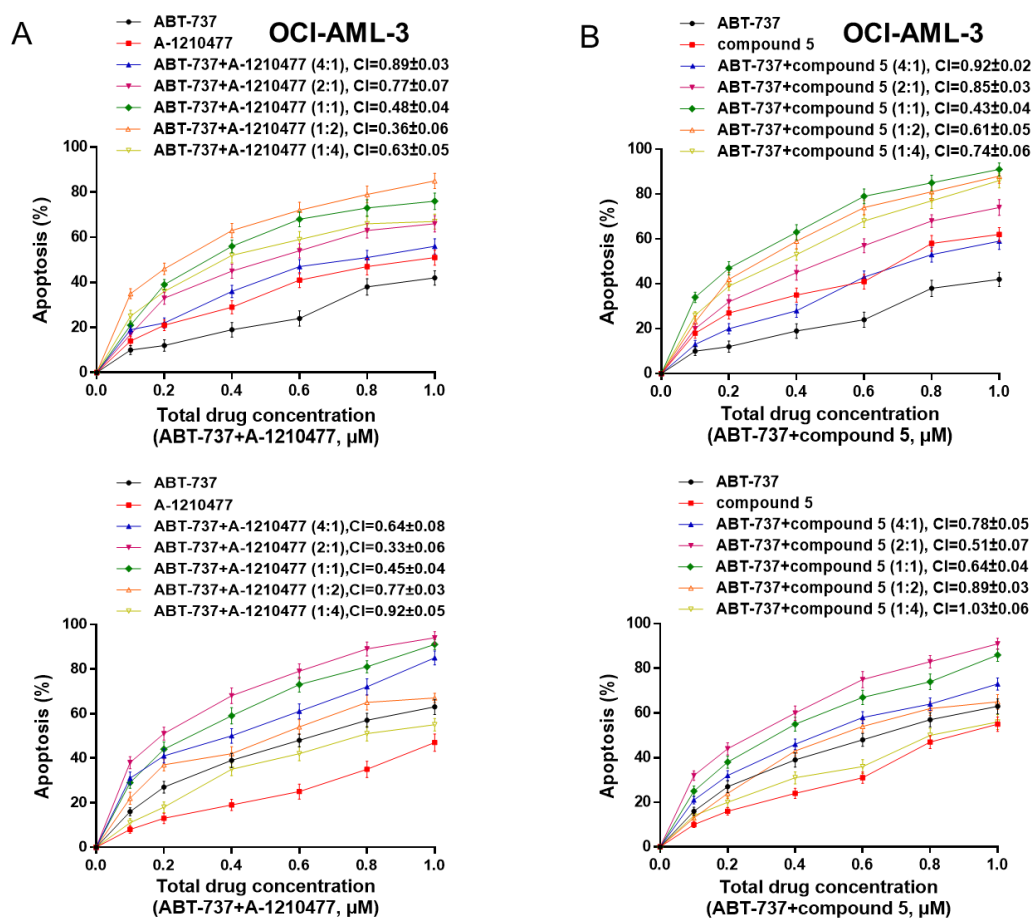


SUPPLEMENTARY MATERIALS



Supplementary Figure S1. The best combination ratios of ABT-737/A-1210477 and ABT-737/compound 5 were experimentally verified in OCI-AML3 and HCT-116. (A, B, C, D) OCI-AML3 and HCT-116 cells were treated by compounds alone or in combination with fixed ratios for 48 h, after which LC₅₀ value was determined by cell apoptosis assay. CI value was calculated by Calcsyn software that utilizes the methodology applied by Chou and Talalay. Data are the mean ± stand deviation (sd) (n=3).

Table S1: Translation of protein interactions into Ordinary Differential Equations.

Protein reactions were translated into a mathematical form. **(A)** Irreversible Bax activation by BH3-only activator protein (Bim and Puma) or by auto-activation. Because BH3-only activator proteins were recycled after activation, the concentration of activator was modelled with no level change in this chemical reaction. Bax concentration change over time was proportional to the product of reactant concentrations. **(B)** Protein turnover of a R_1 was modeled to consist of constant protein production and degradation. The protein degradation rate of R_2 was modeled to be proportional to the actual protein concentration. **(C)** Irreversible inactivation of Bax. Bax concentration change over time was proportional to the product of reactant concentrations. **(D)** Reversible reactions of two proteins R_1 and R_2 forming a complex R_1/R_2 . Protein concentration change was given by balancing binding on and off reactions. **(E)** Reversible reactions of Bax forming pores (MAC). Change of protein concentration was given by balancing forward and backward reactions.

Table S2: Bax activation kinetics by BH3-only activator protein and auto-activation.

The parameter was fitted by our previous report [1].

Table S3: Kinetics for Bax inactivation.

The parameter was fitted by our previous report [1].

Table S4: Degradation rates as used in in the model.

The half-life times of Bcl-2-family proteins were derived from the literature [2-8]. We assumed that the half-life times of most complexes of anti- and pro-apoptotic proteins were as same as that of Bcl-2/tBid

complex (75 minutes) [1]. The degradation constants k_{deg} were calculated by the following equation: $k_{deg} = \ln(2) / (60 * t_{1/2})$.

Table S5: Kinetics for interactions between BH3-only proteins, Bax or BH3 mimetics with anti-apoptotic Bcl-2 proteins.

The interaction constants for Bcl-2 or Mcl-1 binding Bim, Puma, Bax were taken from literature [9-14].

The reaction constant k/k_+ for ABT-737, A-1210477 or compound 5 were derived from previous data of K_d value. We fixed k_- value of 0.0001, and k_+ was obtained by $k_+ = k_- / k_d$.

Table S6: Kinetics for Bax dimerization.

We modelled that activated Bax could form dimerize in the outer membrane of mitochondrial to induce MOMP. The parameter was derived from literature.

Table S1

A		Bax activation by BH3-only activator protein and auto-activation	
$\text{Bim} + \text{InBax} \xrightarrow{k_{\text{Bim} \rightarrow \text{AcBax}}} \text{AcBax} + \text{Bim}$		$d[\text{InBax}]/dt = d[\text{AcBax}]/dt = k_{\text{Bim} \rightarrow \text{AcBax}} \times [\text{Bim}] \times [\text{InBax}]$	
$\text{AcBax} + \text{InBax} \xrightarrow{k_{\text{auto_Act}}} \text{MAC}$		$d[\text{MAC}]/dt = k_{\text{auto_Act}} \times [\text{AcBax}] \times [\text{InBax}]$	
		$d[\text{AcBax}]/dt = d[\text{InBax}]/dt = -k_{\text{auto_Act}} \times [\text{AcBax}] \times [\text{InBax}]$	
B		Protein and protein complex turnover	
$\Phi \xrightleftharpoons[k_{\text{deg}}]{k_{\text{pro}}} \text{R}_1$		$d[\text{R}_1]/dt = k_{\text{prod}} - k_{\text{deg}} \times [\text{R}_1]$	
$\text{R}_2 \xrightarrow{k_{\text{deg}}} \Phi$		$d[\text{R}_2]/dt = -k_{\text{deg}} \times [\text{R}_2]$	
C		Bax inactivation	
$\text{AcBax} \xrightarrow{k_{\text{Ac} \rightarrow \text{In}}} \text{InBax}$		$d[\text{InBax}]/dt = k_{\text{Ac} \rightarrow \text{In}} \times [\text{AcBax}]$	
		$d[\text{AcBax}]/dt = -k_{\text{Ac} \rightarrow \text{In}} \times [\text{AcBax}]$	
D		Protein complex association and dissociation	
$\text{R}_1 + \text{R}_2 \xrightleftharpoons[k_{\text{off}}]{k_{\text{on}}} \text{R}_1/\text{R}_2$		$d[\text{R}_1/\text{R}_2]/dt = k_{\text{on}} \times [\text{R}_1] \times [\text{R}_2] - k_{\text{off}} \times [\text{R}_1/\text{R}_2]$	
		$d[\text{R}_1]/dt = d[\text{R}_2]/dt = k_{\text{off}} \times [\text{R}_1/\text{R}_2] - k_{\text{on}} \times [\text{R}_1] \times [\text{R}_2]$	
E		Bax dimerization to form pores	
$2\text{AcBax} \xrightleftharpoons[k]{k_+} \text{MAC}$		$d[\text{MAC}]/dt = k_+ \times [\text{AcBax}]^2 - k \times [\text{MAC}]$	
		$d[\text{AcBax}]/dt = k \times [\text{MAC}] - k_+ \times [\text{AcBax}]^2$	

Table S2

Biochemical reaction in (A)	k	Value	Comment
$\text{Bim} + \text{InBax} \longrightarrow \text{AcBax} + \text{Bim}$	$k_{\text{Bim} \rightarrow \text{AcBax}}$	1.3E-03	Fitted by our previous data [1]
$\text{AcBax} + \text{InBax} \longrightarrow \text{MAC}$	$k_{\text{auto_Act}}$	3.0E-04	Fitted by our previous data [1]

Table S3

Biochemical reaction in (A)	k	Value	Comment
$\text{AcBax} \longrightarrow \text{InBax}$	$k_{\text{Ac} \rightarrow \text{In}}$	1.11E-03	Fitted by our previous data [1]

Table S4

Biochemical reaction in (B)	k	Value	Comment
Bcl-2 \longrightarrow Φ	k_{deg_Bcl-2}	$9.6E-06\ s^{-1}; t_{1/2} = 1200\ min$	[1]
Mcl-1 \longrightarrow Φ	k_{deg_Mcl-1}	$2.6E-04\ s^{-1}; t_{1/2} = 45\ min$	[6]
Bim \longrightarrow Φ	k_{deg_Bim}	$2.4E-05\ s^{-1}; t_{1/2} = 480\ min$	[4]
Bcl-2/Bim \longrightarrow Φ	$k_{deg_Bcl/Bim}$	$1.5E-04\ s^{-1}; t_{1/2} = 75\ min$	[2]
Mcl-1/Bim \longrightarrow Φ	$k_{deg_Mcl/Bim}$	$7.7E-05\ s^{-1}; t_{1/2} = 150\ min$	[3, 7]
Bcl-2/AcBax \longrightarrow Φ	$k_{deg_Bcl/AcBax}$	$1.5E-04\ s^{-1}; t_{1/2} = 75\ min$	[2]
Mcl-1/AcBax \longrightarrow Φ	$k_{deg_Mcl/AcBax}$	$7.7E-05\ s^{-1}; t_{1/2} = 150\ min$	[3, 7]
Bcl-2/ABT-737 \longrightarrow Φ	$k_{deg_Bcl-2/ABT-737}$	$1.5E-04\ s^{-1}; t_{1/2} = 75\ min$	[2]
Mcl-1/A-1210477 \longrightarrow Φ	$k_{deg_Mcl-1/A-1210477}$	$8.5E-05\ s^{-1}; t_{1/2} = 120\ min$	Fitted by datasets in Figs.
Mcl-1/compound 5 \longrightarrow Φ	$k_{deg_Mcl-1/compound\ 5}$	$4.1E-04\ s^{-1}; t_{1/2} = 20\ min$	3A and 3B

Table S5

Biochemical reaction in (E)	k_{on}	Value	k_{off}	Value	comment
Bcl-2 + Bim \longleftrightarrow Bcl-2/Bim	$k_{on_Bcl2/Bim}$	$3.00E-05$	$k_{off_Bcl2/Bim}$	$1.40E-04$	[9]
Bcl-2 + AcBax \longleftrightarrow Bcl-2/AcBax	$k_{on_Bcl2/AcBax}$	$9.33E-06$	$k_{off_Bcl2/AcBax}$	$1.40E-04$	[11]
Bcl-2 + ABT-737 \longleftrightarrow Bcl-2/ABT-737	$k_{on_Bcl2/ABT}$	$4.69E-06$	$k_{off_Bcl2/ABT}$	$1.0E-04$	[15]
Mcl-1 + Bim \longleftrightarrow Mcl-1/Bim	$k_{on_Mcl-1/Bim}$	$7.80E-04$	$k_{off_Mcl-1/Bim}$	$2.17E-03$	[12]
Mcl-1 + AcBax \longleftrightarrow Mcl-1/AcBax	$k_{on_Mcl-1/AcBax}$	$3.25E-05$	$k_{off_Mcl-1/AcBax}$	$2.60E-04$	[14]
Mcl-1+A-1210477 \longleftrightarrow Mcl-1/A-1210477	$k_{on_Mcl-1/A-1210477}$	$2.86E-05$	$k_{off_Mcl-1/A-1210477}$	$1.0E-04$	[10]
Mcl-1+compound 5 \longleftrightarrow Mcl-1/compound 5	$k_{on_Mcl-1/compound\ 5}$	$6.67E-07$	$k_{off_Mcl-1/compoud\ 5}$	$1.0E-04$	[13]

Table S6

Biochemical reaction in (F)	k_+	Value	k_-	Value	Comment
2AcBax \longleftrightarrow MAC	$k_{AcBax \rightarrow Mac}$	$1.44E-03$	$k_{MAC \rightarrow AcBax}$	$2.0E-02$	[16]

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