Supporting Information for:

Cytochromes P450 1A2 and 3A4 Catalyze the Metabolic Activation of Sunitinib

Gracia M. Amaya,[†] Rebecca Durandis,[†] David S. Bourgeois,[†] James A. Perkins,[†] Arsany A. Abouda,[†] Kahari J. Wines,[†] Mohamed Mohamud,[†] Samuel A. Starks,[†] R. Nathan Daniels,[†‡] and Klarissa D. Jackson[†‡*]

Department of Pharmaceutical Sciences, Lipscomb University College of Pharmacy and Health Sciences; Nashville, Tennessee 37204-3951, and Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146

*To whom correspondence should be addressed: tel, 615-966-7011; fax, 615-966-7163; email, klarissa.jackson@lipscomb.edu

[†] Lipscomb University College of Pharmacy and Health Sciences, Department of Pharmaceutical Sciences
[‡] Vanderbilt University School of Medicine, Department of Pharmacology

Running title:

Sunitinib Metabolic Activation

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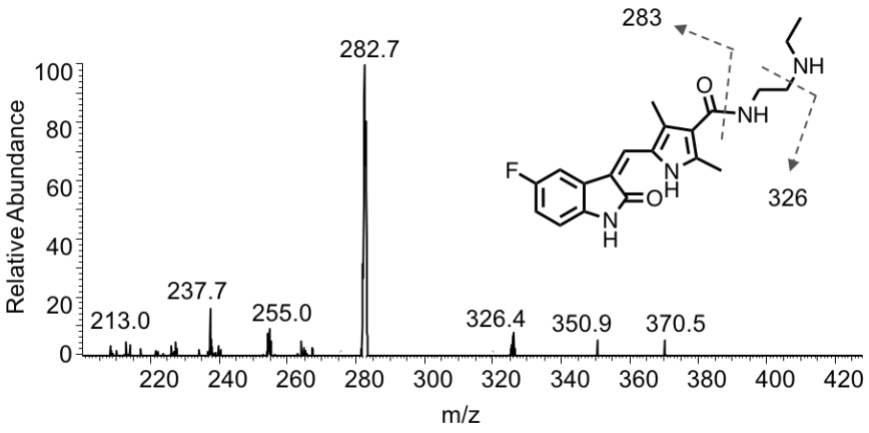
Figure S1: LC-MS/MS Analysis of Sunitinib Metabolites. Sunitinib (10 μM) was incubated with pooled human liver microsomes (0.5 mg/mL) in the presence of an NADPH-regenerating system or UDP-glucuronic acid for 30 min. Control experiments were performed without co-factor. Sunitinib metabolites were analyzed by LC-MS/MS using electrospray ionization in positive ion mode. Shown are representative product ion spectra from MS/MS analysis of the precursor ions (A) *m/z* 371 (*N*-desethylsunitinib, M1), (B) *m/z* 415 (hydroxy-sunitinib, M2), (C) *m/z* 397 (defluorosunitinib, M3), and (D) *m/z* 575 (sunitinib glucuronide, M4), corresponding to the predicted sunitinib metabolites. The fragmentation pattern for each metabolite is consistent with that proposed previously by Speed et al.[1] The predicted structure of each metabolite is shown.

Figure S1

A.



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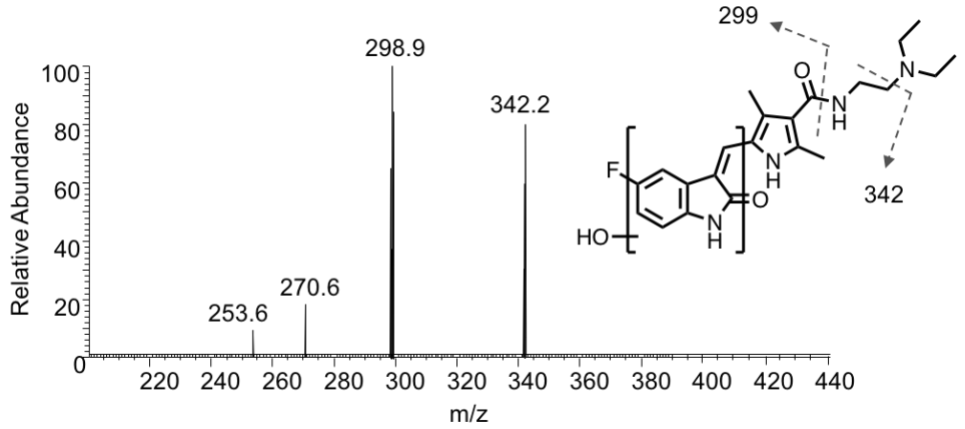
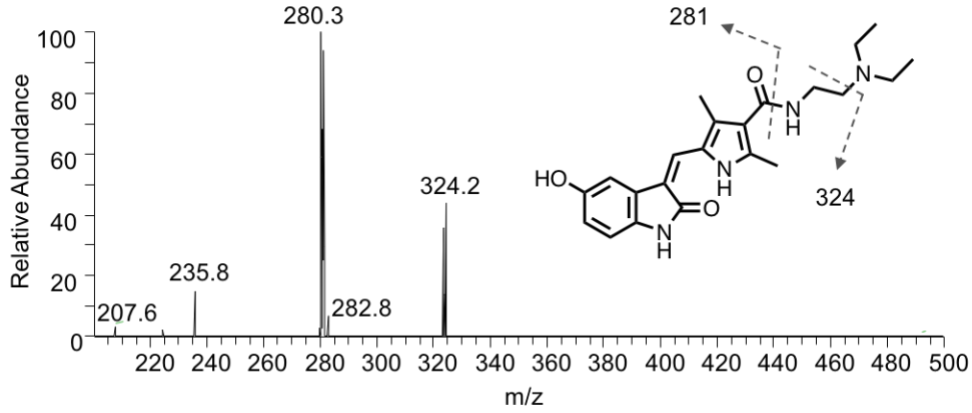

Relative Abundance
m/z
298.9
342.2
270.6
253.6
299
342
F
HO
O
N
H

C.


Relative Abundance
m/z
280.3
324.2
235.8
207.6
282.8
281
324
HO
O
N
H

D.

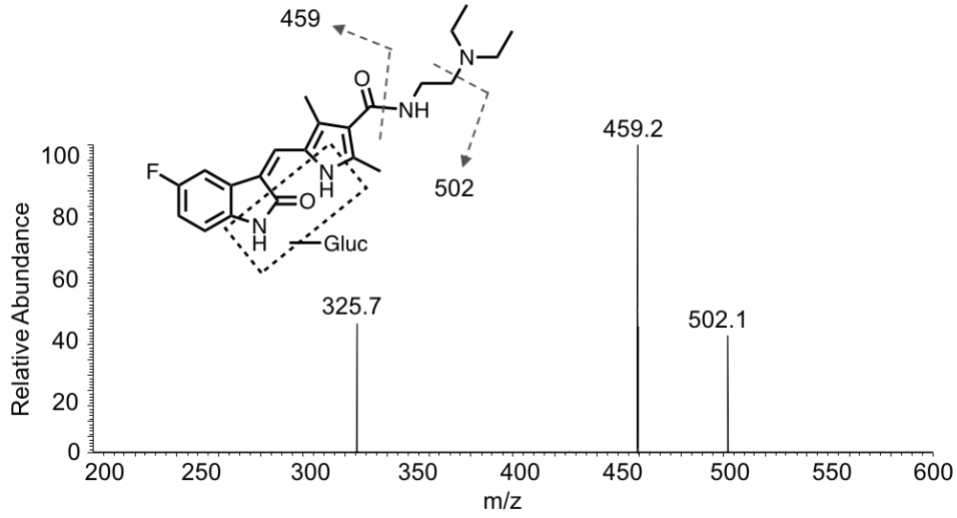
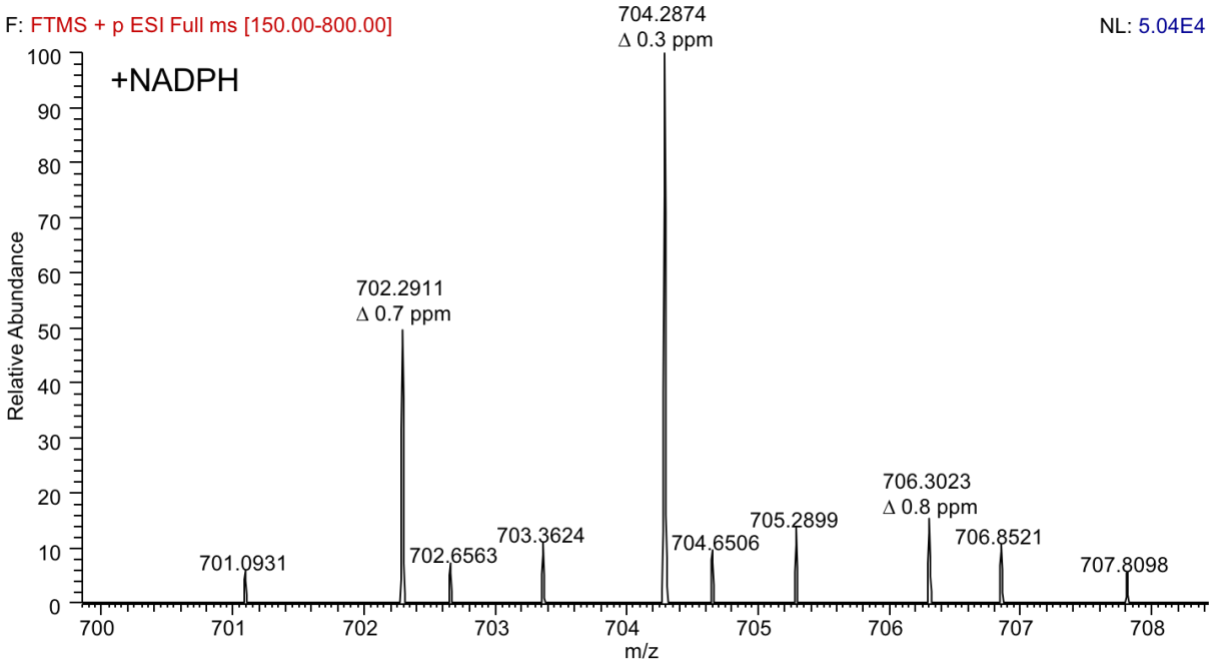

Relative Abundance
m/z
459
502
459.2
325.7
502.1
F
O
NH
N
H
Gluc

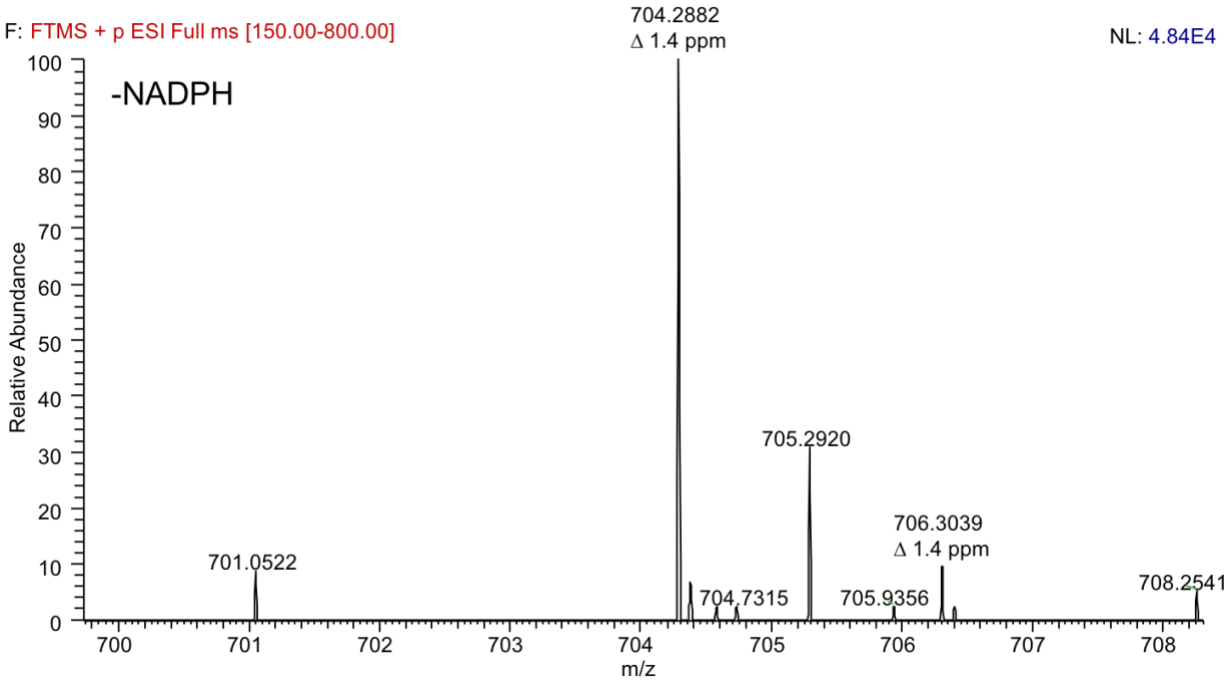
Figure S2: HRMS Analysis of the Putative Quinoneimine-GSH Conjugate (M5) and the Direct Sunitinib-GSH Conjugate (Sun-SG). Sunitinib (50 μM) was incubated with pooled human liver microsomes (0.5 mg/mL) in the presence of GSH (5 mM) and an NADPH regenerating system for 30 min. Control incubations were carried out without NADPH regenerating system. HRMS analysis was performed using a Waters Acquity UPLC coupled to a LTQ Orbitrap XL system (Thermo). Shown are the following annotated spectra: (A) full scan HRMS analysis of GSH conjugates from incubations (A) with NADPH and (B) without NADPH; and (C) higher-energy collisional dissociation MS/MS analysis of *m/z* 704.29 (collision energy 25 V). The calculated exact mass of M5 ($C_{32}H_{44}N_7O_9S^+$) is *m/z* 702.2916; the exact mass of Sun-SG ($C_{32}H_{45}FN_7O_8S^+$) is *m/z* 706.3029; and the exact mass of the predicted GSH conjugate at *m/z* 704 ($C_{32}H_{43}FN_7O_8S^+$) is *m/z* 704.2872. HRMS and MS/MS analysis was performed in ESI positive ion mode.

Figure S2

A.



B.


F: FTMS + p ESI Full ms [150.00-800.00]
704.2882
Δ 1.4 ppm
NL: 4.84E4
-NADPH
Relative Abundance
705.2920
706.3039
Δ 1.4 ppm
701.0522
704.7315
705.9356
708.2541
m/z

C.

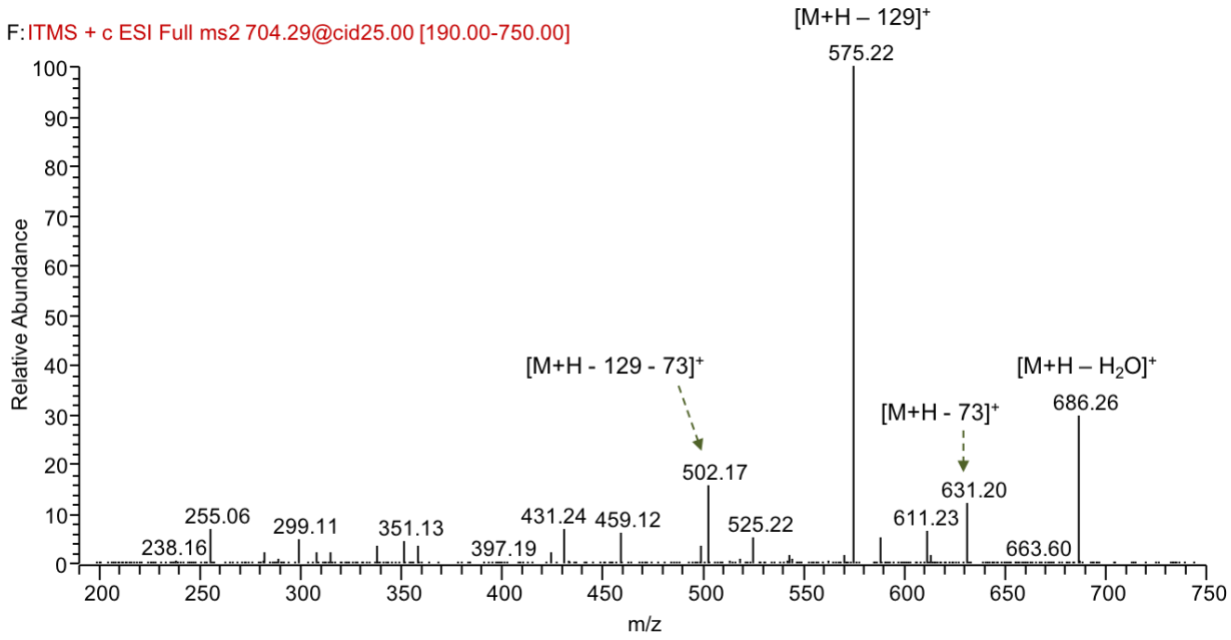

F: ITMS + c ESI Full ms2 704.29@cid25.00 [190.00-750.00]
[M+H – 129]+
575.22
Relative Abundance
[M+H - 129 - 73]+
[M+H – H2O]+
686.26
[M+H - 73]+
502.17
631.20
255.06
299.11
351.13
431.24
459.12
525.22
611.23
238.16
397.19
663.60
m/z

Figure S3: LC-MS/MS Chromatograms of M5 and Sun-SG. Sunitinib (50 μM) was incubated with pooled human liver microsomes (0.5 mg/mL) in the presence of an NADPH-regenerating system and supplemented with GSH (5 mM) for 60 min. Metabolites were analyzed by LC-MS/MS utilizing MRM. Shown are representative LC-MRM chromatograms of the (A) quinoneimine-GSH conjugate, M5 (*m/z* 702 > 586), and (B) GSH conjugates of sunitinib parent drug, Sun-SG (*m/z* 706 > 577).

Figure S3

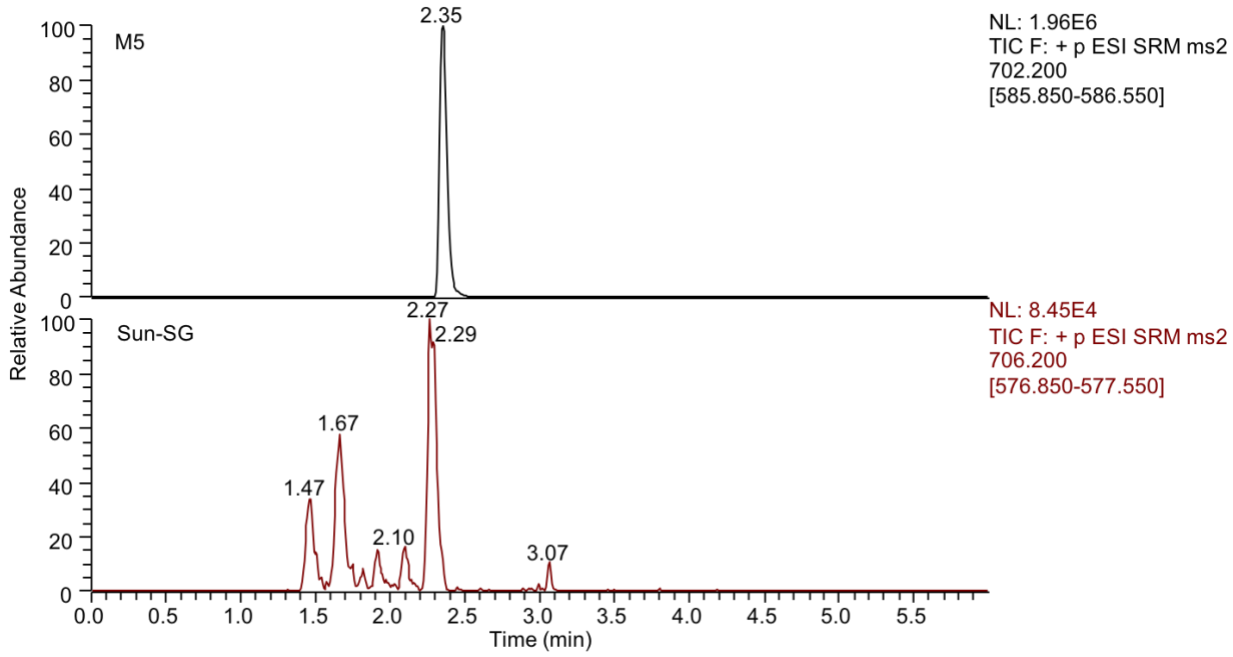
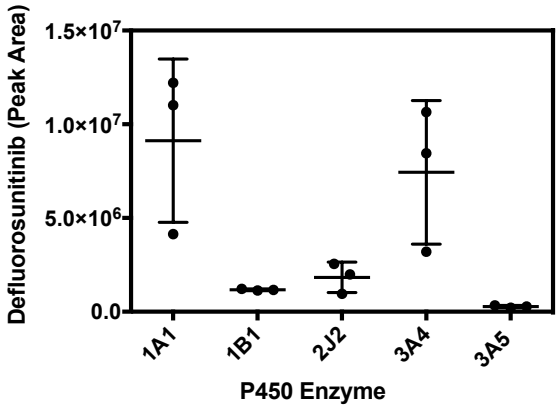


Figure S4: Relative Contributions of Extrahepatic P450s to Sunitinib Metabolism. (A) Defluorosunitinib (M3), and (B) quinoneimine-GSH conjugate (M5). Sunitinib (25 μM) was incubated with selected recombinant human P450 Supersomes™ (20 nM) in the presence of an NADPH-regenerating system and supplemented with GSH (5 mM). Sunitinib metabolites were analyzed by LC-MS/MS analysis utilizing MRM. The MRM transitions for the metabolites indicated were: defluorosunitinib (M3, *m/z* 397 > 281) and quinoneimine-GSH conjugate (M5, *m/z* 702 > 586). The MRM peak areas are shown for each metabolite. The results are shown as the mean ± SD from a single experiment performed in triplicate.

Figure S4

A.



B.

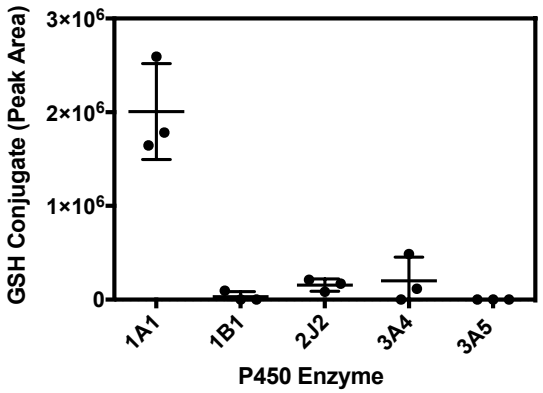
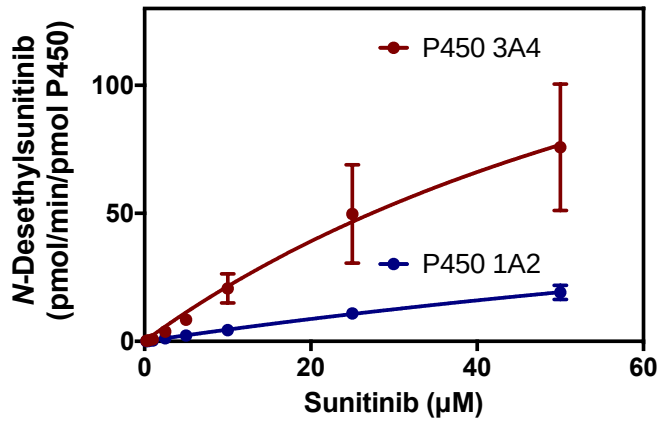


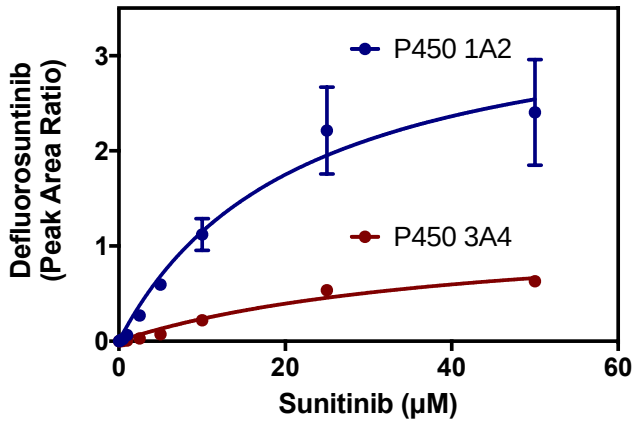
Figure S5: Kinetic Analysis of Sunitinib Metabolism by Recombinant P450 1A2 and 3A4. Increasing concentrations of sunitinib (0.05 - 50 μM) were incubated with P450 1A2 and 3A4 Supersomes™ (20 nM) in the presence of an NADPH-regenerating system and supplemented with GSH (5 mM). Formation of (A) *N*-desethylsunitinib, M1, (B) defluorosunitinib, M3, and (C) quinoneimine-GSH conjugate, M5, were analyzed by LC-MS/MS analysis utilizing MRM. The MRM transitions for the metabolites indicated were: M1 (*m/z* 371 > 283), M3 (*m/z* 397 > 281), M5 (*m/z* 702 > 586). M1 was quantified using an authentic chemical standard. The peak area ratios for M3 and M5 were obtained using the MRM peak area for the analyte of interest divided by peak area for sunitinib-d_4, internal standard (*m/z* 403 > 283). The results are shown as the means ± SD from three independent experiments (n = 3) performed in triplicate each.

Figure S5

A.



B.


Defluorosuntinib
(Peak Area Ratio)
P450 1A2
P450 3A4
Sunitinib (μM)
0
1
2
3
0
20
40
60

C.

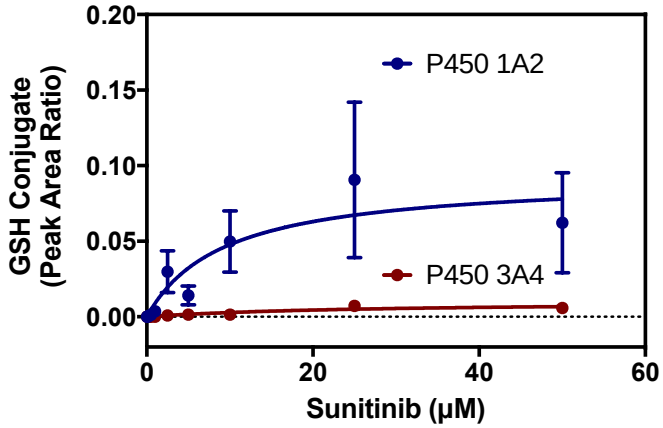
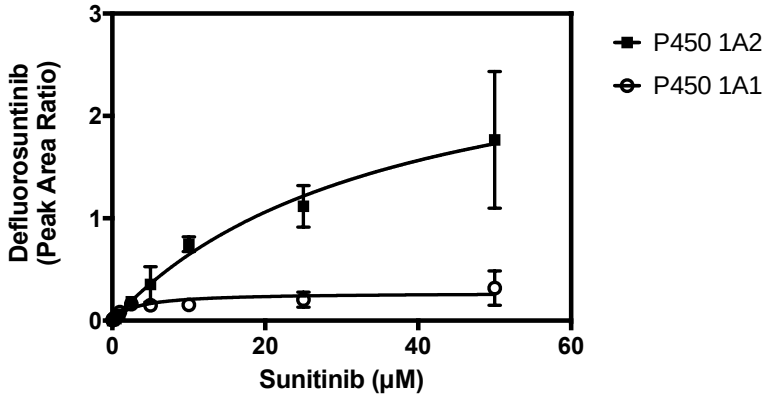

GSH Conjugate
(Peak Area Ratio)
P450 1A2
P450 3A4
Sunitinib (μM)
0.00
0.05
0.10
0.15
0.20
0
20
40
60

Figure S6: Kinetic Analysis of Sunitinib Metabolism by Recombinant P450 1A1 and 1A2.

Sunitinib (0.05 - 50 μM) was incubated with P450 1A1 and 1A2 Supersomes™ (20 nM) in the presence of an NADPH-regenerating system and supplemented with GSH (5 mM). Formation of (A) defluorosunitinib, M3, and (B) quinoneimine-GSH conjugate, M5, were analyzed by LC-MS/MS analysis utilizing MRM. The MRM transitions for the metabolites indicated were: M3 (*m/z* 397 > 281) and M5 (*m/z* 702 > 586). The peak area ratios for M3 and M5 were obtained using the MRM peak area for the analyte of interest divided by peak area for sunitinib-d_4, internal standard (*m/z* 403 > 283). The results are shown as the means ± SD from a single experiment.

Figure S6

A.



B.

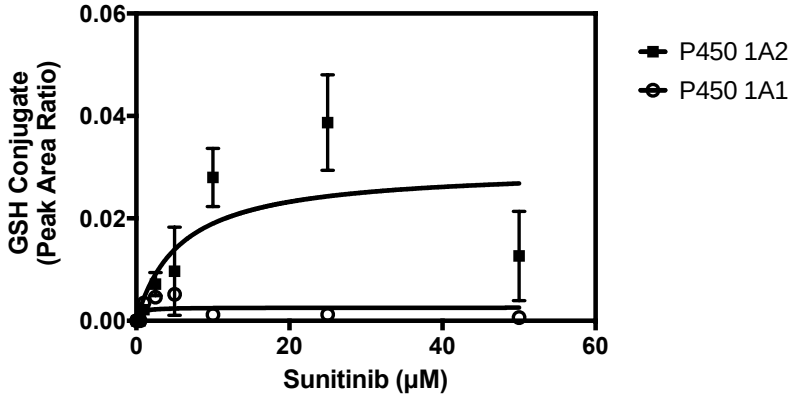
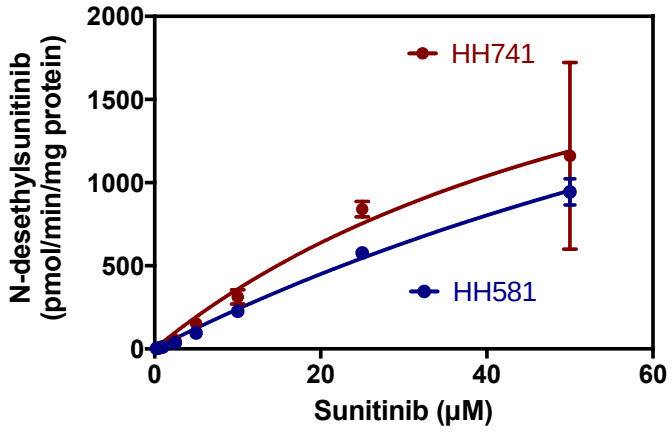


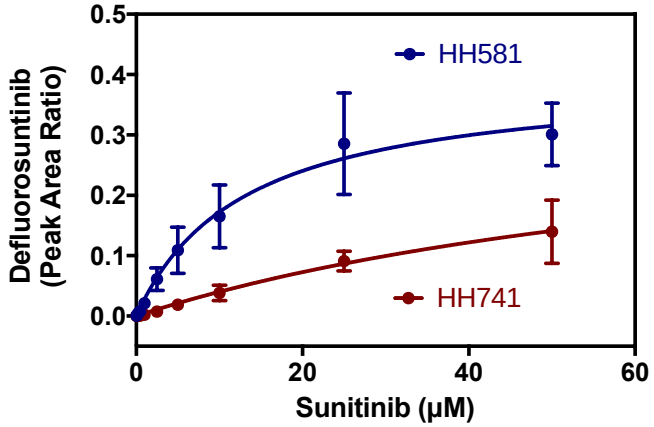
Figure S7: Kinetic Analysis of Sunitinib Metabolism with Single Donor Human Liver Microsomes (HH581 and HH741). P450 1A2 activity was characterized as high for HH581 and low for HH741. (*See "Materials and Methods"*). Sunitinib at increasing concentrations (0.05 - 50 μM) was incubated with single donor human liver microsomes, HH581 and HH741 (0.25 mg protein/mL) in the presence of an NADPH-regenerating system and supplemented with GSH (5 mM). Formation of (A) *N*-desethylsunitinib, M1, (B) defluorosunitinib, M3, and (C) quinoneimine-GSH conjugate, M5, were analyzed by LC-MS/MS analysis utilizing MRM. The MRM transitions for the metabolites indicated were: M1 (*m/z* 371 > 283), M3 (*m/z* 397 > 281), M5 (*m/z* 702 > 586). Formation of M1 was quantified using an authentic chemical standard. The peak area ratios for M3 and M5 were obtained using the MRM peak area for the analyte of interest divided by peak area for sunitinib-d_4, internal standard (*m/z* 403 > 283). The results are shown as the means ± SD from three independent experiments (n = 3) performed in triplicate each.

Figure S7

A.



B.


Defluorosuntinib
(Peak Area Ratio)
0.5
0.4
0.3
0.2
0.1
0.0
0
20
40
60
Sunitinib (μM)
HH581
HH741

C.

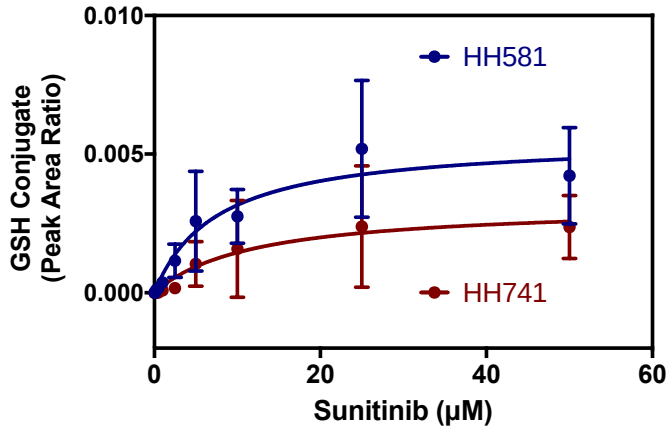
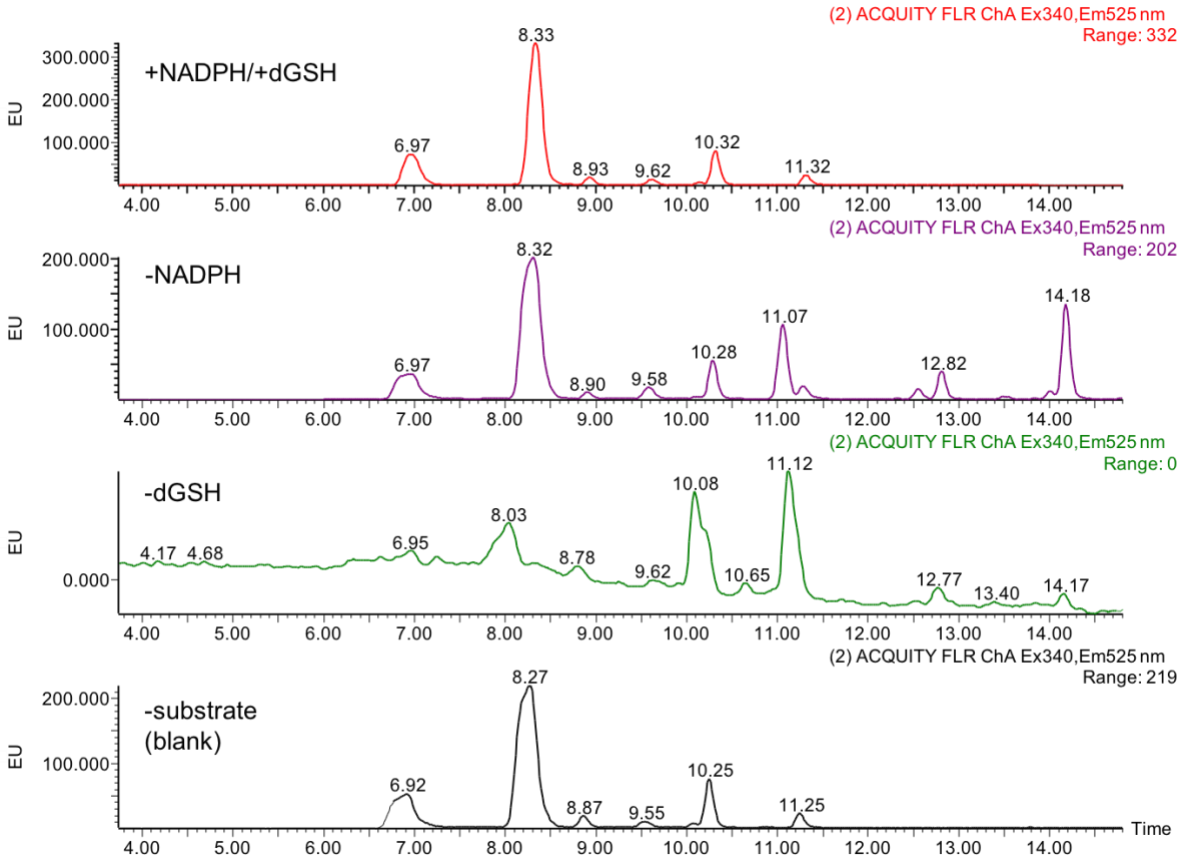

GSH Conjugate
(Peak Area Ratio)
0.010
0.005
0.000
0
20
40
60
Sunitinib (μM)
HH581
HH741

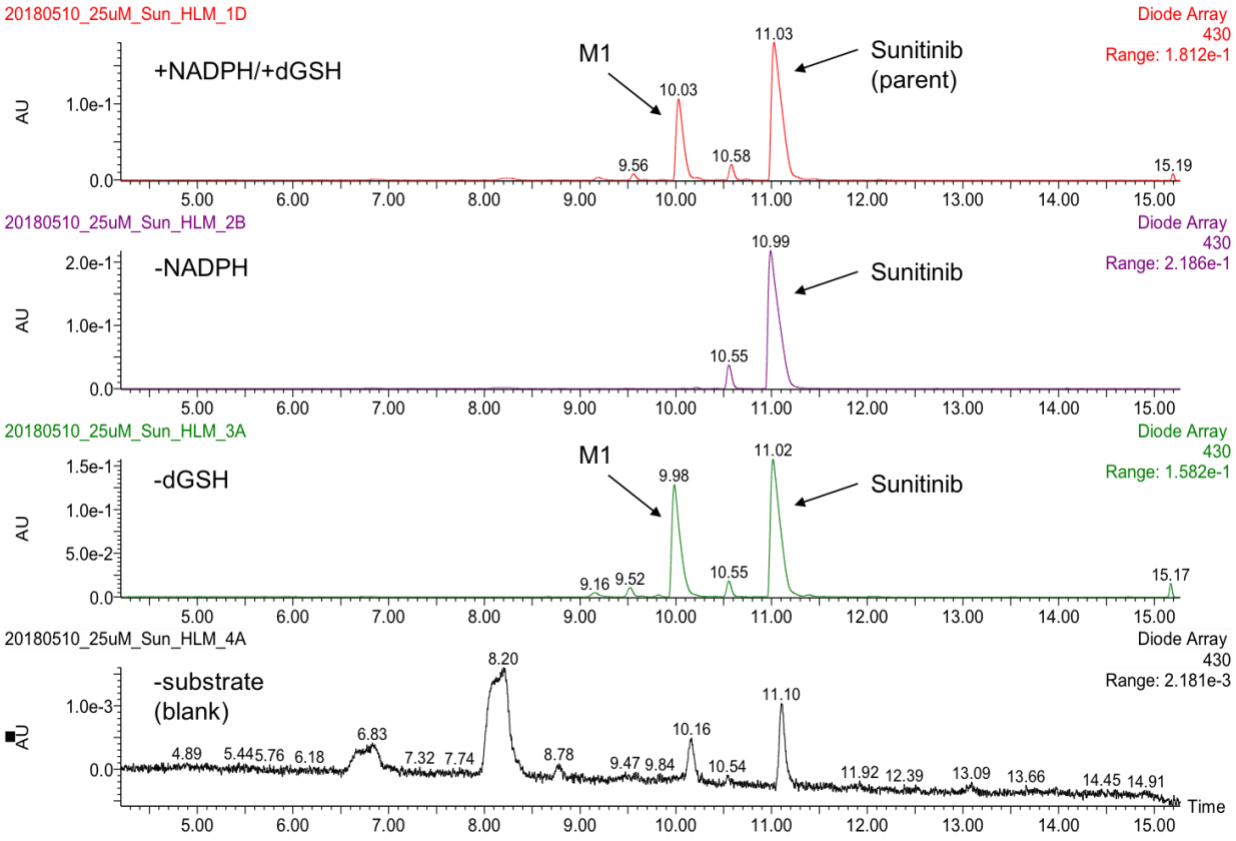
Figure S8: UPLC-UV-Fluorescence Analysis of Sunitinib Metabolic Incubations with Dansyl-GSH. Sunitinib (25 μM) was incubated with pooled human liver microsomes (1 mg/mL) in the presence of dGSH (1 mM, containing 0.01 mM TCEP) and an NADPH regenerating system for 30 min. Control incubations were without NADPH, without dGSH, or without substrate (blank sample). Each reaction condition was performed in triplicate. Samples were analyzed by UPLC-UV-fluorescence detection. Shown is (A) UPLC-fluorescence analysis (excitation 340 nm, emission 525 nm) for detection of dansyl-related products, based on the method of Gan et al.[2] (B) UPLC-UV absorbance analysis at 430 nm for detection of sunitinib-related products. The incubation conditions are indicated: +NADPH/+dGSH, -NADPH, -dGSH, and -substrate (blank).

Figure S8

A.



B.



References

(1) Speed, B., Bu, H. Z., Pool, W. F., Peng, G. W., Wu, E. Y., Patyna, S., Bello, C., and Kang, P. (2012) Pharmacokinetics, distribution, and metabolism of [^{14}C]sunitinib in rats, monkeys, and humans. *Drug Metab. Dispos. 40*, 539-555.

(2) Gan, J., Harper, T. W., Hsueh, M. M., Qu, Q., and Humphreys, W. G. (2005) Dansyl glutathione as a trapping agent for the quantitative estimation and identification of reactive metabolites. *Chem. Res. Toxicol. 18*, 896-903.