Role of Epoxide Hydrolases and Cytochrome P450s on Metabolism of KZR-616, a First-in-Class Selective Inhibitor of the Immunoproteasome

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Legends for Supplemental Figures

Supplemental Figure 1: Metabolism of testosterone (probe substrate) in HLM, MLM and human hepatocytes A) Formation of metabolite 6β-testosterone in the presence or absence of 1-ABT in the incubations of testosterone with: A) 0.5 mg/mL HLM; B) 0.25 mg/mL MLM incubations, and C) 0.5 x10^6 cells/mL human hepatocytes. Data represent mean ± SD from duplicate incubations.

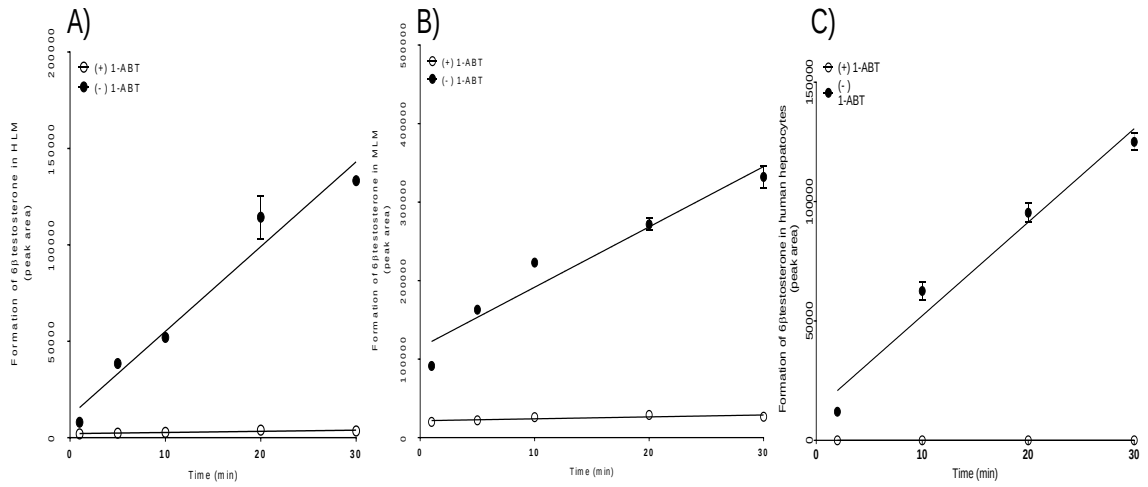
Supplemental Figure 2: Epoxide hydrolysis of *cis*-SO and *trans*-SO in HLM & MLM, human & monkey cytosol and recombinant mEH & sEHs

Epoxide hydrolysis of *cis*-SO (50 µM) in A) HLM (0.5mg/ml), B) MLM (0.25 mg/mL), and in recombinant human mEH (10 µg/mL), respectively. Epoxide hydrolysis of *trans*-SO (50 µM) in D) HLM (0.5 mg/mL), E) MLM (0.25 mg/mL), and F) recombinant human sEH (25 µg/mL), respectively. The diol derivatives of *cis*-SO and *trans*-SO were quantified by using LC-UV methods. Data represent mean ± SD from duplicate incubations.

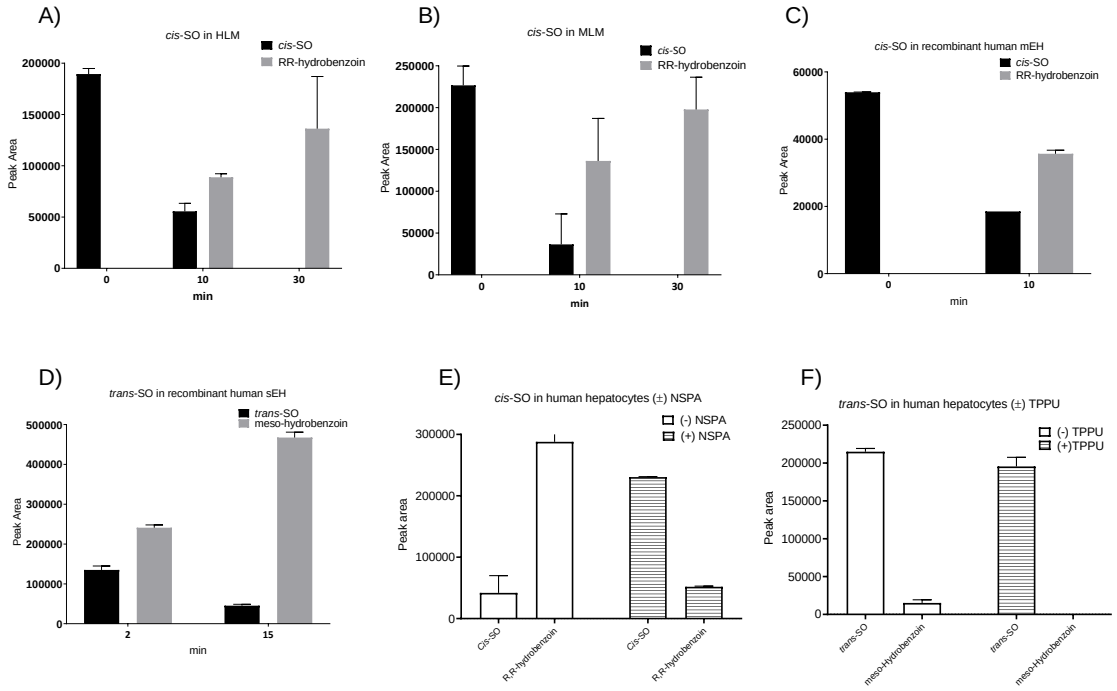
Supplemental Figure 3: Inhibition of epoxide hydrolysis of *cis*-SO in HLMs, MLM, and recombinant human mEH by NSPA Inhibition of mEH activity by NSPA in A) female HLMs (0.5 mg/mL), B) male HLMs (0.5 mg/mL), and C) recombinant human mEH (4 µg/mL), respectively. The concentration of *cis*-SO was 50 µM.

Supplemental Figure 4: Metabolism of *cis*-SO, *trans*-SO and testosterone in monkey hepatocytes A) formation of RR-hydrobenzoin from *cis*-SO (50 µM), B) formation of meso-hydrbenzoin from *trans*-SO (50 µM), C) formation of 6βtestosterone from testosterone (50 µM), respectively. Data represent mean ± SD from duplicate incubations.

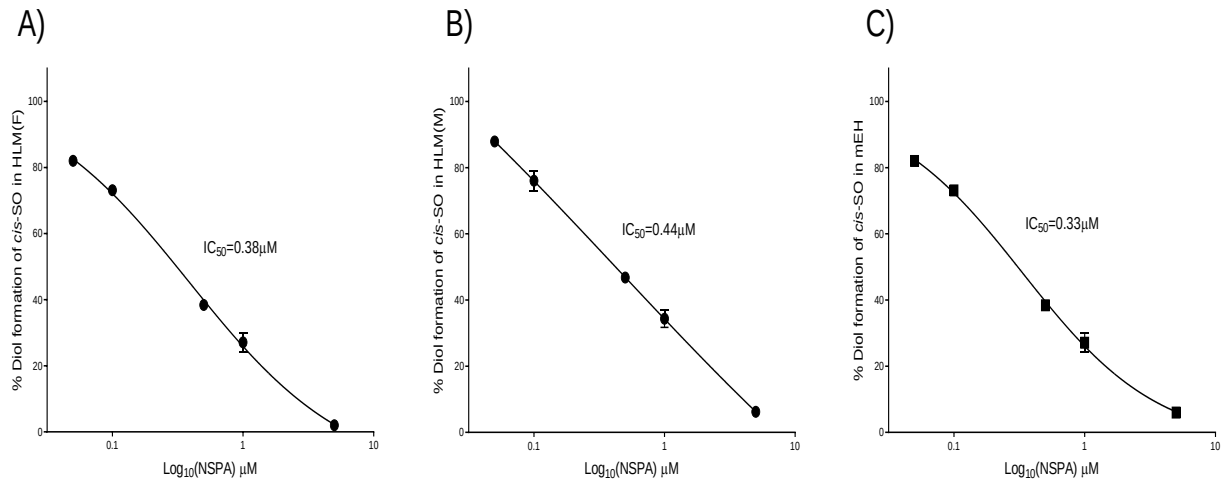
Supplemental Figure 1



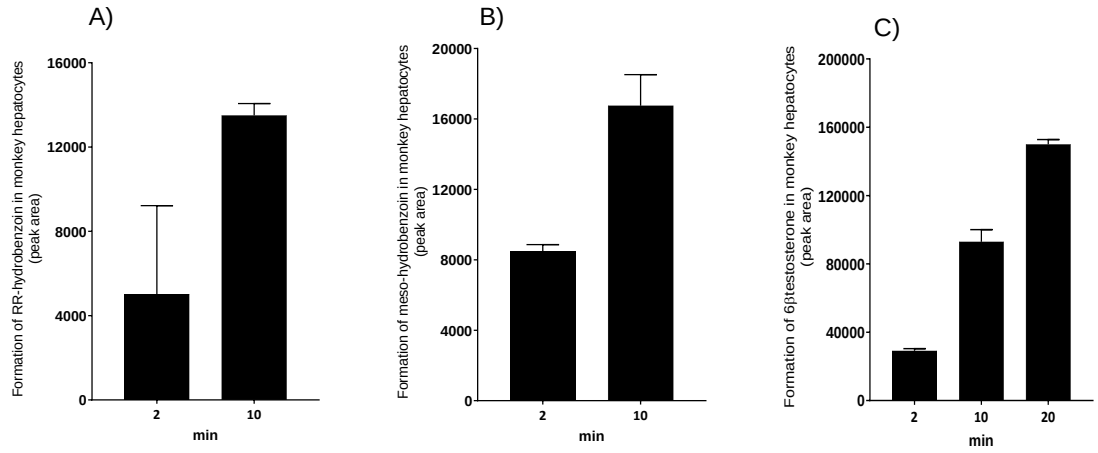
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Table 1. Effect of Selective CYP Inhibitors on the Clearance of KZR-616 by HLM

Incubated compound	Inhibitors	KZR-616 Remaining (%, Mean)			KZR-59587 Formation (%, Mean)		
		0 min	15 min	30 min	0 min	15 min	30 min
KZR-616	DMSO	100	83.1	54.0	1.20	13.3	23.1
	Furafylline	100	74.6	54.1	1.10	13.1	23.4
	Montelukast	100	94.5	72.3	1.10	9.80	21.0
	Sulfaphenazole	100	85.6	52.9	1.40	14.5	22.5
	Benzylnirvanol	100	77.8	54.6	1.30	12.8	20.9
	Quinidine	100	79.6	53.9	1.10	11.9	23.8
	Ketoconazole	100	99.6	98.8	0.900	13.0	31.1