In vitro metabolism of montelukast by Cytochrome P450s (CYPs) and UDP-glucuronosyltransferases (*UGTs*)

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Supplemental Data

Table S1. Correlation of formation rates of montelukast 1,2 diol, 21(R)-OH montelukast, 21(S)-OH montelukast and 25-OH montelukast from montelukast (1 μM) metabolism with the activities of different P450 isoforms and total P450 contents in 14 HLMs

	Montelukast 1,2 diol		**21(R)-OH montelukast**		**21(S)-OH montelukast**		**25-OH montelukast**	
P450s	***r***	***p***	***r***	***p***	***r***	***p***	***r***	***p***
Total P450	0.47	0.091	0.46	0.094	0.46	0.094	0.65	0.012
Cytochrome c	0.02	0.935	0.18	0.542	0.15	0.604	0.06	0.852
Cytochrome b5	-0.09	0.770	-0.23	0.430	-0.24	0.403	-0.28	0.336
CYP1A2	0.21	0.473	-0.01	0.970	-0.08	0.794	0.15	0.604
CYP2A6	0.30	0.302	0.84	0.0002	0.78	0.001	0.68	0.008
CYP2B6	0.48	0.080	0.61	0.020	0.59	0.027	0.65	0.012
CYP2C8	0.63	0.016	0.33	0.246	0.33	0.253	0.60	0.025
CYP2C9	0.37	0.190	0.17	0.562	0.15	0.609	0.30	0.291
CYP2C19	0.82	0.0004	0.84	0.0002	0.84	0.0001	0.94	<0.0001
CYP2D6	0.34	0.239	0.28	0.337	0.35	0.220	0.32	0.259
CYP2E1	0.50	0.066	0.05	0.863	0.01	0.976	0.27	0.357
CYP3A4	0.68	0.007	0.85	0.0001	0.81	0.0004	0.78	0.001
CYP4A11	0.81	0.0004	0.29	0.323	0.33	0.253	0.61	0.020
FMO	0.12	0.689	-0.07	0.815	-0.04	0.886	0.06	0.839

Data were analyzed using the nonparametric correlation test (Spearman *r*). The activity of each isoform was determined using the respective specific substrate probe reaction as indicated by the supplier (see Materials and Methods section). $p < 0.05$ is considered statistically significant.

Figure S1

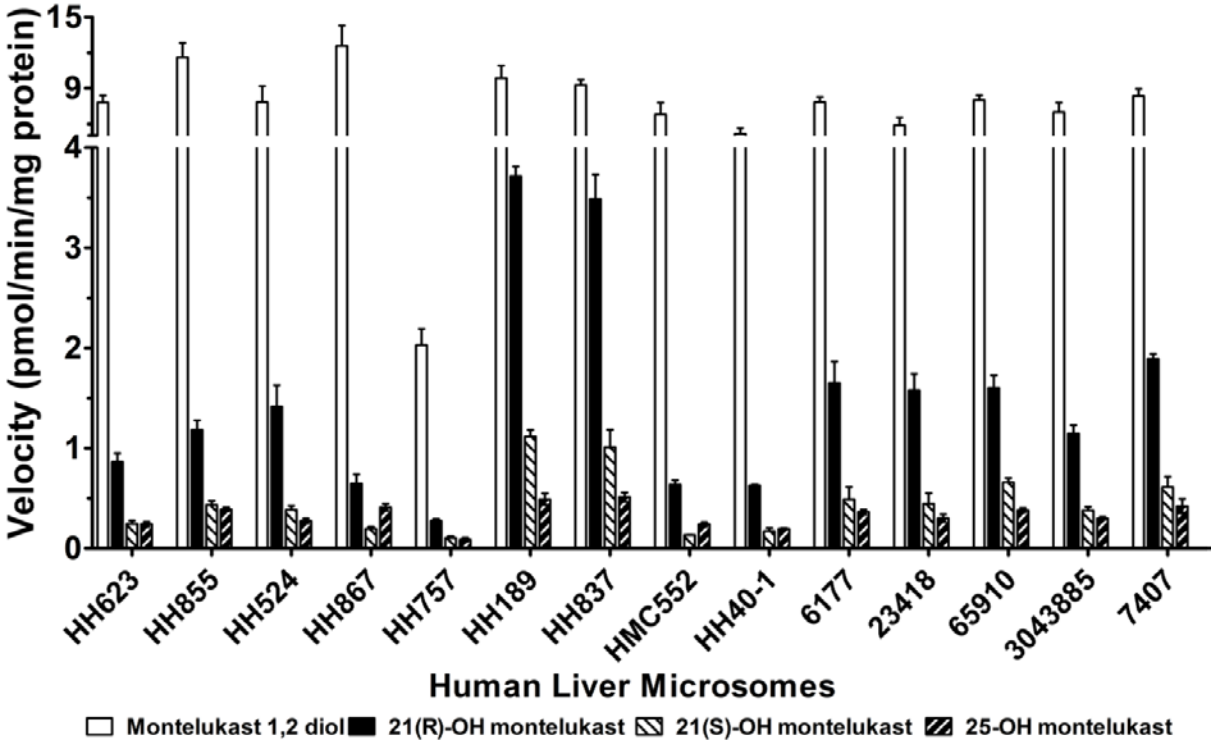

Figure S1. The formation rate of montelukast 1,2 diol, 21(R)-OH montelukast, 21(S)-OH montelukast and 25-OH montelukast in a panel of 14 characterized HLMs. Montelukast (1 μM) was incubated in HLMs (0.25 mg/mL) with a NADPH-generating system (final volume, 250 μL) at 37℃ for 30 min.

Figure S2

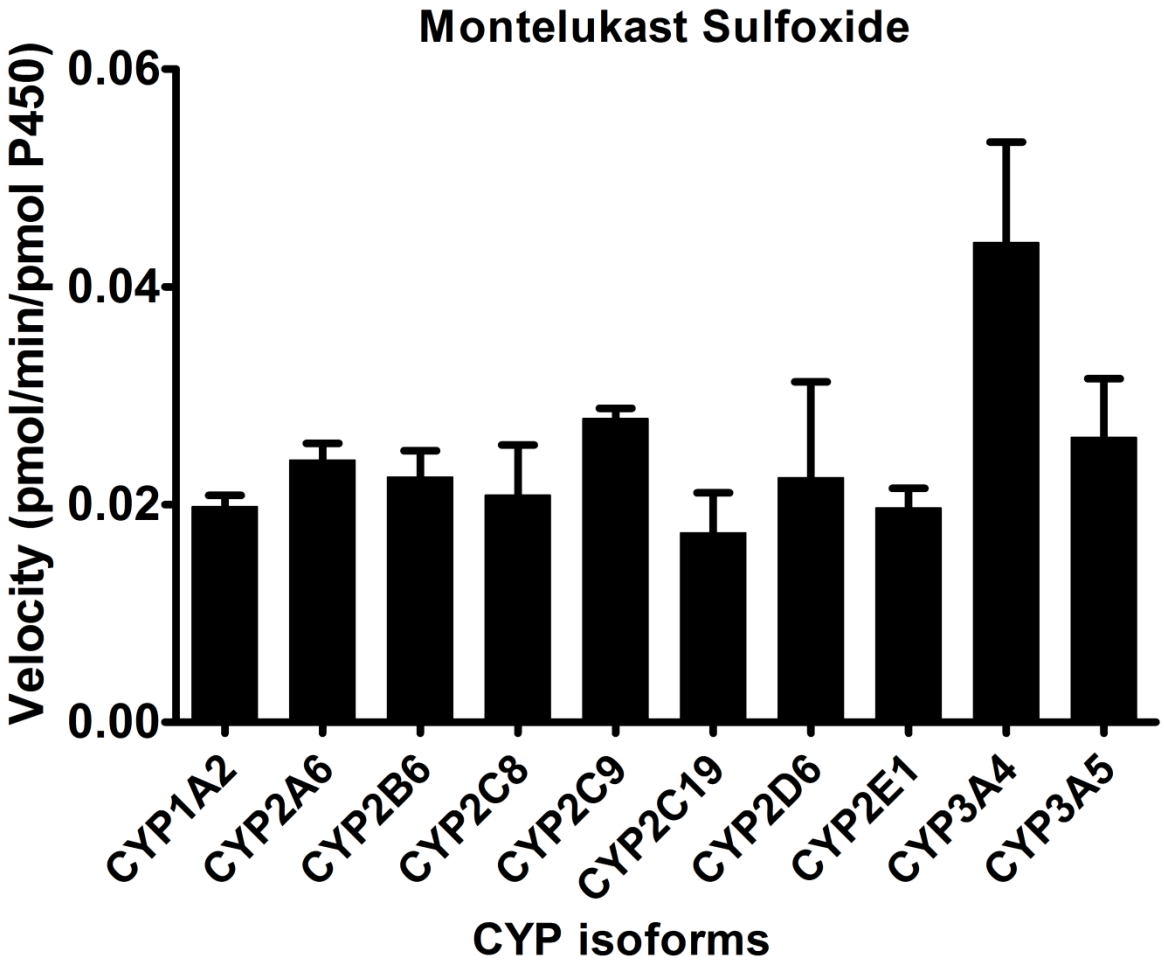

Figure S2. Formation rate of montelukast sulfoxide in a panel of 10 recombinant human CYP P450s. 20 pmol of recombinant P450 was incubated with montelukast (1 μM) and NADPH-generating system (final volume, 250 μL) at 37 ℃ for 30 min. The recombinant human CYPs used were co-expressed without cytochrome b_5.

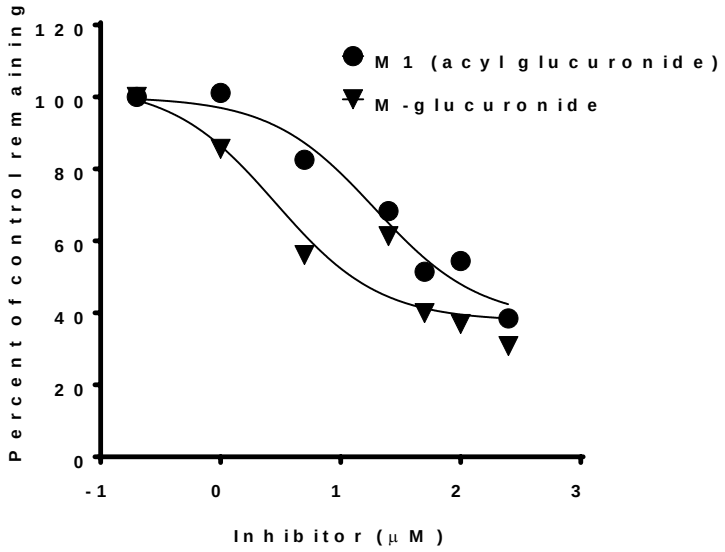

Figure S3. Inhibition of montelukast glucuronidation to montelukast acyl-β-D glucuronide and M-glucuronide by gemfibrozil in HLM. Montelukast ((1μM) was incubated with HLMs (0.25 mg/mL) and UDPGA (5mM) for 30 min at 37℃ in the absence (vehicle control) and in the presence increasing concentration of gemfibrozil (0.5 to 250 μM). Percent activity remaining versus the control activity was calculated. Nonlinear regression was implemented to fit the data to the log(gemfibrozil) vs. percent activity remaining. The absolute and relative IC50s were similar (83.4μM and 37.6μM for the inhibition of acyl-glucuronide and M-glucuronide respectively). Each data represents average of duplicate incubations.