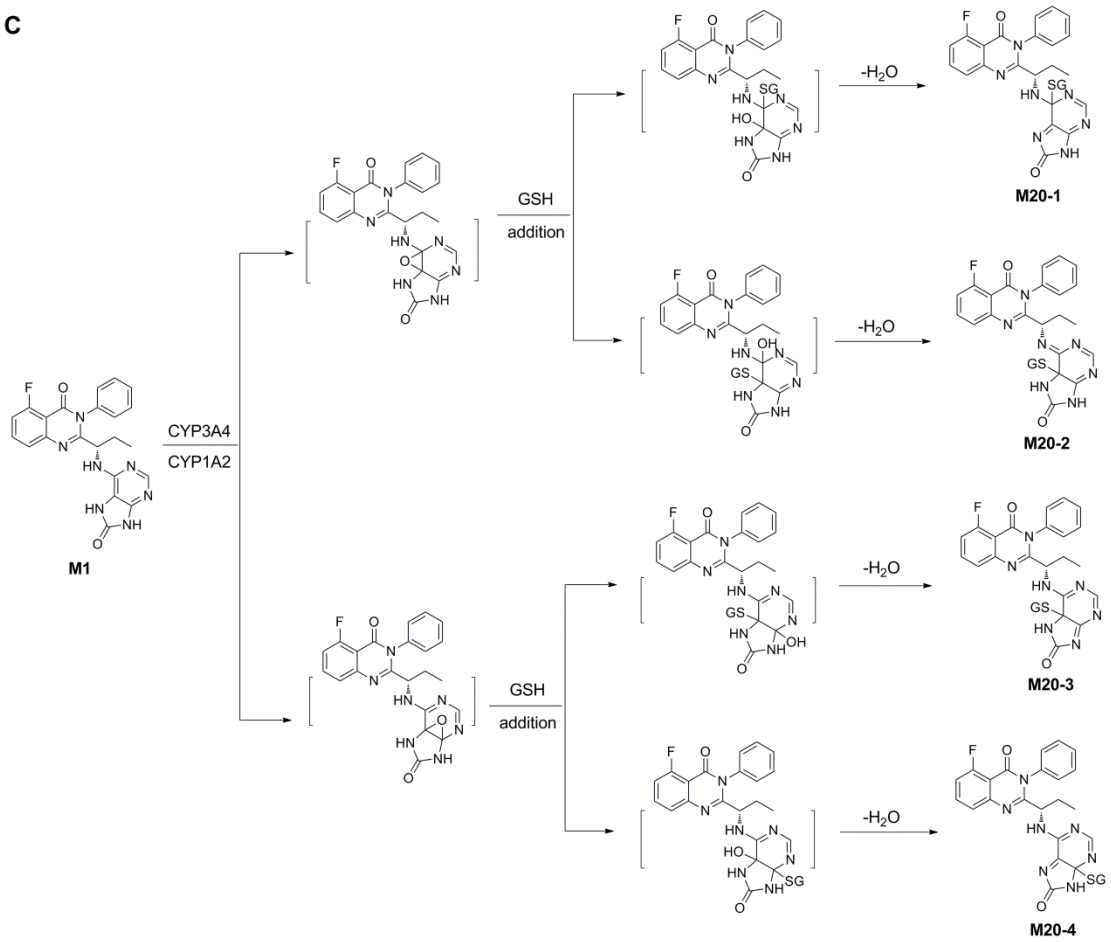


Figure S10. Proposed mechanisms for the formation of ILB GSH adducts M2 (A), M19 (B) and M20 (C).

Table S1. Summary of ILB metabolites. These metabolites were identified in the liver, fecal and urinary samples of mice treated with ILB. M1 and M2 are the major metabolites in mouse liver. Oxidized metabolites M1, M5, M8, M9, M10 and M11 were also detected in the incubation of ILB with HLM. M2, M3, M6, M15, M16, M19 and M20 are novel metabolites of ILB.

RT (min)	Observed m/z [M + H]	Calculated m/z [M + H]	Mass Error (ppm)	Formula	Identification	Metabolite ID	Sources
5.28	416.1628	416.1635	-1.7	$C_{22}H_{19}FN_7O$	ILB	-	L, S, F, U
5.46	432.1584	432.1584	0	$C_{22}H_{19}FN_7O_2$	ILB+O	M1	H, L, S, F, U
4.48	719.2408	719.2360	6.7	$C_{32}H_{35}N_{10}O_8S$	ILB-F+O+GSH	M2	H, L, S, F
5.20	575.1826	575.1825	0.2	$C_{27}H_{27}N_8O_5S$	ILB-F+O+NAC	M3	H, L, S, F, U
4.31	533.1695	533.1719	-4.5	$C_{25}H_{25}N_8O_4S$	ILB-F+O+Cys	M4	H, L, S, F, U
4.68	432.1566	432.1584	-4.2	$C_{22}H_{19}FN_7O_2$	ILB+O	M5	H, L, S, F, U
4.38	590.1959	590.1934	4.2	$C_{27}H_{28}N_9O_5S$	ILB-F+O+Cys-Gly	M6	H, F, U
6.45	460.1534	460.1556	-4.8	$C_{23}H_{22}N_7O_2S$	ILB-F+O+SMe	M7	L, S, F, U
5.86	414.1695	414.1678	4.1	$C_{22}H_{19}N_7O_2$	ILB-F+O	M8	H, L, S, F, U
5.65	432.1585	432.1584	0.2	$C_{22}H_{19}FN_7O_2$	ILB+O	M9	H, L, S, F, U

4.12	432.1563	432.1584	-4.9	$C_{22}H_{19}FN_7O_2$	ILB+O	M10	H, L, S, F, U
4.97	430.1618	430.1628	-2.3	$C_{22}H_{20}N_7O_3$	ILB-F+2O	M11	H, L, F, U
5.01	608.1904	608.1905	-0.2	$C_{28}H_{27}FN_7O_8$	ILB+O+Glu	M12	L, S, F, U
4.83	608.1921	608.1905	2.6	$C_{28}H_{27}FN_7O_8$	ILB+O+Glu	M13	L, U
5.12	592.1926	592.1956	-5.1	$C_{28}H_{27}FN_7O_7$	ILB+Glu	M14	S, F, U
5.38	591.1805	591.1774	5.2	$C_{27}H_{27}N_8O_6S$	ILB-F+2O+NAC	M15	H, F, U
4.50	549.1675	549.1669	1.1	$C_{25}H_{25}N_8O_5S$	ILB-F+2O+Cys	M16	H, F, U
6.55	476.1466	476.1505	-8.2	$C_{23}H_{22}N_7O_3S$	ILB-F+2O+SMe	M17	F, U
4.60	551.1632	551.1625	1.3	$C_{25}H_{24}FN_8O_4S$	ILB+O+Cys	M18	H, L, F, U
4.69	735.2325	735.2309	2.2	$C_{32}H_{35}N_{10}O_9S$	ILB-F+2O+GSH	M19	H, L, F
4.61	737.2250	737.2266	-2.2	$C_{32}H_{34}FN_{10}O_8S$	ILB+O+GSH	M20	H, L, F

+O: monooxidation; -F: defluorination; +SMe: methylthio adduct; Glu: glucuronide; +2O: dioxidation; +Cys: cysteine adduct; +GSH: GSH adduct; +Cys-Gly: cysteine-glycine adduct; +NAC: *N*-acetyl cysteine adduct; H: human liver microsomes; L: mouse liver; S: mouse serum; F: mouse feces; U: mouse urine; RT: retention time; ppm: parts per million.