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Supporting Information

Reply to Correspondence on "Synergy and Antagonism between Allosteric and Active-Site Inhibitors of Abl Tyrosine Kinase"

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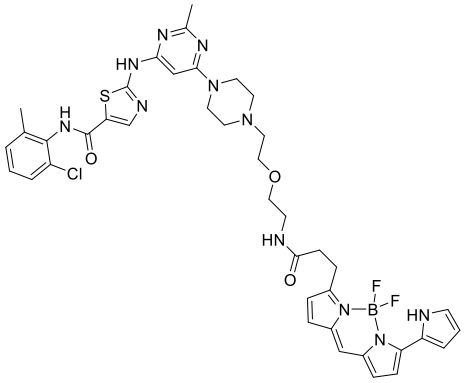
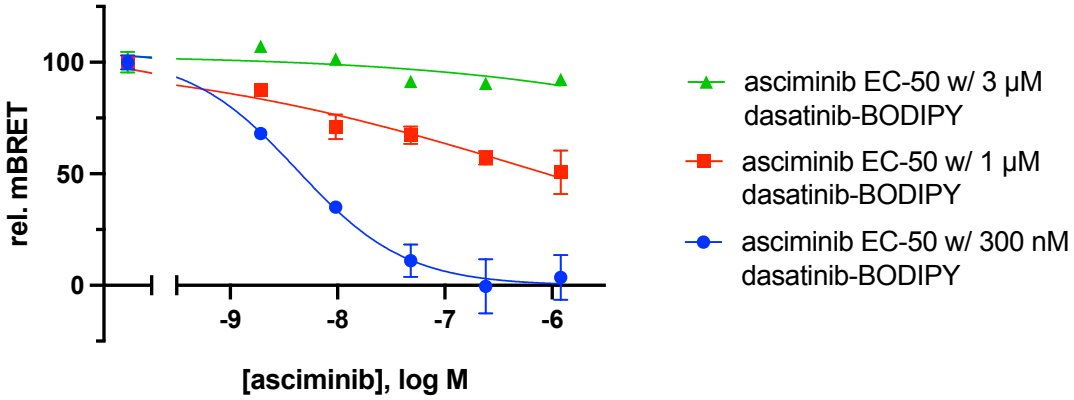
I. NanoBRET assay

The NanoBRET TE assay kit was purchased from Promega and carried out as described in the assay kit. HEK293 cells (ATCC) were used for transfection and intracellular compound binding to Abl was studied using the NanoLuc-Abl1 fusion vector (Promega). Dasatinib-BODIPY was used as the tracer at a concentration of 330 nM, if not otherwise specified. BRET ratios were calculated from the donor signal (415 nm) and acceptor signal (610 nm).

Dasatinib-BODIPY tracer used:

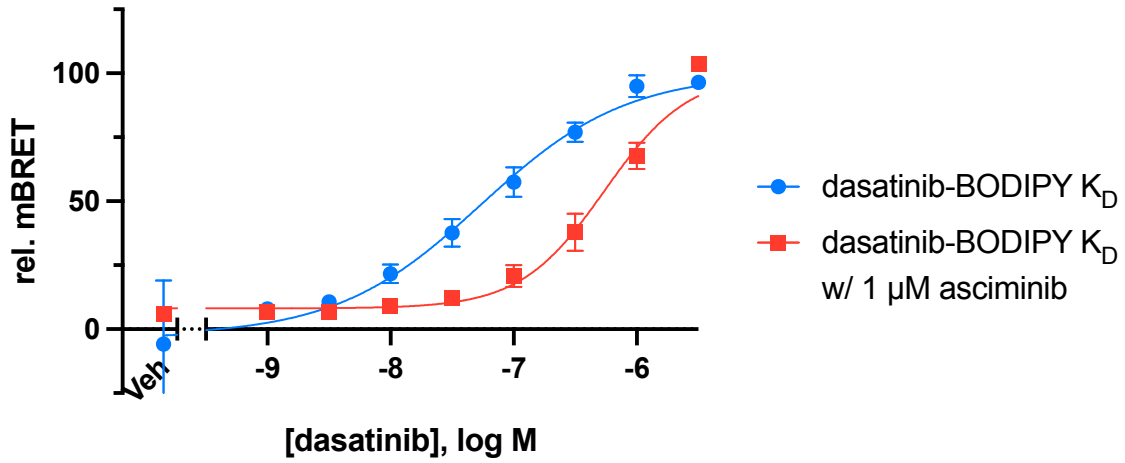


Asciminib EC_{50} values with varied dasatinib-BODIPY:



	dasatinib-BODIPY@ 0.3uM	dasatinib-BODIPY @ 1uM	dasatinib-BODIPY @ 3uM
HillSlope	-0.8581	-0.2445	-0.2575
IC50	4.213e-009	6.837e-007	0.001662

Dasatinib-BODIPY K_D measurements with and without 1 µM asciminib:



	DMSO	1 uM Asciminib
IC50	5.430e-008	5.458e-007

II. KCL22 synergy

***General procedure for cellular characterization*.**

1. Cell culture and seeding: KCL22 cells were cultured in RPMI 1640 media with 10% FBS. An aliquot of the cells was mixed with Trypan Blue solution and the cell number was quantified using a hemocytometer. The cells were plated 100 µL in each well at 30,000 cells/mL so that each well contained 3,000 cells. The cells were plated into sterile, clear bottom 96 well plates and then immediately dosed with compound. Additionally, 3 wells were created containing 100 µL of media with no cells.

2. Dosing: The compounds were made in 100% DMSO at 1,000X the final concentrations that were desired for the assay generally covering a concentration range of 6 log units. These DMSO stocks were diluted 10X in RPMI 1640 media. 1 µL of the compound diluted in media was added to each well for a final concentration of 0.1% DMSO. The wells containing only media were not dosed. In general, each compound concentration was dosed in triplicate wells. The plates were returned to normal culture conditions (per ATCC) for 72 hours.

3. Assay: After 72 hours, the plates were removed from the incubator, and 10 µL of WST-1 reagent was added to each well. The plates were returned to the incubator and the color change was visually monitored for 0.5 – 2 hours. When sufficient color change had occurred, the plates were shaken on a plate shaker for 30 seconds, and absorbance at 450 and 630 nm was read in a Biotek Synergy 4 plate reader. The absorbance at 630 nm was subtracted from the absorbance at 450 nm.

4. Data Analyses: The average absorbance value from wells containing media without cells was subtracted from the absorbance value for all the wells containing cells. The absorbance values were then taken as a percentage of the absorbance for the vehicle wells (0.1% DMSO - no compound). The percent compared to vehicle was then plotted vs. log(Concentration). Data analyses and curve fitting were performed using Graphpad Prism 6. For each compound, there were n = 3 data points for each concentration. For curves that did not reach full inhibition, the bottom was set to -10.

General procedure for cellular synergy.

1. Cell culture and seeding: KCL22 cell line was cultured in RPMI 1640 media with 10% FBS. An aliquot of the cells was mixed with trypan blue solution and the cell number was quantified using a hemacytometer. The cells were plated 100 µL in each well at 30,000 cells/mL so that each well contained 3,000 cells. The cells were plated into sterile, clear bottom 96 well plates and then immediately dosed with compound.

2. Dosing: The compounds dilutions (2X) and combinations were made in 100% DMSO at 1,000X the final concentrations that were desired for the assay. These DMSO stocks were diluted 10X in RPMI 1640 media. 1 µL of the compound diluted in media was added to each well for a final concentration of 0.1% DMSO. The wells containing only media were not dosed. In general, each compound concentration was dosed in triplicate wells. The plates were returned to normal culture conditions (per ATCC) for 72 hours.

3. Assay: After 72 hours, the plates were removed from the incubator and 10 µL of WST-1 reagent was added to each well. The plates were returned to the incubator and the color change was visually monitored for 0.5 – 2 hours. When sufficient color change had occurred, the plates were shaken on a plate shaker for 60 seconds and read in a Biotek Synergy 4 plate reader.

4. Data Analyses: The average absorbance value from wells containing media without cells was subtracted from the absorbance value for all the wells containing cells. The data were then

calculated as a fraction of the vehicle well (1% DMSO) and subtracted from 1 in order to represent the data as the fraction of population affected by the treatment at each given dose. The data were then analyzed using Compusyn to determine the combination indices.

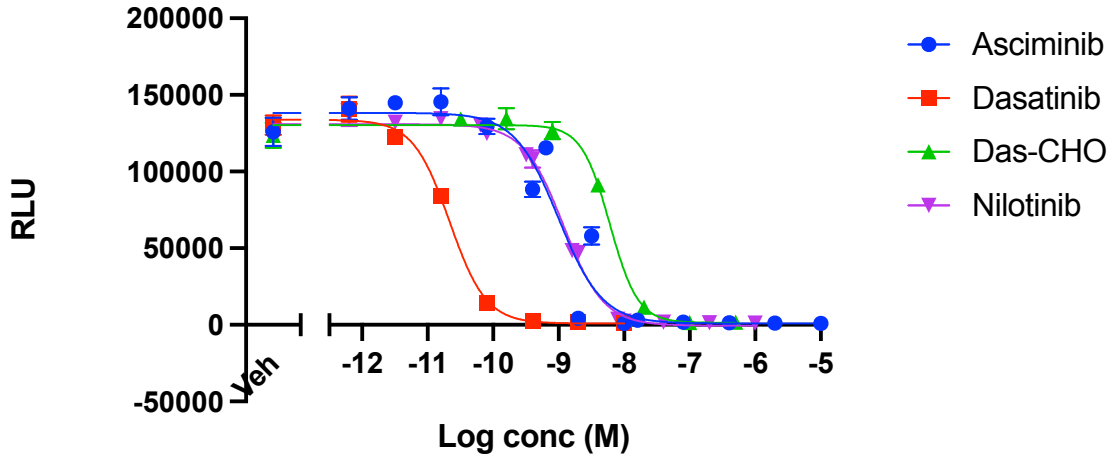
Equation for Determination of Combination Index (CI)

$$CI = \frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2} = \frac{(D)_1}{(D_m)_1\left[{}^{fa}/_{(1-fa)}\right]^{1/m_1}} + \frac{(D)_2}{(D_m)_2\left[{}^{fa}/_{(1-fa)}\right]^{1/m_2}} \quad (1)^1$$

where $(D)_1$ and $(D)_2$ are the doses of drugs 1 and 2, D_m is the dose required to produce the median effect (analogous to IC_{50}, ED_{50}, or LD_{50} values), m is a Hill-type coefficient signifying the sigmoidicity of the dose-effect curve, and f_a is fraction affected[1]

These dose-response curves were used to aid in the selection of optimal doses for the Chou-Talalay synergy experiments.

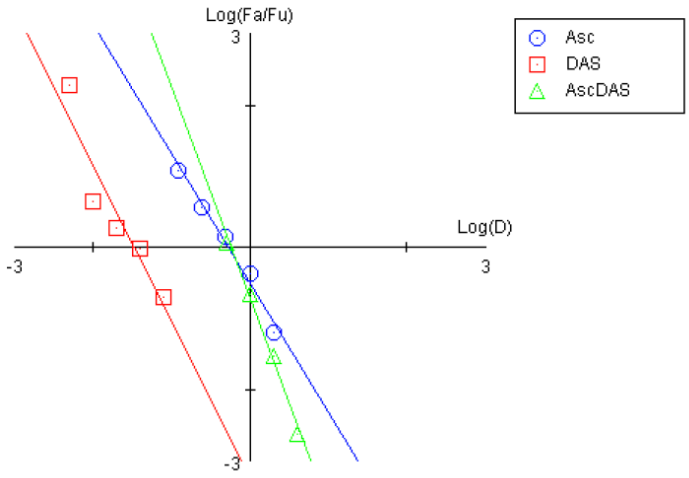
KCL22:



	Asciminib	Dasatinib	Das-CHO	Nilotinib
IC50	9.939e-010	2.168e-011	6.029e-009	1.178e-009

Chou-Talalay synergy analysis for asciminib + dasatinib:

Median-Effect Plot



CI Data for Drug Combo: AscDAS (Asc+DAS [4:0.08])

Fa	CI Value	Total Dose
0.05	0.80243	1.56153
0.1	0.92285	1.21491
0.15	1.00679	1.04005
0.2	1.07526	0.92521
0.25	1.13548	0.83999
0.3	1.19098	0.77198
0.35	1.24385	0.71501
0.4	1.29554	0.66551
0.45	1.34722	0.62126
0.5	1.39995	0.58077
0.55	1.45483	0.54291
0.6	1.51316	0.50681
0.65	1.57659	0.47173
0.7	1.64746	0.43691
0.75	1.72933	0.40154
0.8	1.82827	0.36455
0.85	1.95599	0.32430
0.9	2.14011	0.27763
0.95	2.47638	0.21600
0.97	2.74864	0.18067

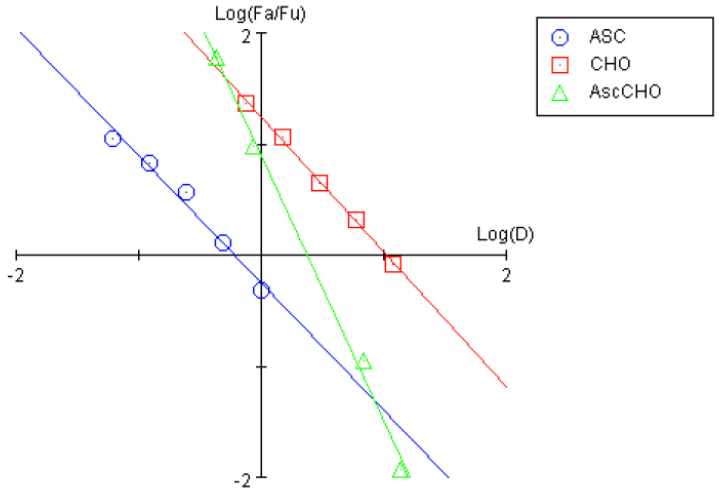
Combo	ED50
AscDAS	1.39995

Data for Fa = 0.5

Drug/Combo	CI value	Dose Asc	Dose DAS
Asc		0.53450	
DAS			0.03402
AscDAS	1.39995	0.56938	0.01139

Chou-Talalay synergy analysis for asciminib + DAS-CHO-II:

Median-Effect Plot



CI Data for Drug Combo: AscCHO (ASC+CHO [4:24])

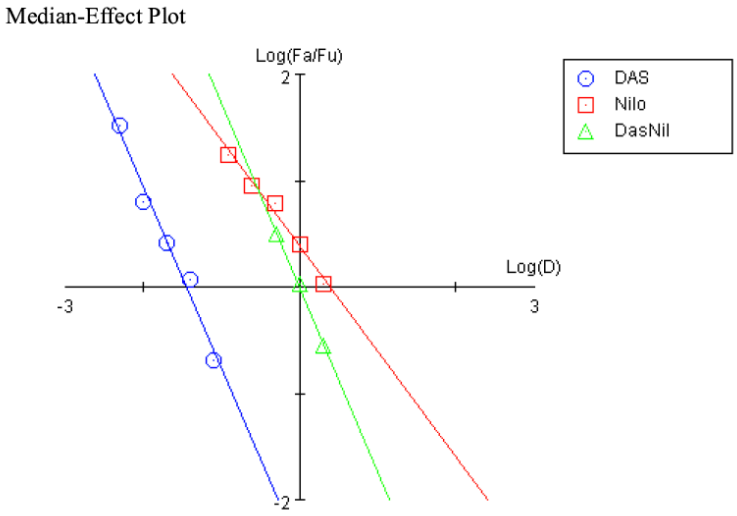
Fa	CI Value	Total Dose
0.05	0.20617	8.29170
0.1	0.28592	6.05824
0.15	0.35013	4.98839
0.2	0.40784	4.30950
0.25	0.46265	3.81902
0.3	0.51653	3.43646
0.35	0.57090	3.12230
0.4	0.62696	2.85442
0.45	0.68590	2.61916
0.5	0.74903	2.40745

Combo	ED50
AscCHO	0.74903

Data for Fa = 0.5

Drug/Combo	CI value	Dose ASC	Dose CHO
ASC		0.61969	
CHO			10.6349
AscCHO	0.74903	0.34392	2.06353

Chou-Talalay synergy analysis for dasatinib + nilotinib:



Fa	CI Value	Total Dose
0.05	0.65394	5.56024
0.1	0.70024	3.61165
0.15	0.73672	2.76496
0.2	0.76912	2.26120
0.25	0.79961	1.91511
0.3	0.82939	1.65642
0.35	0.85927	1.45187
0.4	0.88992	1.28341
0.45	0.92201	1.14027
0.5	0.95625	1.01551

Combo	ED50
DasNil	0.95625

Data for Fa = 0.5

Drug/Combo	CI value	Dose DAS	Dose Nilo
DAS		0.03574	
Nilo			2.49457
DasNil	0.95625	0.01991	0.99560

III. References

(1) Chou, T.-C.; Talalay, P. Quantitative Analysis of Dose-Effect Relationships: The Combined Effects of Multiple Drugs or Enzyme Inhibitors. *Adv. Enzym. Regul.* **1984**, *22*, 27–55.