

## Supplementary Information

# Splice Modulation Synergizes Cell Cycle Inhibition

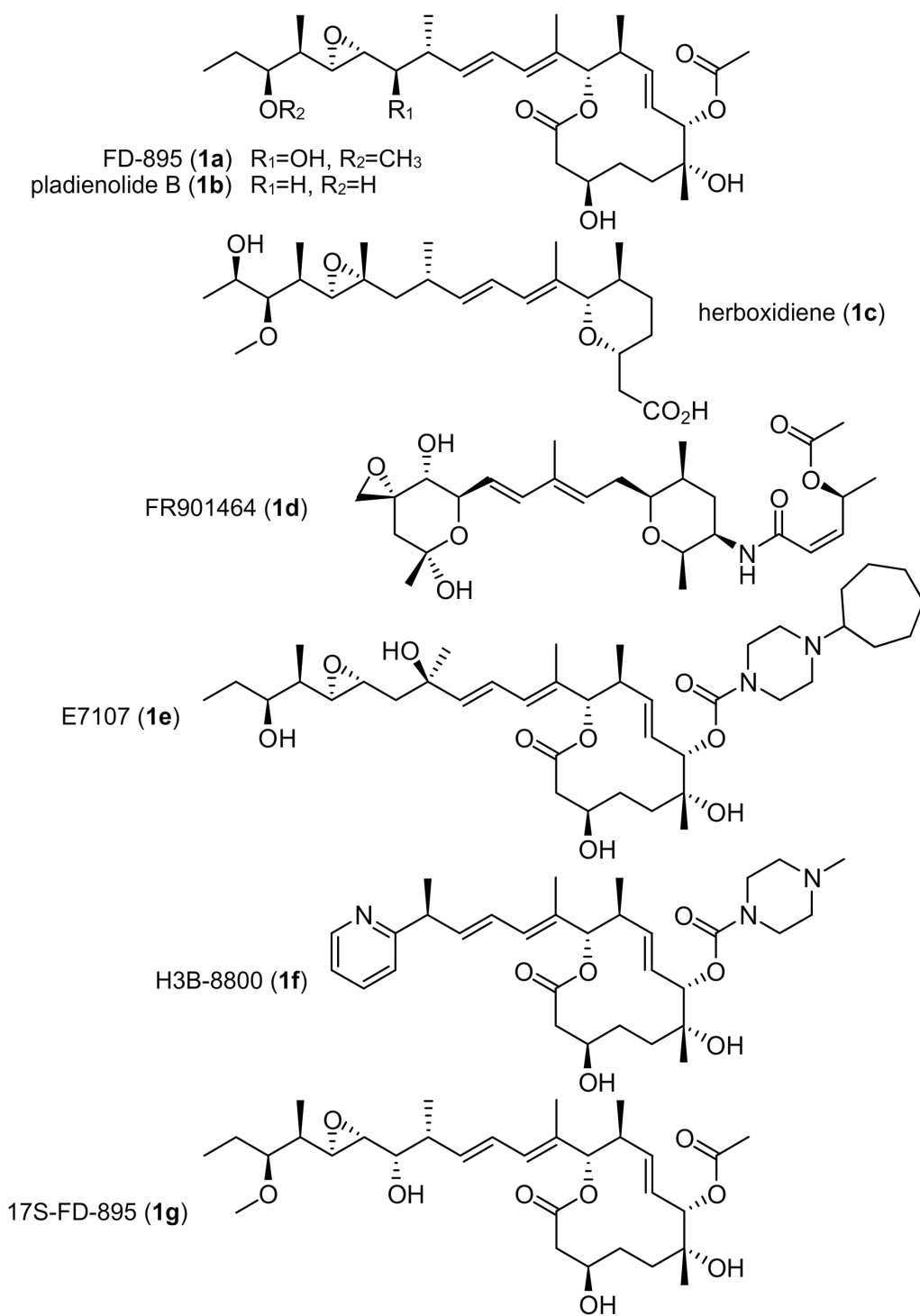
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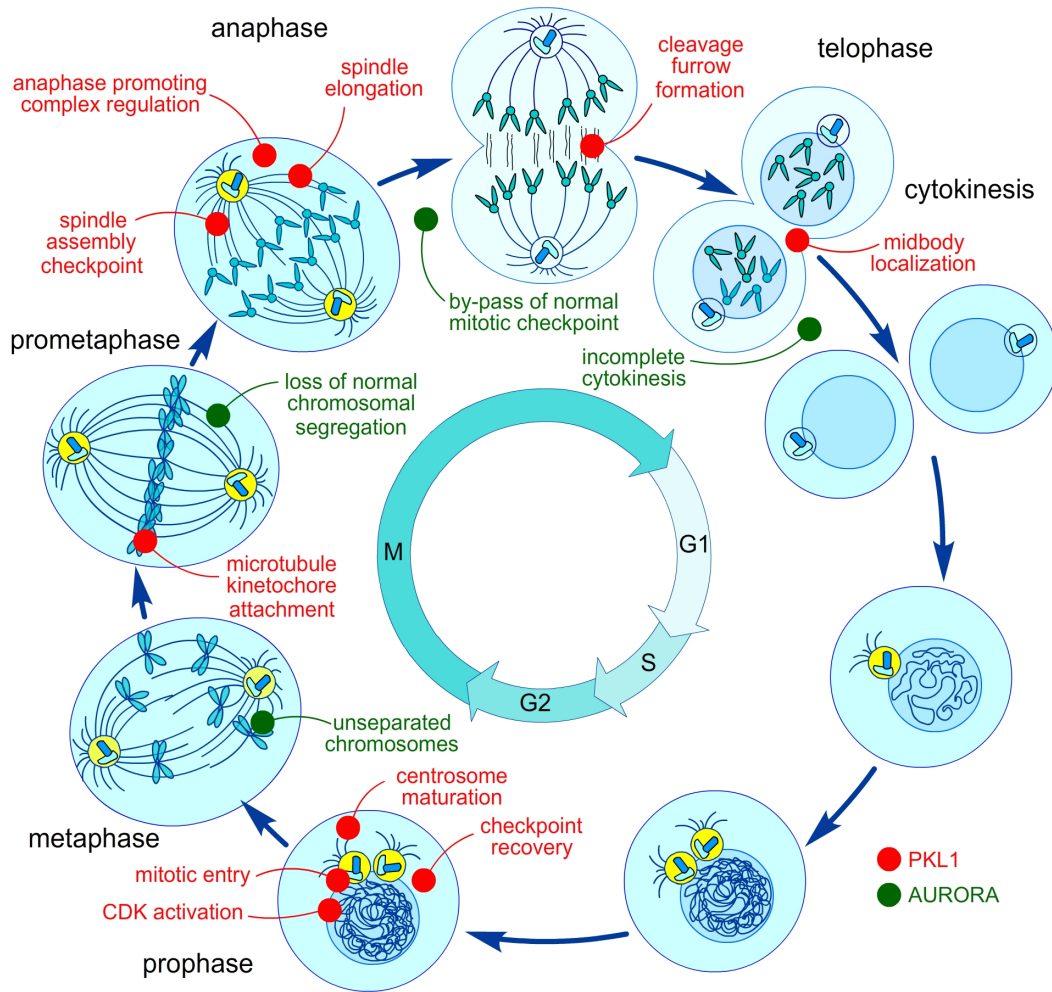
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### Table of Contents

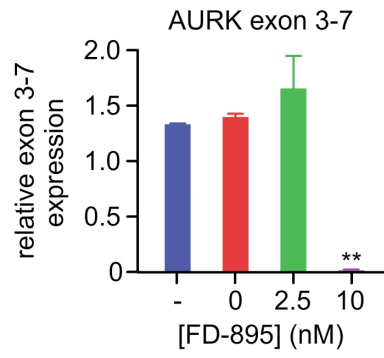
Supplementary Figure S1	S1
Supplementary Figure S2	S2
Supplementary Figure S3	S3
Supplementary Figure S4	S4
Supplementary Table S1	S5
Supplementary Table S2	S6



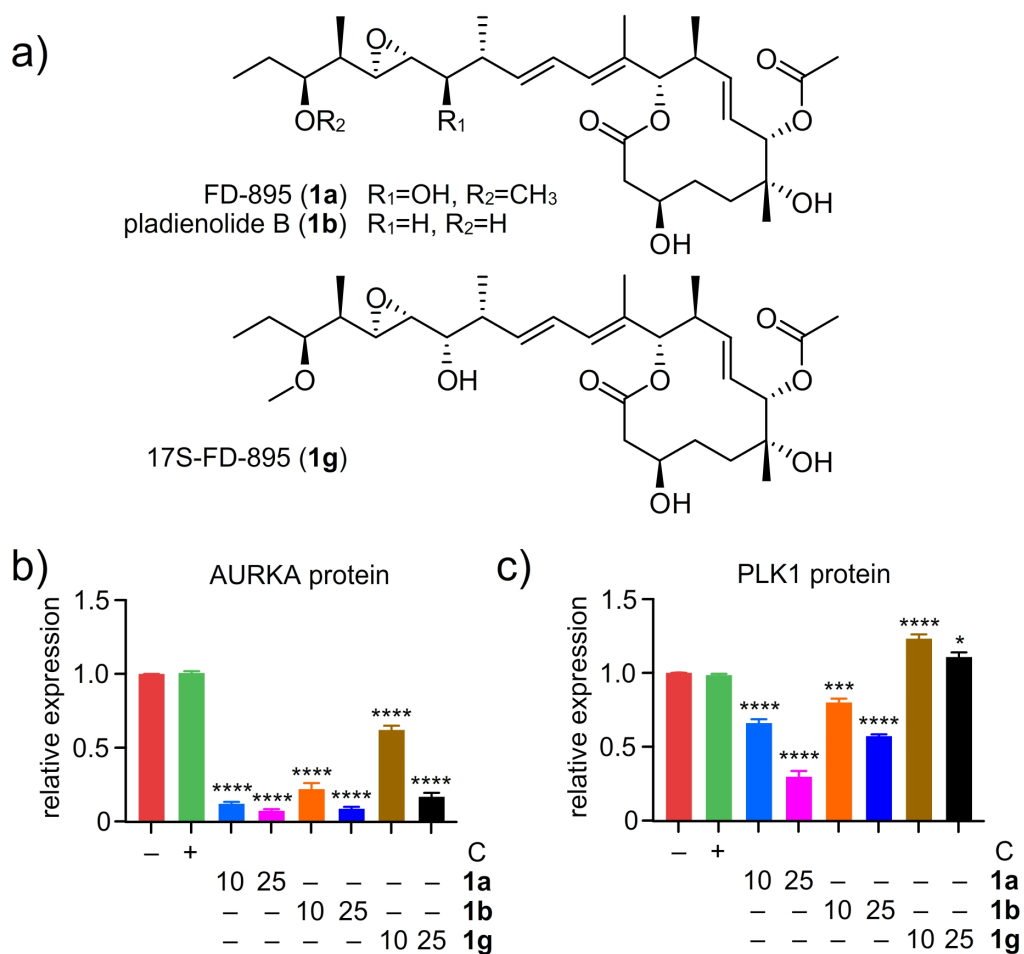
**Supplementary Figure S1.** Expanded set of splicing modulators including natural products FD-895 (**1a**), pladienolide B (**1b**), herboxidiene (**1c**), and FR901464 (**1d**). Structures of clinically-evaluated analogs E7107 (**1e**) and H3B-8800 (**1f**) along with clinical-candidate 17S-FD-895 (**1g**).



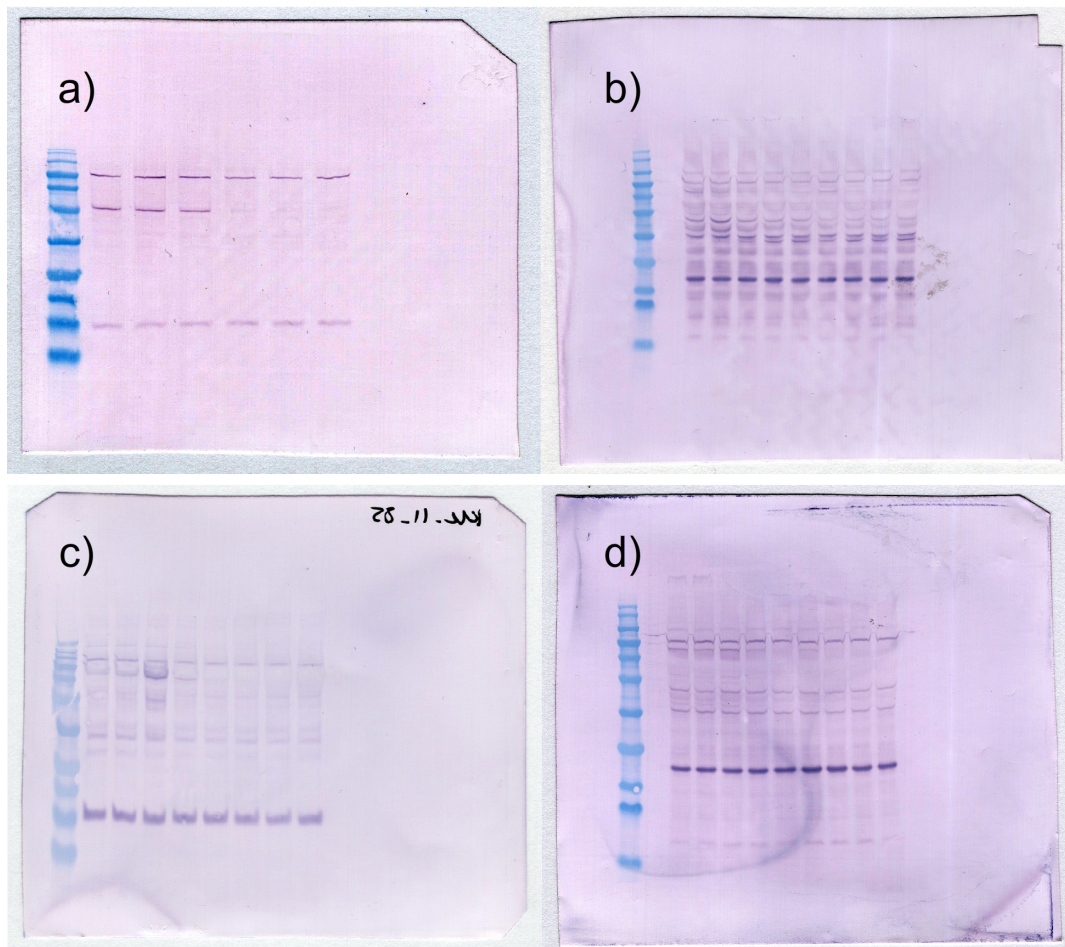
**Supplementary Figure S2.** Roles of polo-like kinase 1 (PLK-1) and aurora kinases (AURK) in the mitotic cycle. Both kinases play an interactive role in regulating each stage of passage through mitosis with effects arising from inhibition or reduced expression of PLK-1 (in red) and aurora kinases (in green).



**Supplementary Figure S3.** AURKA gene regulation by FD-895. Increasing concentrations of FD-895 led to a decrease in the inclusion of exons 3-7 in AURKA RNA.



**Supplementary Figure S4.** Splice modulator selectivity. FD-895 (**1a**) and pladienolide B (**1b**) reduced AURKA and PLK-1 protein expression in a concentration-dependent manner. 17S-FD-895 (**1g**) reduced AURKA expression and modestly increased PLK-1 expression. Experiments were conducted in biological triplicate. Statistics were calculated using a standard one-way ANOVA; p-values were represented so that \* signifies  $p < 0.05$ , \*\* signifies  $p < 0.01$ , \*\*\* signifies  $p < 0.001$ , and \*\*\*\* signifies  $p < 0.0001$ .



**Supplementary Figure S5.** Full scale scans of representative western blots used in this manuscript. **a)** Representative western blot used in Figure 3d. **b)** Representative western blot used in Figure 3e. **c)** Representative western blot used in Figure 4c. **d)** Representative western blot used in Figure 4d.

**Supplementary Table 1.** Sequences of the qPCR primers used in this study.

<b>qPCR primers</b>		
<b>Primer</b>	<b>Location</b>	<b>5' Sequence 3'</b>
<i>GAPDH</i> -FP	Exon 3	TGGTCACCAGGGCTGCTT
<i>GAPDH</i> -RP	Exon 4	AGCTTCCCGTTCTCAGCCTT
<i>AURKA</i> -FP	Intron 2	CCACCTTCGGCATCCTAAT
<i>AURKA</i> -RP	Intron 2	TCCAAGTGGTGCATATTCC
<i>AURKB</i> -FP	Intron 2	GGAGAGCTTAAAATTGCAG
<i>AURKB</i> -RP	Intron 2	TGCAGCTCTTCTGCAGCTC
<i>PLK1</i> -FP		CTCAACACGCCTCATCCTC
<i>PLK1</i> -RP		GTGCTCGCTCATGTAATTG
FP: forward primer; RP reverse primer		

**Supplementary Table 2.** Confidence intervals of GI<sub>50</sub> values for cytotoxicity studies.\*

Compounds	Cell Line	GI <sub>50</sub> value (nM)	Standard error (nM)	Confidence interval
<b>1a</b>	HCT116	0.5-1.7	0.1-0.4	0.4-2.6
<b>1a</b>	Caov3	1.6-2.0	0.46-0.59	0.94-3.46
<b>1a</b>	HeLa	3.7-4.5	0.92-1.26	2.16-8.24
<b>2a</b>	HCT116	430.0	440.7	3453.0 - 5231.0
<b>2a + 1 nM 1a</b>	HCT116	517.0	281.0	136.9 - 1886.0
<b>2a + 5 nM 1a</b>	HCT116	0.05	0.05	0.01 - 0.18
<b>2a</b>	Caov3	5500	621	4303 - 6938
<b>2a + 1 nM 1a</b>	Caov3	2800	450	1906 - 3979
<b>2a + 5 nM 1a</b>	Caov3	0.08	0.06	0.01 - 0.22
<b>2a</b>	HeLa	10100	3371	5527 - 20150
<b>2a + 1 nM 1a</b>	HeLa	4000	1084	2368 - 6667
<b>2a + 5 nM 1a</b>	HeLa	0.20	0.10	0.01 - 0.42
<b>2b</b>	HCT116	2100	186	1737 - 2492
<b>2b + 1 nM 1a</b>	HCT116	250.0	35.7	180.3 - 351.6
<b>2b + 5 nM 1a</b>	HCT116	0.05	0.04	0.01 - 0.13
<b>2b</b>	Caov3	6000	402	5233 - 6942
<b>2b + 1 nM 1a</b>	Caov3	900	230	468 - 1765
<b>2b + 5 nM 1a</b>	Caov3	0.07	0.06	0.01 - 0.19
<b>2b</b>	HeLa	19500	4112	13180 - 31166
<b>2b + 1 nM 1a</b>	HeLa	16300	4178	10281 - 28836
<b>2b + 5 nM 1a</b>	HeLa	0.30	0.16	0.01 - 0.74
<b>3a</b>	HCT116	160.0	52.9	67.0 - 444.6
<b>3a + 1 nM 1a</b>	HCT116	0.90	0.33	0.33 - 2.28
<b>3a + 5 nM 1a</b>	HCT116	0.004	0.002	0.001 - 0.008
<b>3a</b>	Caov3	25.9	9.6	6.2 - 79.8
<b>3a + 1 nM 1a</b>	Caov3	12.8	5.4	1.7 - 48.8
<b>3a + 5 nM 1a</b>	Caov3	0.20	0.05	0.07 - 0.30
<b>3a</b>	HeLa	13200	2662	8927 - 20456
<b>3a + 1 nM 1a</b>	HeLa	10700	2369	7036 - 17217

\* Data from these studies was obtained from and reported in Figure 2



**Supplementary Table 3.** Combination Index values for cytotoxicity studies.\*

Figure	[1a] (nM)	[2a] (nM)	[2b] (nM)	[2b] (nM)	CI value
2a	1.0	1.0			2.35
	1.0	10.0			12.4
	1.0	50.0			28.4
	1.0	100.0			24.5
	1.0	500.0			12.2
	1.0	1,000.0			4.34
	1.0	5,000.0			0.190
	1.0	10,000.0			0.166
2a	5.0	1.0			0.0605
	5.0	10.0			0.0741
	5.0	50.0			0.0525
	5.0	100.0			0.0403
	5.0	500.0			0.0530
	5.0	1,000.0			0.0681
	5.0	5,000.0			0.627
	5.0	10,000.0			0.833
2b	1.0	1.0			12,700,000
	1.0	10.0			76,600,000
	1.0	50.0			404,000
	1.0	100.0			1,600
	1.0	500.0			1,790,000
	1.0	1,000.0			3,300
	1.0	5,000.0			10.7
	1.0	10,000.0			0.0962
2b	5.0	1.0			0.000218
	5.0	10.0			0.000329
	5.0	50.0			0.000348
	5.0	100.0			0.000372
	5.0	500.0			0.000567
	5.0	1,000.0			0.000810
	5.0	5,000.0			0.00275
	5.0	10,000.0			0.00519
2c	1.0	1.0			498,000,000
	1.0	10.0			30,400
	1.0	50.0			1,790,000
	1.0	100.0			1,790,000
	1.0	500.0			1,790,000
	1.0	1,000.0			76,600,000
	1.0	5,000.0			78.6
	1.0	10,000.0			0.0000407
2c	5.0	1.0			0.00811
	5.0	10.0			0.00642
	5.0	50.0			0.00642
	5.0	100.0			0.0156

	5.0	500.0			0.0157
	5.0	1,000.0			0.00234
	5.0	5,000.0			0.0000237
	5.0	10,000.0			0.0000108
2d	1.0		1.0		4,580
	1.0		10.0		6.75
	1.0		50.0		2.56
	1.0		100.0		2.30
	1.0		500.0		0.519
	1.0		1,000.0		0.268
	1.0		5,000.0		0.260
	1.0		10,000.0		0.0409
2d	5.0		1.0		0.0761
	5.0		10.0		0.0823
	5.0		50.0		0.0686
	5.0		100.0		0.0787
	5.0		500.0		0.0769
	5.0		1,000.0		0.0890
	5.0		5,000.0		0.181
	5.0		10,000.0		0.450
2e	1.0		1.0		587,000
	1.0		10.0		426,000
	1.0		50.0		154,000
	1.0		100.0		54,800
	1.0		500.0		2,090
	1.0		1,000.0		223
	1.0		5,000.0		8.74
	1.0		10,000.0		4.04
2e	5.0		1.0		0.00242
	5.0		10.0		0.01263
	5.0		50.0		0.00593
	5.0		100.0		0.00882
	5.0		500.0		0.0251
	5.0		1,000.0		0.0187
	5.0		5,000.0		0.0123
	5.0		10,000.0		0.0120
2f	1.0		1.0		307,000,000,000.
	1.0		10.0		307,000,000,000.
	1.0		50.0		307,000,000,000.
	1.0		100.0		307,000,000,000.
	1.0		500.0		1,108,000,000.
	1.0		1,000.0		307,000,000,000.
	1.0		5,000.0		1,108,000,000.
	1.0		10,000.0		8.53
2f	5.0		1.0		0.0591
	5.0		10.0		0.0344
	5.0		50.0		0.437

	5.0		100.0		0.135
	5.0		500.0		0.0156
	5.0		1,000.0		0.0156
	5.0		5,000.0		0.000324
	5.0		10,000.0		0.0000536
2g	1.0			1.0	4.03
	1.0			10.0	1.57
	1.0			50.0	1.49
	1.0			100.0	1.66
	1.0			500.0	0.807
	1.0			1,000.0	0.501
	1.0			5,000.0	0.00378
	1.0			10,000.0	0.000414
2g	5.0			1.0	0.0254
	5.0			10.0	0.00704
	5.0			50.0	0.00415
	5.0			100.0	0.00712
	5.0			500.0	0.0185
	5.0			1,000.0	0.00472
	5.0			5,000.0	0.0170
	5.0			10,000.0	0.0000336
2h	1.0			1.0	35,700,000
	1.0			10.0	240,000,000
	1.0			50.0	922,000
	1.0			100.0	4,470,000
	1.0			500.0	2,610
	1.0			1,000.0	4,330
	1.0			5,000.0	10.4
	1.0			10,000.0	0.0938
2h	5.0			1.0	0.000188
	5.0			10.0	0.000412
	5.0			50.0	0.000309
	5.0			100.0	0.000295
	5.0			500.0	0.000447
	5.0			1,000.0	0.000879
	5.0			5,000.0	0.00385
	5.0			10,000.0	0.00455
2i	1.0			1.0	307,000,000,000
	1.0			10.0	6,140,000
	1.0			50.0	76,600,000
	1.0			100.0	76,600,000
	1.0			500.0	1,790,000
	1.0			1,000.0	307,000,000,000
	1.0			5,000.0	144,000
	1.0			10,000.0	1.59
2i	5.0			1.0	0.0156
	5.0			10.0	0.0234

	5.0			50.0	0.135
	5.0			100.0	0.0496
	5.0			500.0	0.00811
	5.0			1,000.0	0.00811
	5.0			5,000.0	0.000142
	5.0			10,000.0	0.0000167

\*Data from these studies was obtained from and reported in Figure 2