

Efficient screening of P450 BM3 mutants for their metabolic activity and diversity towards a wide set of drug-like molecules in chemical space

Jelle Reinen, Jolanda S. van Leeuwen, Yongmin Li, Lifang Sun, Peter D. J. Grootenhuis, Caroline J. Decker, John Saunders, Nico P. E.

Vermeulen and Jan N. M. Commandeur

Drug Metabolism and Disposition

Supplemental Table 3 Effects of different mutations on the substrate depletion of nine drugs compared to their corresponding mutation templates.

BM3 mutant	Amitriptyline	Buspirone	Cilostazol	Citalopram	Diltiazem	Irbesartan	Ondansetron	Propafenone	Repaglinide
M01 ^a	58.7 ± 7.9	55.9 ± 8.7	7.2 ± 3.3	2.0 ± 6.4	0.1 ± 4.0	15.7 ± 2.5	10.9 ± 5.6	2.1 ± 1.5	0.3 ± 2.2
M02 ^a	52.8 ± 10.3	90.8 ± 2.2	27.3 ± 1.8	1.4 ± 2.8	0.9 ± 4.8	1.1 ± 3.2	12.3 ± 3.2	3.2 ± 4.4	5.5 ± 2.7
M05 ^a	88.9 ± 2.0	61.6 ± 3.7	24.2 ± 3.9	29.5 ± 3.9	1.0 ± 5.2	36.5 ± 3.0	1.1 ± 3.8	3.5 ± 5.3	7.9 ± 5.6
M11 ^a	70.0 ± 2.5	24.5 ± 9.9	5.3 ± 3.3	6.6 ± 1.8	2.2 ± 1.9	14.7 ± 6.5	4.1 ± 0.9	10.4 ± 6.4	11.4 ± 1.8
MT32 (L437E) ^{bc}	+9.7 ± 2.7	+23.0 ± 11.7	+57.1 ± 3.4	+40.5 ± 2.3	+21.8 ± 7.0	+57.6 ± 10.3	+2.2 ± 2.5	-3.2 ± 8.0	+0.4 ± 6.3
MT33 (L437N) ^{bc}	-5.3 ± 4.5	-21.1 ± 10.8	-0.9 ± 7.3	+5.1 ± 4.1	+29.2 ± 2.8	+17.2 ± 10.3	+7.6 ± 1.1	-5.3 ± 9.3	-0.3 ± 3.4
MT34 (A74E) ^{bc}	+10.9 ± 4.5	-18.4 ± 15.3	+13.4 ± 8.7	-0.7 ± 2.7	+1.7 ± 4.1	+0.5 ± 6.5	+4.1 ± 3.0	+1.6 ± 6.7	-6.6 ± 2.9
MT35 (L437S) ^{bc}	+24.0 ± 3.1	+8.0 ± 10.9	+28.6 ± 3.7	+39.8 ± 5.5	+41.3 ± 3.8	+53.8 ± 7.7	-0.8 ± 4.8	+2.1 ± 7.0	+24.7 ± 4.3
MT36 (L437T) ^{bc}	+2.5 ± 3.2	-6.8 ± 11.0	+23.8 ± 4.3	+24.0 ± 6.0	+1.7 ± 4.8	+31.6 ± 8.8	+0.6 ± 1.0	-5.3 ± 6.5	-6.5 ± 3.8

MT37 (A74D) ^{bc}	+3.5 ± 10.8	-5.7 ± 12.9	-1.6 ± 3.6	+4.1 ± 4.4	+6.2 ± 4.3	+0.0 ± 4.3	-3.5 ± 2.5	+14.7 ± 7.3	-11.4 ± 1.8
MT38 (S72D) ^{bc}	+21.7 ± 2.8	-11.8 ± 10.4	+69.9 ± 4.5	+44.0 ± 2.3	+16.9 ± 2.7	+23.1 ± 3.7	+12.4 ± 4.2	+15.9 ± 7.6	-7.2 ± 3.5
MT41 (A74E) ^{bd}	+0.1 ± 16	-16.6 ± 15.3	+28.4 ± 4.6	+26.1 ± 7.5	+13.3 ± 5.2	+36.2 ± 3.3	+0.1 ± 6.8	+16.6 ± 4.4	-0.6 ± 2.2
MT43 (S72D) ^{bd}	-11.1 ± 9.3	+0.6 ± 8.9	+79.9 ± 5.3	+17.8 ± 6.6	+10.6 ± 4.1	-6.8 ± 7.7	+26.2 ± 7.5	+19.9 ± 2.3	+1.7 ± 5.2
MT44 (S72E) ^{bd}	-4.4 ± 11.0	-23.0 ± 9.7	+67.6 ± 6.8	+22.0 ± 6.7	+15.7 ± 5.2	+6.2 ± 7.9	+3.2 ± 6.2	+21.9 ± 4.5	+0.4 ± 3.0

The value of the mutant displaying the highest amount of substrate depletion has been highlighted in **bold** for each compound.

^a The substrate depletion is calculated by using the averaged peak area of the parent at 90 min and at time zero. Values represent the mean ± standard deviation of three replicates and are expressed in percentages of the averaged peak area of the parent at time zero.

^b Effects on substrate depletion efficiency have been calculated by subtracting the depletion measured for the corresponding mutation template from the depletion measured for the mutant in question. Standard deviations have been incorporated in this calculation.

^{bc} The M11 mutant was used as mutation template.

^{bd} The M01 mutant was used as mutation template.