

Supplementary Table S1. Output Parameter Values for Live Cell Multiparametric Hepatotoxicity Assay

Output parameter	Concentration (μM)	n (control)	n (compound)	Average ± SD (max)	Average ± SD (min)	Z'-Factor	Assay window
Positive W2 (number of Calcein AM-positive cells)							
Phenylbutasone	100	8	8	2,056 ± 76	147 ± 57	0.79	14.0
Staurosporine	10	8	8	1,812 ± 125	138 ± 118	0.56	13.1
Doxorubicin	10	8	8	1,828 ± 157	10 ± 11	0.72	189.1
Pimozide ^a	100	20	8	2,750 ± 413	23 ± 13	0.53	119.6
Chloroquine ^a	100	20	8	2,750 ± 413	34 ± 15	0.53	82.1
Seneciophylline ^a	100	20	8	2,750 ± 413	243 ± 62	0.43	11.3
rac perhexiline maleate ^a	100	20	4	2,750 ± 413	1.3 ± 1.9	0.55	2,199.8
Tamoxifen ^a	100	20	4	2,750 ± 413	6.8 ± 5.9	0.54	407.4
Promethazine · HCl ^a	100	20	4	2,750 ± 413	15.5 ± 13.5	0.53	177.4
% Positive W2 (% of Calcein AM-positive cells)							
Phenylbutasone	100	8	8	98 ± 0.3	91.6 ± 2.6	-0.28	1.1
Staurosporine	10	8	8	99 ± 0.4	49.9 ± 6.5	0.57	2.0
Doxorubicin	10	8	8	98 ± 0.8	59.4 ± 34.5	-1.72	1.7
Pimozide	100	20	8	93 ± 3.3	6.1 ± 4.9	0.71	15.2
Chloroquine	100	20	8	93 ± 3.4	29.6 ± 13.4	0.21	3.1
Seneciophylline	100	20	8	93 ± 3.4	82.9 ± 7.5	-2.20	1.1
rac perhexiline maleate	100	20	4	93 ± 3.4	0.2 ± 0.4	0.88	390.4
Tamoxifen	100	20	4	93 ± 3.4	0.9 ± 0.8	0.86	103.8
Promethazine · HCl	100	20	4	93 ± 3.4	41.6 ± 6.3	0.43	2.2
Positive W3 (number of cells with intact mitochondria)							
Phenylbutasone	100	8	8	1,778 ± 34	93 ± 22	0.90	19.2
Staurosporine	10	8	8	1,588 ± 96	50 ± 73	0.67	31.6
Doxorubicin	10	8	8	1,623 ± 130	12 ± 11	0.74	131.6
Pimozide	100	20	8	1,191 ± 147	56 ± 77	0.41	20.0
Chloroquine	100	20	8	1,191 ± 147	0.3 ± 0.5	0.63	4,765.6
Seneciophylline	100	20	8	1,191 ± 147	171 ± 45	0.43	7.0
rac perhexiline maleate	100	20	4	1,191 ± 147	0.3 ± 0.5	0.63	4,765.6
Tamoxifen	100	20	4	1,191 ± 147	0.8 ± 0.5	0.63	1,588.5
Promethazine · HCl	100	20	4	1,191 ± 147	0.3 ± 0.5	0.63	4,765.6

(continued)

Supplementary Table S1. (Continued)

Output parameter	Concentration (μM)	n (control)	n (compound)	Average ± SD (max)	Average ± SD (min)	Z'-Factor	Assay window
Total cells							
Phenylbutasone	100	8	8	2,095 ± 81	161 ± 61	0.78	13.1
Staurosporine	10	8	8	1,838 ± 128	191 ± 105	0.58	9.6
Doxorubicin	10	8	8	1,857 ± 152	13 ± 11	0.73	142.8
Pimozide	100	20	8	2,987 ± 446	660 ± 180	0.19	4.5
Chloroquine	100	20	8	2,987 ± 446	117 ± 22	0.51	25.6
Seneciophylline	100	20	8	2,987 ± 446	290 ± 55	0.44	10.3
rac perhexiline maleate	100	20	4	2,987 ± 446	722 ± 230	0.11	4.1
Tamoxifen	100	20	4	2,987 ± 446	731 ± 211	0.13	4.1
Promethazine · HCl	100	20	4	2,987 ± 446	48 ± 25	0.52	61.9
Positive W2 mean stain area (cell spreading)							
Phenylbutasone	100	8	8	910 ± 47	246 ± 24	0.68	3.7
Staurosporine	10	8	8	997 ± 72	185 ± 13	0.69	5.4
Doxorubicin	10	8	8	910 ± 90	1,042 ± 1,487	-5.74	0.9
Pimozide	100	20	8	548 ± 94	172 ± 15	0.13	3.1
Chloroquine	100	20	8	548 ± 94	243 ± 18	-0.10	2.3
Seneciophylline	100	20	8	548 ± 94	1,560 ± 138	0.31	2.8
rac perhexiline maleate	100	20	4	548 ± 94	80 ± 92	-0.19	6.9
Tamoxifen	100	20	4	548 ± 94	111 ± 75	-0.16	4.9
Promethazine · HCl	100	20	4	548 ± 94	304 ± 46	-0.72	1.8
Positive W2 mean stain integrated intensity							
Phenylbutasone	100	8	8	74,216,160 ± 8,587,114	8,055,733 ± 1,027,195	0.56	9.2
Staurosporine	10	8	8	70,186,701 ± 12,035,372	5,121,566 ± 486,415	0.42	13.7
Doxorubicin	10	8	8	57,453,660 ± 14,452,184	256,376,639 ± 477,143,244	-6.41	0.2
Pimozide	100	20	8	7,974,532 ± 1,601,910	1,513,469 ± 227,804	0.15	5.3
Chloroquine	100	20	8	7,974,532 ± 1,601,910	2,522,166 ± 902,099	-0.38	3.2
Seneciophylline	100	20	8	7,974,532 ± 1,601,910	54,458,219 ± 6,225,276	0.49	6.8
rac perhexiline maleate	100	20	4	7,974,532 ± 1,601,910	702,935 ± 907,532	-0.04	11.3
Tamoxifen	100	20	4	7,974,532 ± 1,601,910	835,341 ± 619,332	0.07	9.5
Promethazine · HCl	100	20	4	7,974,532 ± 1,601,910	5,477,076 ± 974,086	-2.09	1.5

(continued)

Supplementary Table S1. (Continued)

Output parameter	Concentration (μM)	n (control)	n (compound)	Average ± SD (max)	Average ± SD (min)	Z'-Factor	Assay window
Positive W2 mean stain average intensity							
Phenylbutasone	100	8	8	35,024 ± 2,727	13,852 ± 1,191	0.44	2.5
Staurosporine	10	8	8	29,660 ± 3,933	11,790 ± 1,651	0.06	2.5
Doxorubicin	10	8	8	26,514 ± 4,858	10,964 ± 6,046	-1.10	2.4
Pimozide	100	20	8	6,126 ± 357	3,688 ± 371	0.10	1.7
Chloroquine	100	20	8	6,126 ± 357	4,333 ± 1,205	-1.61	1.4
Seneciophylline	100	20	8	6,126 ± 357	14,980 ± 308	0.77	2.4
rac perhexiline maleate	100	20	4	6,126 ± 357	1,847 ± 2,365	-0.91	3.3
Tamoxifen	100	20	4	6,126 ± 357	2,353 ± 1,656	-0.60	2.6
Promethazine · HCl	100	20	4	6,126 ± 357	7,640 ± 1,247	-2.18	1.2
Positive W3 mean stain area							
Phenylbutasone	100	8	8	531 ± 41	196 ± 32	0.34	2.7
Staurosporine	10	8	8	607 ± 46	167 ± 25	0.52	3.6
Doxorubicin	10	8	8	518 ± 66	94,943 ± 109,469	-2.48	0.0
Pimozide	100	20	8	233 ± 32	333 ± 169	0.69	6.9
Chloroquine	100	20	8	233 ± 32	42 ± 84	-0.82	5.6
Seneciophylline	100	20	8	233 ± 32	464 ± 24	0.27	2.0
rac perhexiline maleate	100	20	4	233 ± 32	31 ± 62	-0.40	7.5
Tamoxifen	100	20	4	233 ± 32	112 ± 84	-1.87	2.1
Promethazine · HCl	100	20	4	233 ± 32	0.3 ± 0.5	0.58	932.3
All nuclei mean area (W1)							
Phenylbutasone	100	8	8	121 ± 2	93 ± 4	0.28	1.3
Staurosporine	10	8	8	142 ± 6	85 ± 4	0.46	1.7
Doxorubicin	10	8	8	132 ± 6	161 ± 207	-21.77	0.8
Pimozide	100	20	8	121 ± 8	113 ± 5	-18.00	1.1
Chloroquine	100	20	8	121 ± 8	110 ± 16	-5.39	1.1
Seneciophylline	100	20	8	121 ± 8	146 ± 7	-0.84	1.2
rac perhexiline maleate	100	20	4	121 ± 8	105 ± 7	-1.85	1.2
Tamoxifen	100	20	4	121 ± 8	90 ± 13	-0.94	1.4
Promethazine · HCl	100	20	4	121 ± 8	106 ± 6	-1.84	1.1

(continued)

Supplementary Table S1. (Continued)

Output parameter	Concentration (μM)	<i>n</i> (control)	<i>n</i> (compound)	Average \pm SD (max)	Average \pm SD (min)	Z'-Factor	Assay window
All nuclei mean average intensity (W1)							
Phenylbutasone	100	8	8	970 \pm 26	1,494 \pm 49	0.57	1.5
Staurosporine	10	8	8	828 \pm 27	1,278 \pm 105	0.12	1.5
Doxorubicin	10	8	8	800 \pm 41	978 \pm 1,441	-23.98	1.2
Pimozide	100	20	8	10,460 \pm 1,138	15,206 \pm 5,107	-2.90	1.5
Chloroquine	100	20	8	10,460 \pm 1,139	6,422 \pm 977	-0.57	1.6
Seneciophylline	100	20	8	10,460 \pm 1,139	6,207 \pm 170	0.08	1.7
rac perhexiline maleate	100	20	4	10,460 \pm 1,139	12,421 \pm 912	-2.14	1.2
Tamoxifen	100	20	4	10,460 \pm 1,139	11,859 \pm 1,046	-3.69	1.1
Promethazine · HCl	100	20	4	10,460 \pm 1,139	9,357 \pm 745	-4.13	1.1

Images acquired with 10x objective. Data combined from 2 separate experiments. W1 = Hoechst; W2 = Calcein AM; W3 = MitoTracker Orange.

²⁰ common control wells were used for these six compounds.

SD, standard deviation.