Drug clearance by aldehyde oxidase: can we avoid clinical failure?

Supplementary material

Supplementary Table 1. Multiple reaction monitoring transitions, declustering potential, collision energies and cell exit potential of compounds used for IVIVC.

	MRM transition	DP	CE (V)	CXP (V)
AMG-900	504.163>316.000	211	47	26
Bosutinib	530.244>141.100	186	31	28
Capmatinib	413.153>382.000	110	35	32
Carbazeran	361.360>272.200	71	29	16
4OH-carbazeran	377.350>287.900	101	25	18
Fasudil	292.129>99.000	146	37	20
Idelalisib	416.263>176.100	166	39	26
Imatinib	494.342>394.100	141	37	36
JNJ38877605	378.234>129.100	201	89	22
Lenvatinib	427.185>369.800	151	39	32
Lu AF09535	407.241>270.100	171	19	40
O_6-benzylguanine	242.236>199.300	101	23	16
PF-06273340	480.177>255.100	196	45	37

PF-05190457	513.194>209.000	141	49	30
PF-945863	824.563>158.000	231	57	14
Ruxolitinib	307.240>186.000	181	37	28
XK469	345.000>273.000	120	30	20
Zaleplon	306.126>236.100	161	37	34
Zoniporide	321.222>262.000	131	23	18

MRM, Multiple Reaction Monitoring; DP, Declustering Potential, CE, Collision Energy; CXP, Cell Exit Potential; V, Volt

Supplementary Table 2. Mass-to-charge (m/z) ratios and retention time (RT) of all compounds analyzed for metabolite profiling. Data are reported for substrates and their metabolites considered for analysis, i.e. the metabolite(s) sensitive to hydralazine inhibition (AO substrates) or the probe metabolites for the other enzymes (acetaminophen, benzydamine, 6-nitroquinazolinone, 4-methylumbelliferone, sulfamethazine and trifluoperazine). Data for bosutinib and ruxolitinib, which were used as negative controls as they are not AO substrates, are not reported. All metabolites are mono-oxidations except where noted.

Substrate and metabolites analyzed	m/z	RT (min)
AMG-900	504.159	6.52
M1	520.155	5.64
Capmatinib	413.152	4.56
M1	429.148	3.84
Carbazeran	361.187	4.24
M1	377.182	4.40
Fasudil	292.111	2.45
M1	308.106	1.85
Idelalisib	416.163	4.89
M1	432.158	4.71
Imatinib	494.266	4.96
M1	510.261	4.37
M2	510.261	4.47
JNJ38877605	417.122	4.45

M1	433.117	3.82
Lenvatinib	427.116	4.45
M1	443.111	3.90
Lu AF09535	407.219	6.05
M1	423.213	4.17
M2	439.208	4.43
M3 (di-oxidation)	423.213	5.07
O_6-benzylguanine	242.103	5.00
M1	258.097	5.76
PF-06273340	480.154	6.37
M1	496.149	6.18
PF-05190457		5.51
M1		4.81
PF-945863	824.480	4.42
M1	840.475	4.22
M2	840.475	4.45
XK469[a]	343.049	9.13
M1[a]	359.044	7.57
Zaleplon	306.135	4.75
M1	322.131	3.06

M2	322.131	3.85
Zoniporide	321.146	3.91
M1	337.141	3.40
Acetaminophen[a]	150.056	1.17
M1 (glucuronidation)[a]	326.089	3.61
M2 (sulfation)[a]	230.013	4.05
Benzydamine	310.191	4.68
M1 (N-oxide)	326.186	3.81
6-nitroquinazolinone[a]	174.031	2.95
M1[a]	190.026	2.69
4-methylumbelliferone	177.054	3.50
M1 (glucuronidation)	353.086	1.05
Trifluoperazine	408.171	6.03
M1 (glucuronidation)	584.204	5.24
Sulfamethazine	279.090	2.76
M1 (N-acetylation)	321.101	2.64

[a] detected in negative ion mode

Supplementary Table 3. Predicted and observed blood clearance (CLb) used for the *in-vitro/in-vivo* correlation of all AO substrates shown in Supplementary Figure 1. Unbound, total, hepatocyte CL_{int} was scaled up with the Well-Stirred model (Yang et al., 2007) as described in Materials and Methods. Observed CLb values are the same as those reported in Table 6 and are acquired from the literature.

Compound	Number in Supplementary Figure 1	Predicted CLb (mL/min/kg)	Observed CLb (route)
AMG-900	1	0.3 ± 0.1	1.1 (po)
Capmatinib	2	1.1 ± 0.2	≤2.1 (po)[a]
Carbazeran	3	11.7 ± 1.5	46.2 (iv)
Fasudil	4	18.9 ± 0.2	47.6 (iv)
Idelalisib	5	1.1 ± 0.1	3.6 (po)[b]
Imatinib	5b	2.8 ± 0.3	3.7 (iv)
JNJ38877605	6	1.6 ± 0.3	16.1 (po)[b]
Lenvatinib	7	0.1 ± 0.0	1.7 (po)[b]
O_6-benzylguanine	8	1.8 ± 0.5	12.3 (iv)
PF-06273340	9	1.7 ± 0.2	32.8 (po)
PF-05190457	10	1.8 ± 0.2	10.5 (po)[b]
PF-945863	11	8.2 ± 1.7	93 (po)
Zaleplon	12	5.5 ± 1.7	18.3 (iv)
Zoniporide	13	5.8 ± 0.7	16.7 (iv)

fa ([a]) or F ([b]) were used to adjust oral CLb/F as explained in Materials and Methods

Supplementary Fig 1. *In-vitro/in-vivo* correlation between total, scaled, hepatocyte CL_{int} (predicted blood clearance, CLb) and observed CLb for the AO substrates used in this study. Unbound hepatocyte CL_{int} was scaled up with the Well-Stirred model (Yang et al., 2007). Circles: iv CLb; squares: po CLb/F adjusted by F (black squares) or oral absorption (open square); triangles: po CLb/F unadjusted. N=4 for all compounds except idelalisib and zaleplon (n=3). Lu AF09535 cannot be shown as its CLb_{-obs} is out of scale; clearance for XK469 could not be scaled as its hepatocyte CL_{int} was below the assay sensitivity. Correspondence between compounds and numbers can be seen in Supplementary Table 3.