Supporting Information

P450 3A Catalyzed O-dealkylation of Lapatinib Induces Mitochondrial Stress and Activates Nrf2

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Table S1: Primer sequences for Nrf-2 target genesPrimer	**Forward (5´-3´)**	**Reverse (5´-3´)**
GAPDH	GGGTGTGAACCATGAGAAG	GTCCTTCCACGATACCAAAG
HO-1	CCAAGTTCAAGCAGCTCTAC	CCTCAAAGAGCTGGATGTTG
NQO-1	CAGCGGCTTTGAAGAAGA	GCAGGGTCCTTCAGTTTA
UGT1A1	AGAGGTGACTGTCCAGGACC	TAGGCTTCAAATTCCTGGGA
SOD1	GCAGGTCCTCACTTTAATCC	GCCACACCATCTTTGTCA
SOD2	GGGTTGGCTTGGTTTCAA	GTGCTCCCACACATCAATC

I. Analytical Procedures:

1. Prepared plasma quantitation standards at 2, 10, 20, 40, 100, 200, 500, 1000 and 2000 ng/ml and QC solutions at 10, 100 and 1000 ng/mL by spiking 25 µL mixed lapatinib and OD-lapatinib standard or QC acetonitrile stock solutions to 225 µL blank plasma samples, mixed well.

2. Prepared liver quantitation standards at 1, 5, 10, 20, 50, 100, 250, 500 and 1000 ng/ml and QC solutions at 5, 50 and 500 ng/mL by spiking 10 µL mixed lapatinib and OD-lapatinib standard or QC acetonitrile stock solutions to 190µL blank liver tissue matrix, mixed well. Blank liver matrix was generated by spiking 900ul water to each 100 mg blank liver and homogenized.

3. All plasma samples were diluted either 10 or 50-fold (5 µL sample + 45 or 245 µL blank plasma) before analysis. Liver samples were first diluted 10-fold by spiking 900ul water to each 100 mg sample and homogenized, then further diluted another 50- fold using blank liver matrix. The total dilutions for liver samples were 500-fold.

4. For plasma and liver samples respectively, transferred 25 µL each of standards, QCs or samples to a 96 well filter plate, added 100 µL internal standard solution (5ng/mL Carbamazepine in acetonitrile), mixed well then filtered. The filtrates were ready for analysis.

5. All samples were analyzed by LC-MS/MS (API 5500) and quantified using an internal calibration method. MRMs(+) (582.1/366.1 or 473.2/349.8, m/z) were used to scan for lapatinib or OD-lapatinib. Carbamazepine (238/195.1, m/z) was used as internal standard.

6. LLOQ and calibration linear range for lapatinib or OD-lapatinib plasma sample analysis were 2 ng/ml and 2 – 2000 ng/ml, respectively. LLOQ and calibration linear range for lapatinib or OD-lapatinib liver sample analysis were 5ng/ml and 5 – 1000 ng/ml, respectively.

II. Instrument Settings

Table S2: LC (Shimadzu UFLC XR) conditions of lapatinib or OD-lapatinib

Compound	lapatinib or OD-lapatinib	I.S.
Column	Thermo Betasil C18, 50x2.1 mm, 5u	
Mobile phase	A: Water with 0.1% Formic Acid B: Acetonitrile with 0.1% Formic Acid	
Flow rate (ml/min)	0.35	
Temperature (°C)	35	
Injection volume (µl)	5	
t_R (min)	2.33 or 2.14	2.52

Table S3: Gradient elution conditions

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.2	90	10
0.5	90	10
2.0	5	95
3.0	5	95
4.0	90	10
5.9	90	10

Table S4: MS (API 5500) conditions of lapatinib or OD-lapatinib

Compound	lapatinib or OD-lapatinib	I.S.
MRM (+)	582.1/366.1 or 473.2/349.8	238/195.1
CAD	7	
Curtain GAS	20	
Ion Source Gas1	40	
Ion Source Gas2	40	
IS	5500	
TEM	500	
CE	55	42
DP	170	126
EP	10	
CXP	14	14