

Supplementary material

Aminobenzotriazole (ABT) Inhibits and Induces Several Key Drug Metabolizing Enzymes Complicating its Utility as a Pan CYP Inhibitor for Reaction Phenotyping

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Table S1. Experimental summary for ABT DDI assessments

Experimental endpoint	Systems	Isoforms investigated
Inhibition	H μ rel, Stromal only cells, human hepatocytes, and recombinant enzymes	UGTs (1A1, 1A3, 1A4, 1A6, 1A9, 2B7, 2B15) SULTs (1A1, 1A3, 1B1, 1C2, 1C4, 1E1, 2A1, 2B1)
Induction	H μ rel, plated hepatocytes (RIS donor), stromal only cells	CYPs (1A2, 2B6, 2C8, 2C9, 3A4, UGTs 1A1, 1A6)

Table S2. Donor demographics for hepatocytes used in studies

Parameter	Donor designation		
	2017942-01	HH1103	HU1064HuP
Vendor	LifeNet Health	IVAL 999Elite	H μ rel
Gender	Female	Female	Male (3), Female (2)
Race	Caucasian	Caucasian / Hispanic	Caucasian (5)
Cause of death	CVA/Stroke	Anoxia/CVA	CVA (2)/Anoxia (3)
Age (years)	55	44	40-68

Table S3. Induction parameter estimates for commonly used CYP inhibitors

Isoform	ABT		erythromycin		azamulin		atipamezole		ketoconazole		quinidine		ZY-12201	
	EC50 (µM)	E _{max} (fold)	EC50 (µM)	E _{max} (fold)	EC50 (µM)	E _{max} (fold)	EC50 (µM)	E _{max} (fold)	EC50 (µM)	E _{max} (fold)	EC50 (µM)	E _{max} (fold)	EC50 (µM)	E _{max} (fold)
CYP1A2	227	9.65	1.55	2.54	No induction		10.7	11.3	0.114	2.72	3.96	2.98	5.3	5.01
CYP2B6	80.0	7.75	0.719	3.16	4.97	9.42	4.48	5.42	Not tested		No induction		2.1	4.82
CYP2C8	211	3.59	No induction		2.92	4.80	Not tested		Not tested		Not tested		Not tested	
CYP2C9	No induction		No induction		1.35	3.69	Not tested		Not tested		Not tested		Not tested	
CYP3A4	0.455	2.06	2.75	2.87	2.30	8.73	5.33	3.86	0.23	2.14	5.80	3.15	3.9	7.69
UGT1A1	158	3.19	No induction		2.89	2.78	Not tested		Not tested		Not tested		Not tested	
UGT1A6	No induction		No induction		No induction		Not tested		Not tested		Not tested		Not tested	
Hepatocyte model used	H _p rel		H _p rel		H _p rel		RIS qualified donor HH1103							

Table S4. Induction parameter estimates for positive controls used in induction studies in H_prel.

Enzyme	Rifampin		Phenobarbital	Omeprazole
	EC ₅₀ (µM)	E _{max} (fold)	E _{max} (fold)	E _{max} (fold)
CYP1A2	NA	NA	1.8	48.5
CYP2B6	Not determined	10.2	24.5	9.10
CYP2C8	0.0642	5.10	Not determined	Not determined
CYP2C9	0.0554	4.02	Not determined	Not determined
CYP3A4	0.056	8.78	8.4	4.0
UGT1A1	0.0381	2.77	Not determined	Not determined
UGT1A6	No induction		Not determined	Not determined

Table S5. Metabolite structure proposal

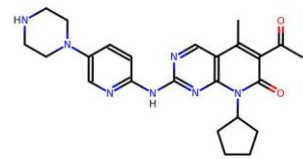
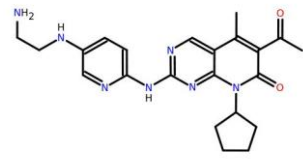
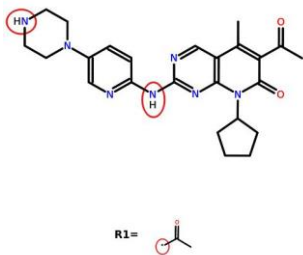

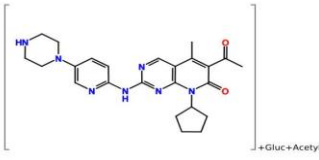
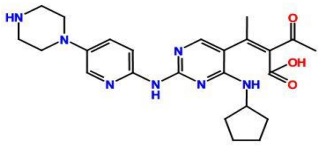
Metabolite					
Peak name	Structure proposal	rt	m/z	z	ppm
Substrate		6.195	448.2448	1	1.5
M422 RT=5.93		5.932	422.2292	1	1.6
M490 RT=7.51	 <p>R1 = </p>	7.514	490.2554	1	1.1
M666 RT=6.28	 <p>+Gluc+Acetyl</p>	6.275	666.2863	1	2.8
M466 RT=5.51		5.512	466.2553	1	1.8

Table S6. Extent of inhibition observed for various pathways in the presence of ARV-471

metabolite designation	% of total metabolites	
	alone	+471
N-dealkylation	24.5	0
+glucuronide	12.4	2.2
+glucuronide + O	26.3	0.2
+glucuronide + acetyl	19.9	0.8
N-sulfation	0.31	1.7
Sulfate	5.2	13.0
Acetyl	0.0	82.1
N-dealkylation + hydrolysis	11.4	0.0

Figure S1 Determination of stability of ABT (A), contribution of stromal cells to observed metabolite formation (B) and impact of ABT on 4-MU glucuronidation in H_{pre}l® co-culture model (C)

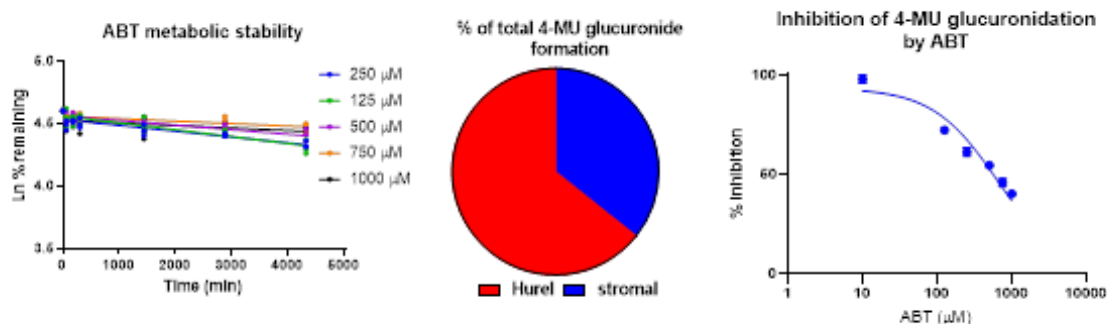


Figure S2 Evaluation of ABT as an inhibitor of UGTs in recombinant systems (A) and pooled human hepatocytes (B)

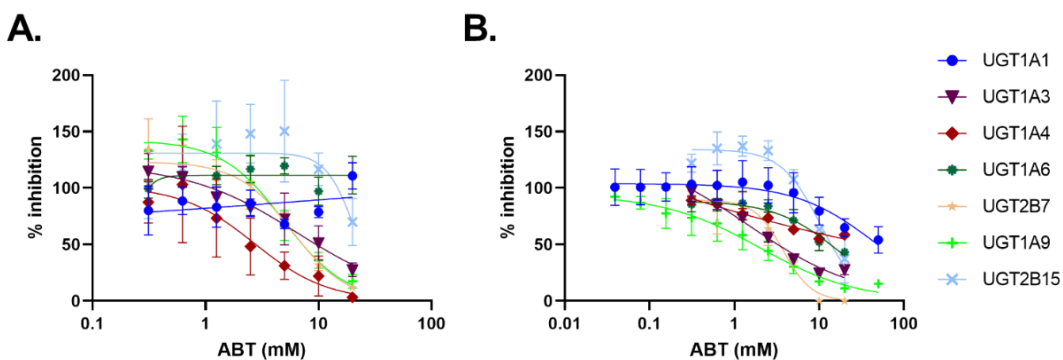


Figure S3 Comparison of palbociclib depletion at physiologically relevant or supratherapeutic concentration used in the metabolite profiling study

