

## SUPPORTING INFORMATION

### Correlating basal gene expression across chemical sensitivity data to screen for novel synergistic interactors of HDAC inhibitors in pancreatic carcinoma

Nemanja Djokovic <sup>1, \*</sup>, Ana Djuric <sup>2</sup>, Dusan Ruzic <sup>1</sup>, Tatjana Srdic-Rajic <sup>2</sup> and Katarina Nikolic <sup>1,\*</sup>

<sup>1</sup> Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11221 Belgrade, Serbia

<sup>2</sup> Department of Experimental Oncology, Institute for Oncology and Radiology of Serbia, Pasterova 14, 11000 Belgrade, Serbia

\* Correspondence: nemanja.djokovic@pharmacy.bg.ac.rs (N.D.); katarina.nikolic@pharmacy.bg.ac.rs (K.N.)

**Supplementary Note S1.** Detailed results of experimental evaluation of synergisms with concentration annotations.

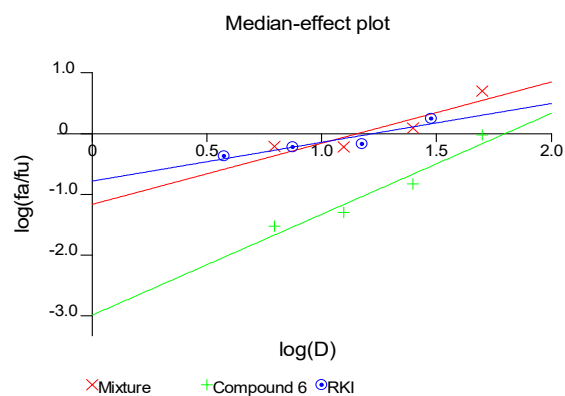
**Legend:**

Symbol	Description
+++++	Very strong synergism
+++	Synergism
++	Moderate synergism
+	Slight synergism
±	Nearly additive
–	Slight antagonism
--	Moderate antagonism
---	Antagonism
----	Strong antagonism
-----	Very strong antagonism

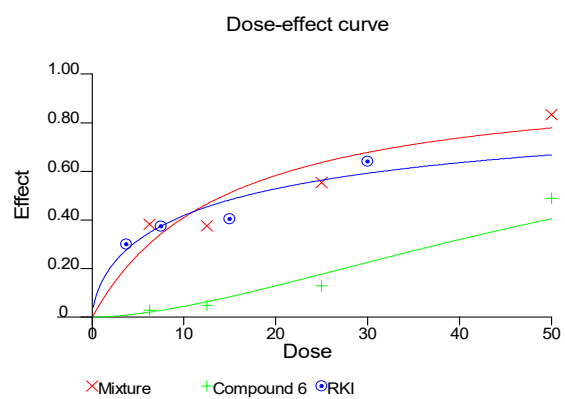
**MIA PaCa-2 cell line**

Drug	Drug concentration			
	I	II	III	IV
HDACi ( <b>6b</b> )	48.60±7.45	13.58±2.33	4.80±1.01	2.91±0.87
ROCKi (RKI-1447)	64.18±8.12	40.49±5.55	37.49±3.02	30.84±7.33
<b>6b</b> + RKI-1447	83.28±10.34	55.48±7.51	37.69±5.38	38.31±4.56
Combination Index (CI)	0.445	0.981	1.253	0.605
Interaction <b>6b</b> + RKI-1447	+++	+	--	++

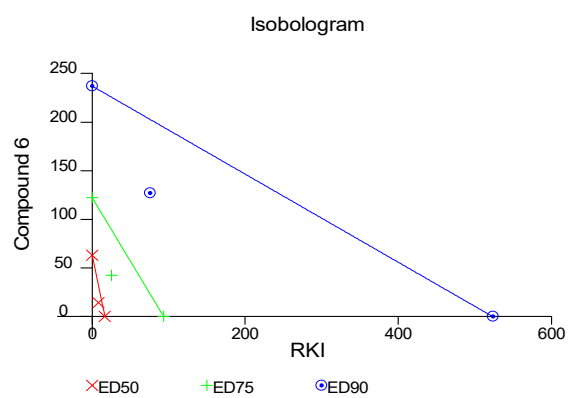
Compound 6 (μM): I=50; II=25; III=12.5; IV=6.25  
 RKI (μM): I=30; II=15; III=7.5; IV=3.75



**Figure S1.** The median-effect plot for tested synergism between 6b and RKI-1447 on MIA PaCa-2 cell line. In this plot,  $f_a$  is the fraction of cells affected, while the  $f_u$  is the fraction of cells unaffected, D is the dose of a drug.



**Figure S2.** Dose-effect curve for tested synergism between 6b and RKI-1447 on MIA PaCa-2 cell line.

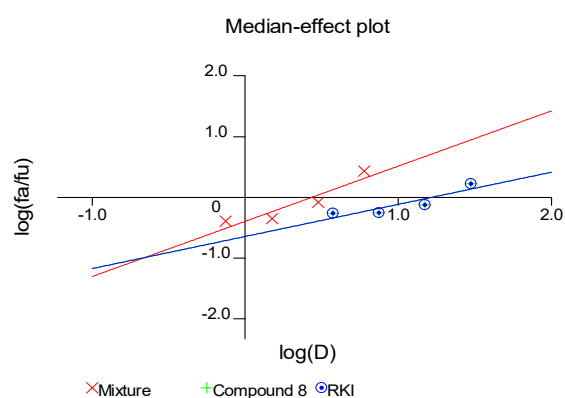


**Figure S3.** Isobologram for synergism between 6b and RKI-1447 on MIA PaCa-2 cell line.

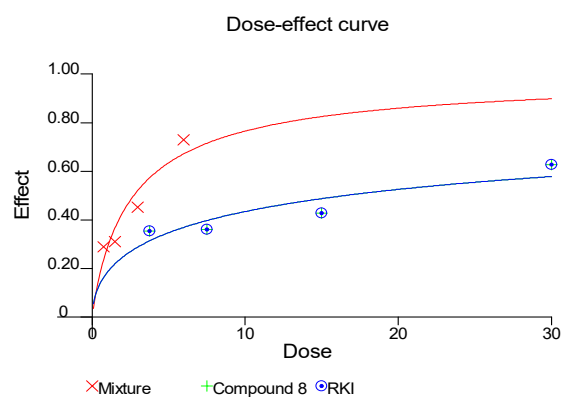
# MIA PaCa-2 cell line

Drug	Drug concentration			
	I	II	III	IV
HDACi ( <b>8b</b> )	27.30±4.44	1.83±0.70	0.94±0.45	0.57±0.19
ROCKi (RKI-1447)	62.94±10.11	42.93±6.99	36.07±7.51	35.46±4.09
<b>8b</b> + RKI-1447	73.02±7.44	45.32±5.14	31.14±5.21	28.91±2.34
Combination Index (CI)	0.333	1.564	2.469	1.505
Interaction <b>8b</b> + RKI-1447	+++	---	---	---

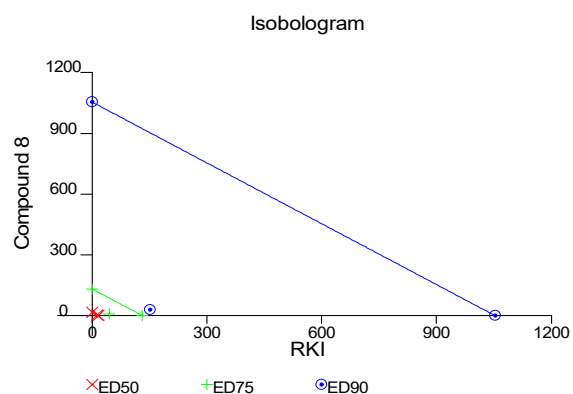
Compound 8 (μM): I=6; II=3; III=1.5; IV=0.75  
 RKI (μM): I=30; II=15; III=7.5; IV=3.75



**Figure S4.** The median-effect plot for tested synergism between 8b and RKI-1447 on MIA PaCa-2 cell line. In this plot, *fa* is the fraction of cells affected, while the *fu* is the fraction of cells unaffected, D is the dose of a drug.



**Figure S5.** Dose-effect curve for tested synergism between 8b and RKI-1447 on MIA PaCa-2 cell line.



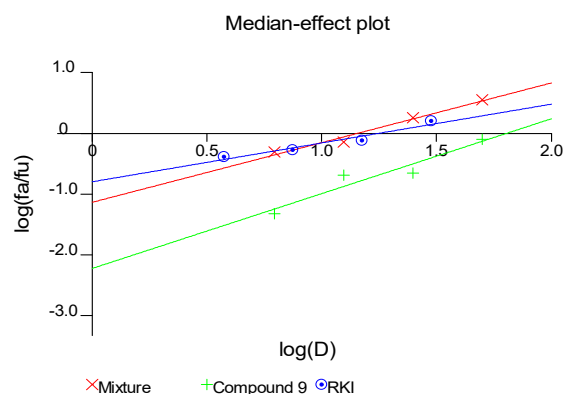
**Figure S6.** Isobologram for synergism between 8b and RKI-1447 on MIA PaCa-2 cell line.

#### MIA PaCa-2 cell line

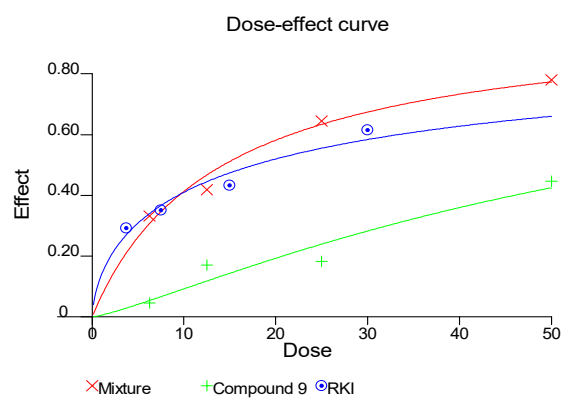
Drug	Drug concentration			
	I	II	III	IV
HDACi ( <b>9b</b> )	44.65±3.39	18.25±2.22	1.71±0.67	0.46±0.20
ROCKi (RKI-1447)	61.64±7.78	43.28±5.38	35.02±3.25	29.16±2.16
<b>9b</b> + RKI-1447	78.03±7.18	64.37±6.78	41.91±5.98	33.32±4.21
Combination Index (CI)	0.513	0.577	0.964	0.803
Interaction <b>9b</b> + RKI-1447	+++	+++	±	++

Compound 9 (μM): I=50; II=25; III=12.5; IV=6.25

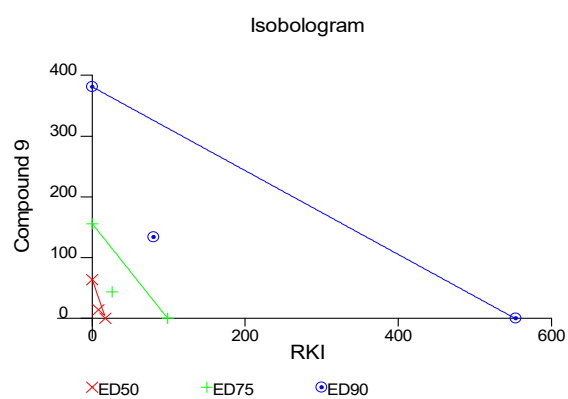
RKI (μM): I=30; II=15; III=7.5; IV=3.75



**Figure S7.** The median-effect plot for tested synergism between 9b and RKI-1447 on MIA PaCa-2 cell line. In this plot,  $fa$  is the fraction of cells affected, while the  $fu$  is the fraction of cells unaffected,  $D$  is the dose of a drug.



**Figure S8.** Dose-effect curve for tested synergism between 9b and RKI-1447 on MIA PaCa-2 cell line.

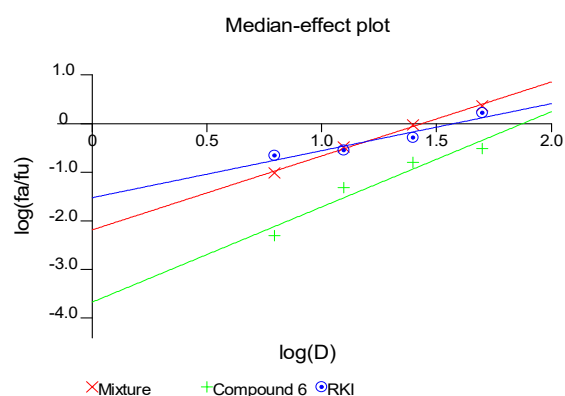


**Figure S9.** Isobologram for synergism between 9b and RKI-1447 on MIA PaCa-2 cell line.

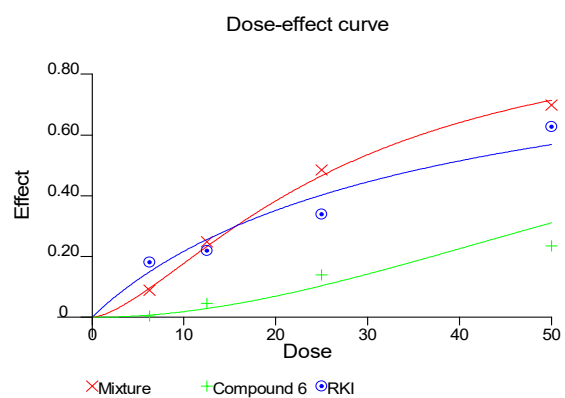
# Panc-1 cell line

Drug	Drug concentration			
	I	II	III	IV
HDACi ( <b>6b</b> )	23.61±3.26	1.39±0.55	0.47±0.21	0.5±0.2
ROCKi (RKI-1447)	62.76±11.23	33.89±8.92	22.02±7.22	18.11±5.90
<b>6b</b> + RKI-1447	69.79±10.45	48.51±6.34	24.98±3.13	8.92±4.21
Combination Index (CI)	0.992	1.050	1.334	2.117
Interaction <b>6b</b> + RKI-1447	±	±	--	---

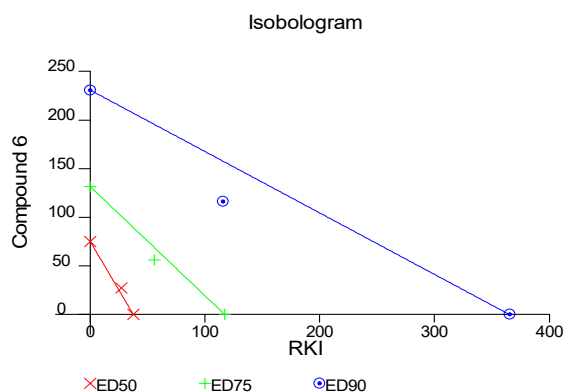
Compound 6 (μM): I=50; II=25; III=12.5; IV=6.25  
RKI (μM): I=50; II=25; III=12.5; IV=6.25



**Figure S10.** The median-effect plot for tested synergism between 6b and RKI-1447 on Panc-1 cell line. In this plot, *fa* is the fraction of cells affected, while the *fu* is the fraction of cells unaffected, *D* is the dose of a drug.



**Figure S11.** Dose-effect curve for tested synergism between 6b and RKI-1447 on Panc-1 cell line.



**Figure S12.** Isobologram for synergism between 6b and RKI-1447 on Panc-1 cell line.

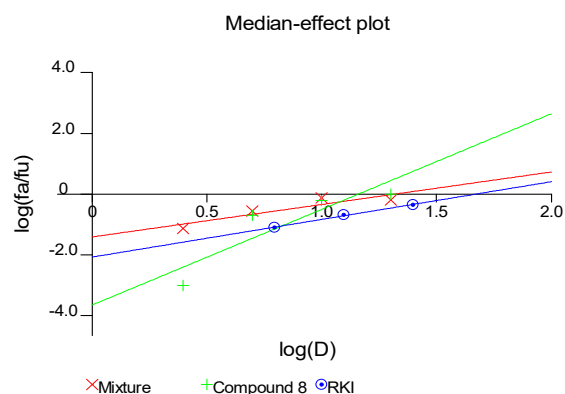
#### Panc-1 cell line

Drug	Drug concentration			
	I	II	III	IV
HDACi ( <b>8b</b> )	49.78±6.77	38.21±5.89	17.13±2.11	0.1±0.05
ROCKi (RKI-1447)	58.78±5.55	30.77±3.45	17.27±2.28	7.41±2.56
<b>8b</b> + RKI-1447	63.94±6.33	42.96±5.79	21.99±3.45	6.79±3.06
Combination Index (CI)	3.133	1.433	1.266	1.474
Interaction <b>8b</b> + RKI-1447	— — —	— —	— —	— — —

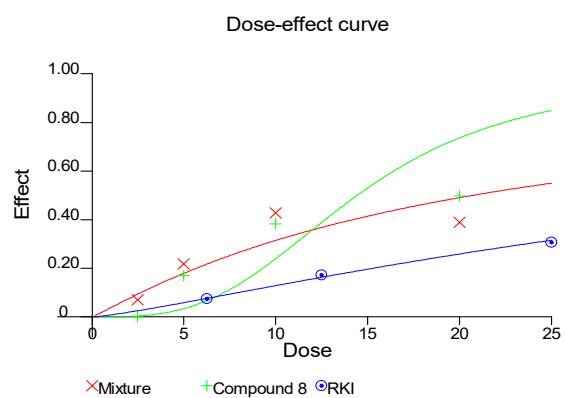
Compound 8 (μM): I=20; II=10; III=5.0; IV=2.5

RKI (μM): I=50; II=25; III=12.5; IV=6.25

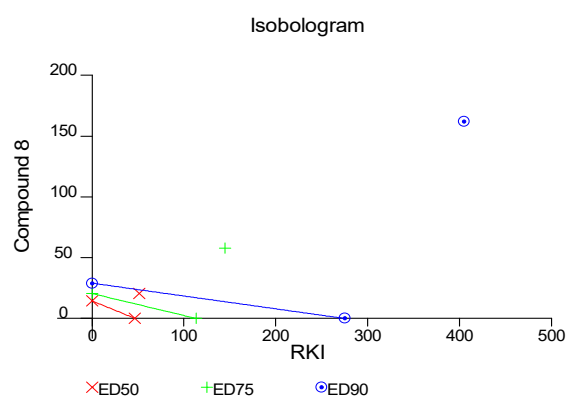




**Figure S13.** The median-effect plot for tested synergism between 8b and RKI-1447 on Panc-1 cell line. In this plot,  $f_a$  is the fraction of cells affected, while the  $f_u$  is the fraction of cells unaffected,  $D$  is the dose of a drug.



**Figure S14.** Dose-effect curve for tested synergism between 8b and RKI-1447 on Panc-1 cell line.

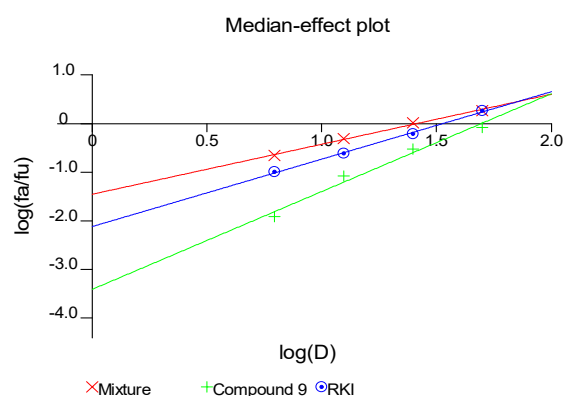


**Figure S15.** Isobologram for synergism between 8b and RKI-1447 on Panc-1 cell line.

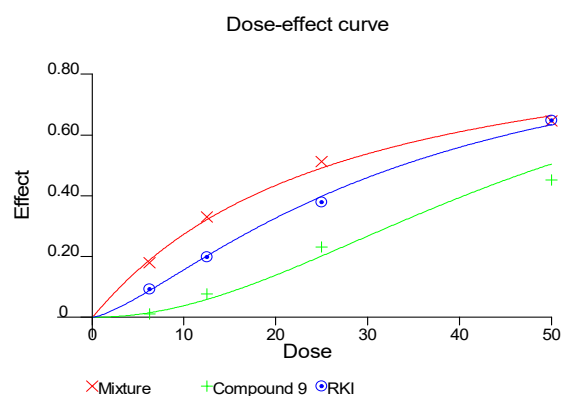
# Panc-1 cell line

Drug	Drug concentration			
	I	II	III	IV
HDACi ( <b>9b</b> )	45.18±4.55	23.14±7.42	7.56±3.71	1.12±0.89
ROCKi (RKI-1447)	64.82±6.41	37.92±5.34	19.76±2.30	9.41±1.06
<b>9b</b> + RKI-1447	64.68±5.01	51.26±7.20	33.14±6.10	18.01±4.02
Combination Index (CI)	1.705	1.207	0.975	0.823
Interaction <b>9b</b> + RKI-1447	---	--	±	+

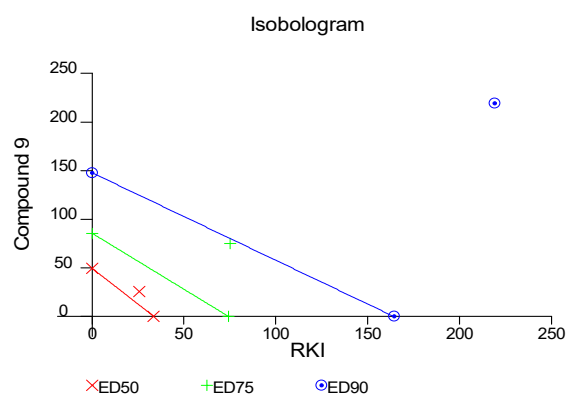
Compound 9 (μM): I=50; II=25; III=12.5; IV=6.25  
RKI (μM): I=50; II=25; III=12.5; IV=6.25



**Figure S16.** The median-effect plot for tested synergism between **9b** and RKI-1447 on Panc-1 cell line. In this plot, *fa* is the fraction of cells affected, while the *fu* is the fraction of cells unaffected, D is the dose of a drug.



**Figure S17.** Dose-effect curve for tested synergism between 9b and RKI-1447 on Panc-1 cell line.

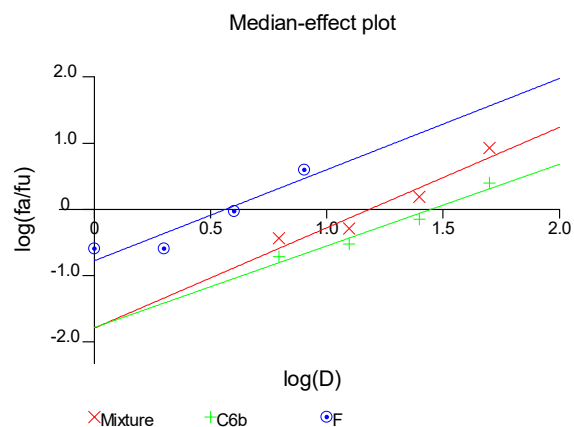


**Figure S18.** Isobologram for synergism between 9b and RKI-1447 on Panc-1 cell line.

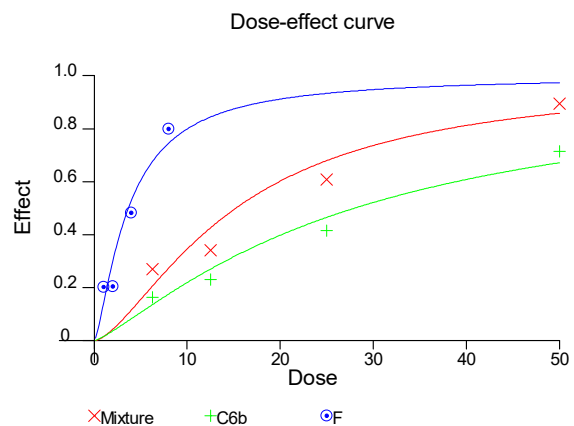
### MIA PaCa-2 cell line

Drug	Drug concentration			
	I	II	III	IV
HDACi ( <b>6b</b> )	71.49±11.91	41.62±11.91	23.06±9.75	16.27±5.34
Fingolimod	79.98±10.36	48.31±12.04	20.57±4.85	20.33±5.37
<b>6b</b> + Fingolimod	89.28±4.05	60.81±20.19	34.11±14.58	26.96±7.59
Combination Index (CI)	0.780	1.422	1.648	1.070
Interaction <b>6b</b> + Fingolimod	++	--	---	±

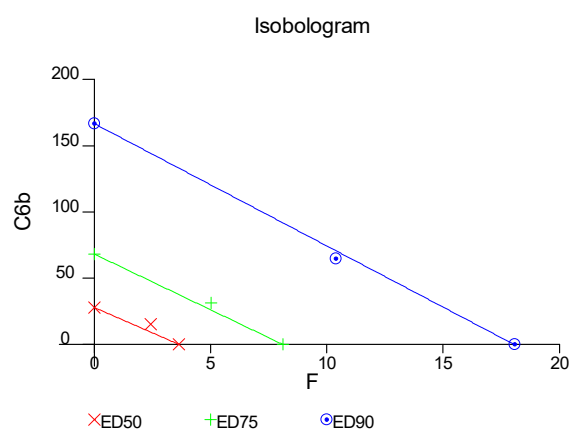
Compound 6 (μM): I=50; II=25; III=12.5; IV=6.25  
Fingolimod (μM): I=8; II=4; III=2; IV=1



**Figure S19.** The median-effect plot for tested synergism between 6b and fingolimod on MIA PaCa-2 cell line. In this plot,  $fa$  is the fraction of cells affected, while the  $fu$  is the fraction of cells unaffected,  $D$  is the dose of a drug.



**Figure S20.** Dose-effect curve for tested synergism between 6b and fingolimod on MIA PaCa-2 cell line.



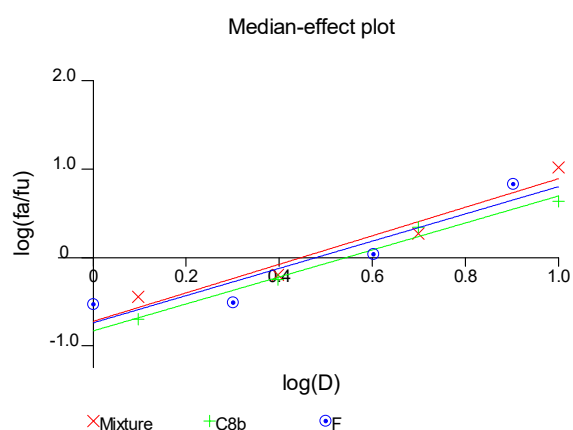
**Figure S21.** Isobologram for synergism between 6b and fingolimod on MIA PaCa-2 cell line.

# MIA PaCa-2 cell line

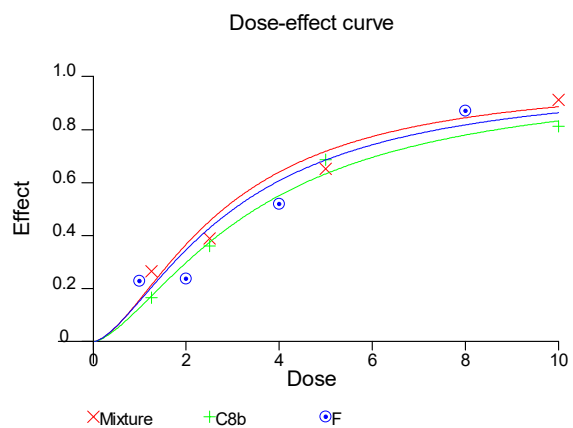
Drug	Drug concentration			
	I	II	III	IV
HDACi ( <b>8b</b> )	81.22±0.96	68.72±2.69	36.08±3.29	16.72±4.28
Fingolimod	87.22±2.01	52.02±2.47	23.73±3.92	22.83±1.89
<b>8b</b> + Fingolimod	91.31±10.08	65.06±1.92	38.98±5.26	26.55±2.94
Combination Index (CI)	1.188	1.836	1.852	1.338
Interaction <b>8b</b> + Fingolimod	— — —	— — —	— — —	— —

Compound 8 ( $\mu\text{M}$ ): I=10; II=5; III=2.5; IV=1.25

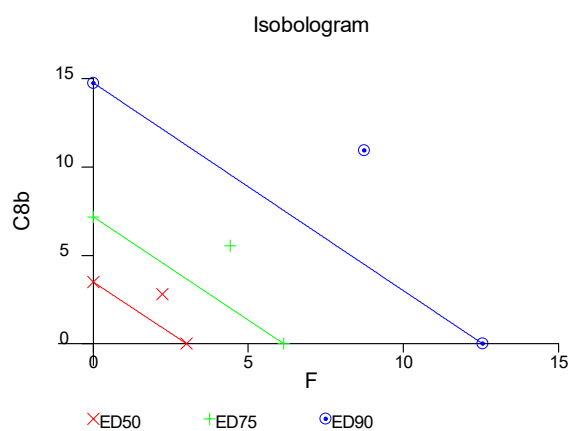
Fingolimod ( $\mu\text{M}$ ): I=8; II=4; III=2; IV=1



**Figure S22.** The median-effect plot for tested synergism between 8b and fingolimod on MIA PaCa-2 cell line. In this plot,  $f_a$  is the fraction of cells affected, while the  $f_u$  is the fraction of cells unaffected, D is the dose of a drug.



**Figure S23.** Dose-effect curve for tested synergism between 8b and fingolimod on MIA PaCa-2 cell line.

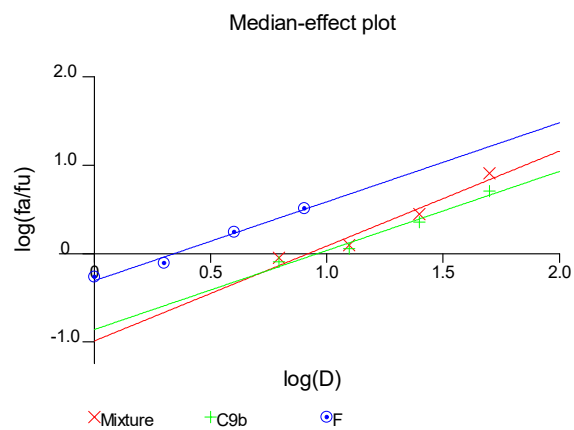


**Figure S24.** Isobologram for synergism between 8b and fingolimod on MIA PaCa-2 cell line.

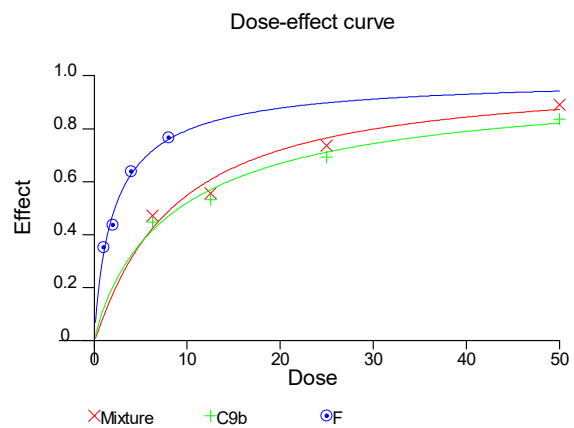
**MIA PaCa-2 cell line**

Drug	Drug concentration			
	I	II	III	IV
HDACi ( <b>9b</b> )	83.61±0.27	69.42±11.34	53.31±17.80	44.84±11.21
Fingolimod	76.58±4.48	63.82±6.81	43.78±12.65	35.34±6.99
<b>9b</b> + Fingolimod	89.11±1.57	73.59±12.40	55.57±18.16	47.22±9.51
Combination Index (CI)	0.870	1.449	1.773	1.293
Interaction <b>9b</b> + Fingolimod	+	— —	— — —	— —

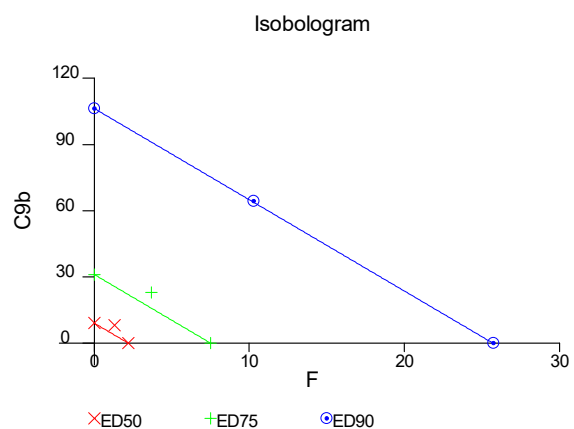
Compound 9 (μM): I=50; II=25; III=12.5; IV=6.25  
Fingolimod (μM): I=8; II=4; III=2; IV=1



**Figure S25.** The median-effect plot for tested synergism between 9b and fingolimod on MIA PaCa-2 cell line. In this plot,  $fa$  is the fraction of cells affected, while the  $fu$  is the fraction of cells unaffected,  $D$  is the dose of a drug.



**Figure S26.** Dose-effect curve for tested synergism between 9b and fingolimod on MIA PaCa-2 cell line.

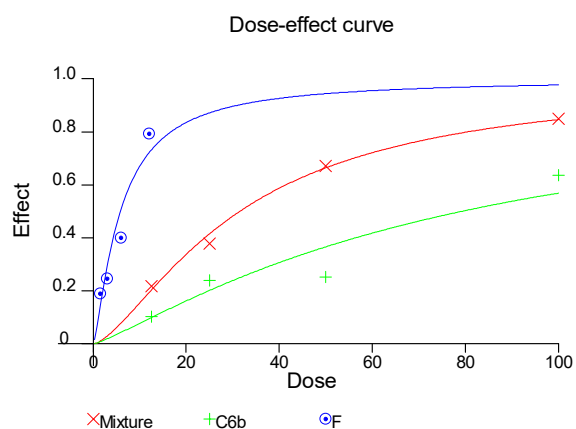


**Figure S27.** Isobologram for synergism between 9b and fingolimod on MIA PaCa-2 cell line.

# Panc-1 cell line

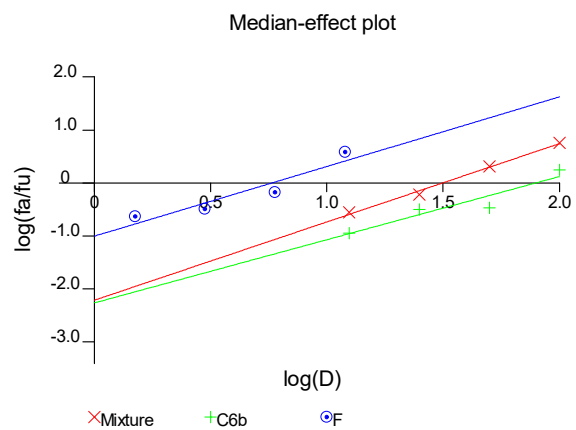
Drug	Drug concentration			
	I	II	III	IV
HDACi ( <b>6b</b> )	63.68±4.70	25.29±6.71	23.93±6.23	10.30±1.96
Fingolimod	79.18±1.45	40.09±4.97	24.56±1.24	18.99±0.95
<b>6b</b> + Fingolimod	84.96±2.05	67.22±5.98	37.87±5.71	21.98±0.78
Combination Index (CI)	0.847	0.945	1.232	1.141
Interaction <b>6b</b> + Fingolimod	++	±	--	-

Compound 6 (μM): I=100; II=50; III=25; IV=12.5  
Fingolimod (μM): I=12; II=6; III=3; IV=1.5

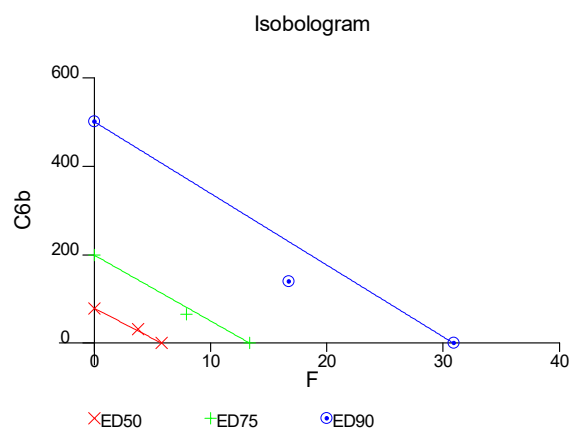


**Figure S28.** The median-effect plot for tested synergism between 6b and fingolimod on Panc-1 cell line. In this plot,  $fa$  is the fraction of cells affected, while the  $fu$  is the fraction of cells unaffected,  $D$  is the dose of a drug.





**Figure S29.** Dose-effect curve for tested synergism between 6b and fingolimod on Panc-1 cell line.

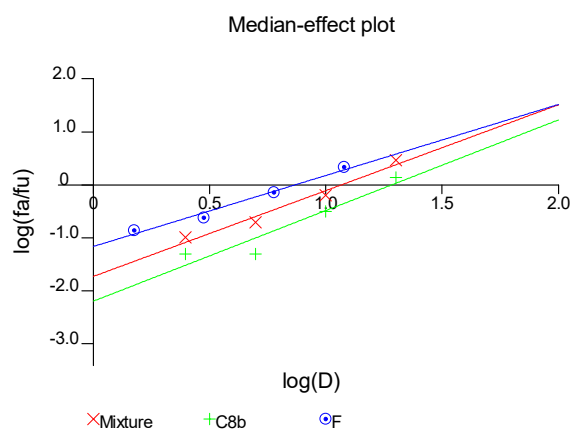


**Figure S30.** Isobologram for synergism between 6b and fingolimod on Panc-1 cell line.

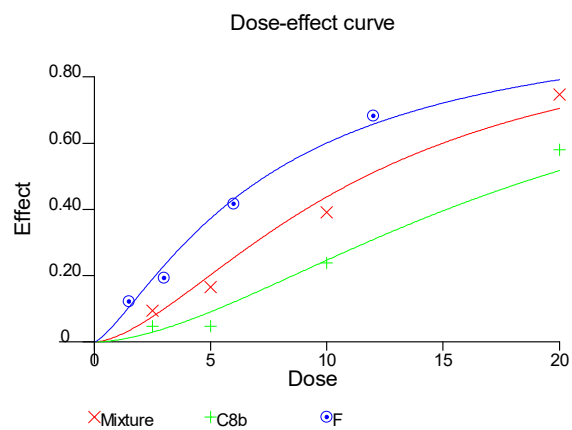
# Panc-1 cell line

Drug	Drug concentration			
	I	II	III	IV
HDACi ( <b>8b</b> )	58.10±0.24	23.86±9.24	4.77±2.69	4.68±2.33
Fingolimod	68.24±4.62	41.81±5.37	19.44±10.97	12.27±8.75
<b>8b</b> + Fingolimod	74.74±5.61	30.91±6.84	16.60±7.22	9.42±5.57
Combination Index (CI)	1.267	1.806	2.027	1.592
Interaction <b>8b</b> + Fingolimod	— —	— — —	— — — —	— — —

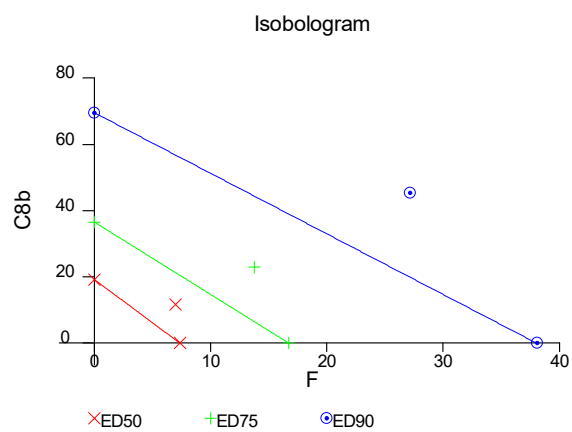
Compound 8 (μM): I=20; II=10; III=5.0; IV=2.5  
Fingolimod (μM): I=12; II=6; III=3; IV=1.5



**Figure S31.** The median-effect plot for tested synergism between 8b and fingolimod on Panc-1 cell line. In this plot, *fa* is the fraction of cells affected, while the *fu* is the fraction of cells unaffected, *D* is the dose of a drug.



**Figure S32.** Dose-effect curve for tested synergism between 8b and fingolimod on Panc-1 cell line.

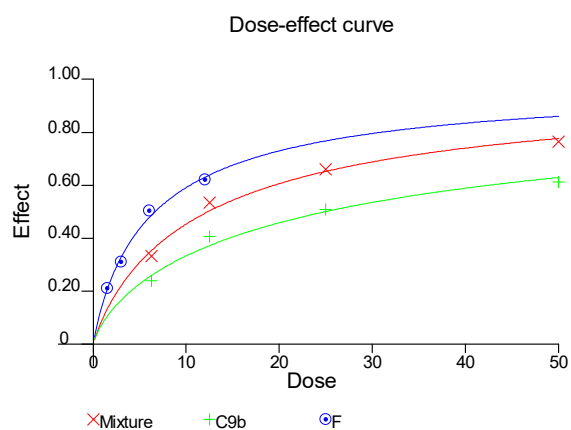


**Figure S33.** Isobologram for synergism between 8b and fingolimod on Panc-1 cell line.

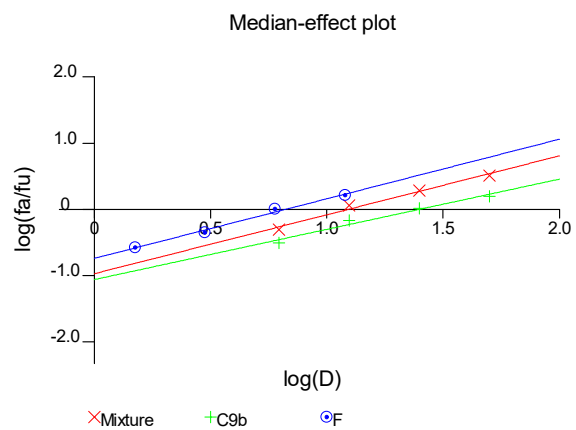
# Panc-1 cell line

Drug	Drug concentration			
	I	II	III	IV
HDACi ( <b>9b</b> )	61.28±6.91	50.93±3.83	40.71±3.74	24.03±5.73
Fingolimod	62.10±4.94	50.04±3.20	31.21±11.85	21.17±3.33
<b>9b</b> + Fingolimod	76.50±5.14	66.12±10.76	53.41±18.12	33.34±10.07
Combination Index (CI)	0.911	0.848	0.810	1.120
Interaction <b>9b</b> + Fingolimod <sup>b</sup>	±	++	++	—

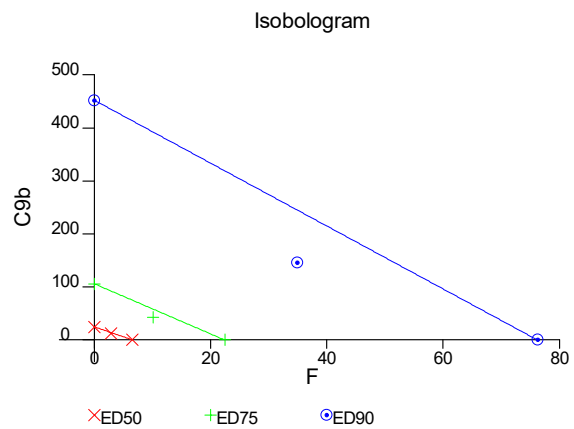
Compound 9 (μM): I=50; II=25; III=12.5; IV=6.25  
Fingolimod (μM): I=12; II=6; III=3; IV=1.5



**Figure S34.** The median-effect plot for tested synergism between 9b and fingolimod on Panc-1 cell line. In this plot, *fa* is the fraction of cells affected, while the *fu* is the fraction of cells unaffected, D is the dose of a drug.



**Figure S35.** Dose-effect curve for tested synergism between 9b and fingolimod on Panc-1 cell line.



**Figure S36.** Isobologram for synergism between 9b and fingolimod on Panc-1 cell line.