Supporting information – Foster, et al.

Supplementary Table 1: Rimonabant EC_{50} values in the THLE cell lines

THLE cell line	Mean (μM)	SD	n
1A2	8.5	2.8	4
2C9	4.5**	0.6	6
2C19	6.0	1.6	3
2D6	9.0	1.7	3
3A4	1.3***	0.2	7
Null[1]	8.4	2.0	8

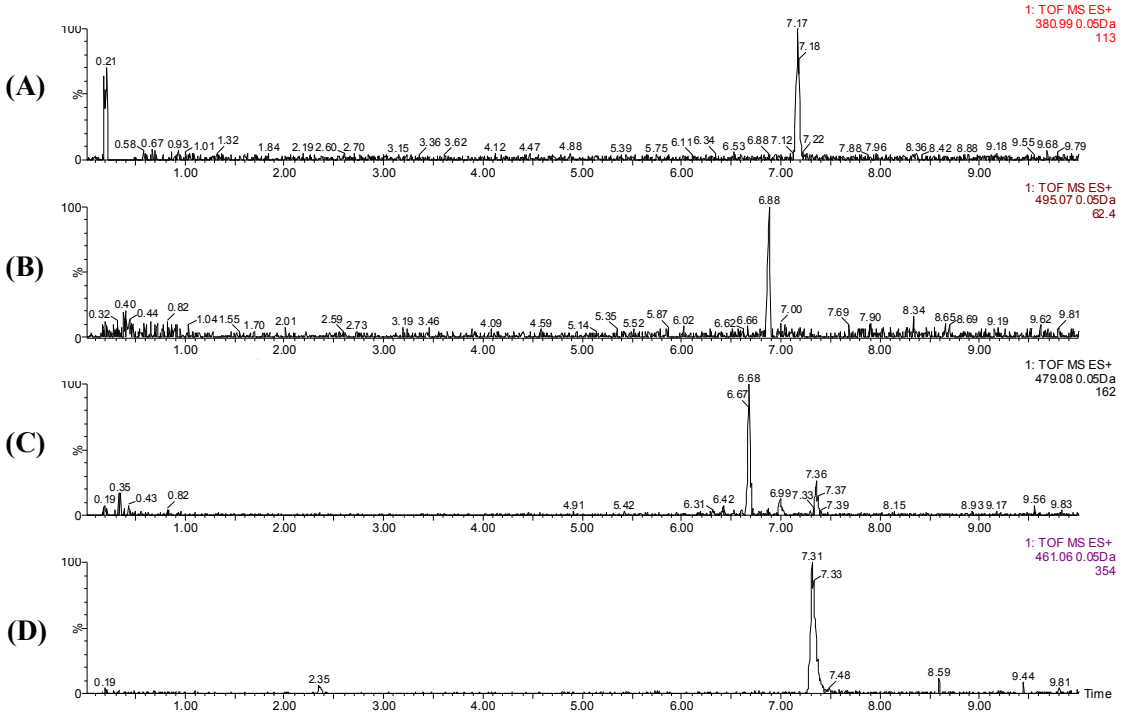
[1] where the EC_{50} value exceeded the top concentration tested the EC_{50} value was set to the top concentration (10 μM). ** P<0.01; *** p<0.001 statistically significant difference from the THLE-Null EC_{50}; paired t-test

Supplementary Table 2: The effect of Ritonavir on the Rimonabant EC_{50} values in the THLE-3A4 and Null cell lines

THLE cell line	Ritonavir (0.3 μM)	Mean (μM)	SD	n
3A4	-	1.3	0.2	4
Null1	-	8.4	2.0	4
3A4	+	4.3*	1.1	4
Null[1]	+	8.4	1.5	4

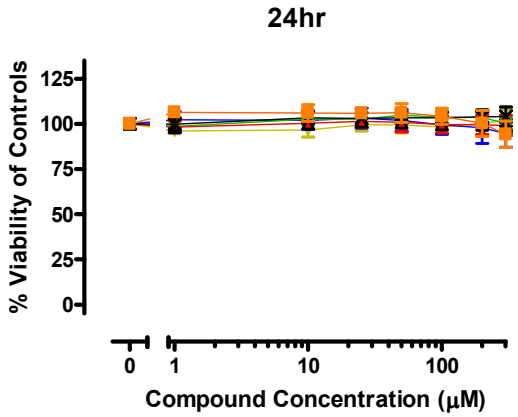
[1] where the EC_{50} value exceeded the top concentration tested the EC_{50} value was set to the top concentration (10 μM). * p<0.05 statistically significant difference from EC_{50} in THLE-3A4 cells in absence of inhibitor; paired t-test

Supplementary Figure 1 LC-MS analysis of supernatants from THLE-3A4 cells incubated with [^{14}C]-rimonabant



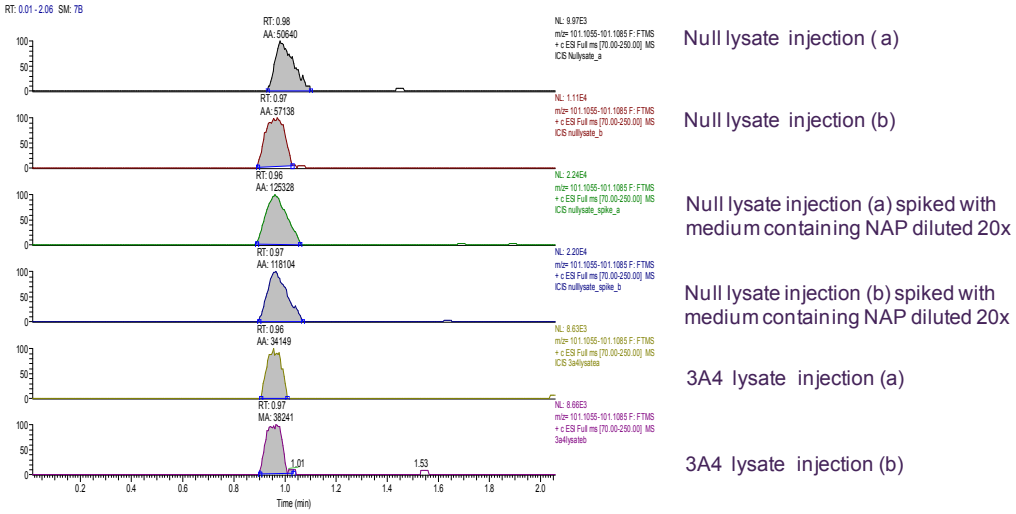
Extracted ion chromatograms for (A) amide cleavage product 6 (MH+ 380.9959), (B) +32 (MH+ 495.0752), (C) +16 (MH+ 479.0802) and (D) -2 Da (M+ 461.0697) metabolites.

Supplementary Figure 2 Toxicity of NAP in the THLE cells



Evaluation of the toxicity of NAP in the THLE-1A2, 2C9, 2C19, 2D6, 3A4 and Null cells. The cells were exposed to 0 – 300 µM NAP for 24 hours; ▼1A2, ◇ 2C9, ■2C19, ● 2D6, △ 3A4, x Null. Data are mean ± S.D. from 3 experiments each performed in triplicate.

Supplementary Figure 3 Intracellular NAP determination



Evaluation of the intracellular penetration of NAP. Twenty four hours post-dosing with 300 µM NAP the medium was removed, the cells washed with 40 mL HBSS-/-, scraped into 1 mL de-ionised water and then lysed by sonication prior to analysis by LCMS. Data are from

duplicate injections ((a) and (b)). THLE-Null lysate samples were spiked with 5 μL 300 μM NAP diluted 20 fold (0.75 μM final concentration) to confirm the identification of the peak and to give an approximate concentration in the initial samples in the absence of a standard curve (area increased by ~ 2 fold).

Supplementary Figure 4 Structure of Ritonavir