Supplemental Information

Molecular Features of CA-074 pH-Dependent Inhibition of Cathepsin B

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Figure S1. Irreversible mechanism of CA-074Me inhibition of cathepsin B at pH 4.6, pH 5.5, and pH 7.2. CA-074Me at pH 4.6 (panel a), pH 5.5 (panel b), and pH 7.2 (panel c) was evaluated for irreversible or reversible inhibition of cathepsin B. Cathepsin B was pre-incubated with inhibitor at 10 times the IC_{50} concentration (consisting of CA-074Me at 90 μM at pH 4.6, 137 μM at pH 5.5, and 75 nM at pH 7.2), followed by dilution to 1/10 the IC_{50} concentration, addition of substrate (Z-F-R-AMC), and measurement of activity in time-course assays. Inhibition of cathepsin B following dilution indicates the irreversible inhibitory mechanism of CA-074Me.

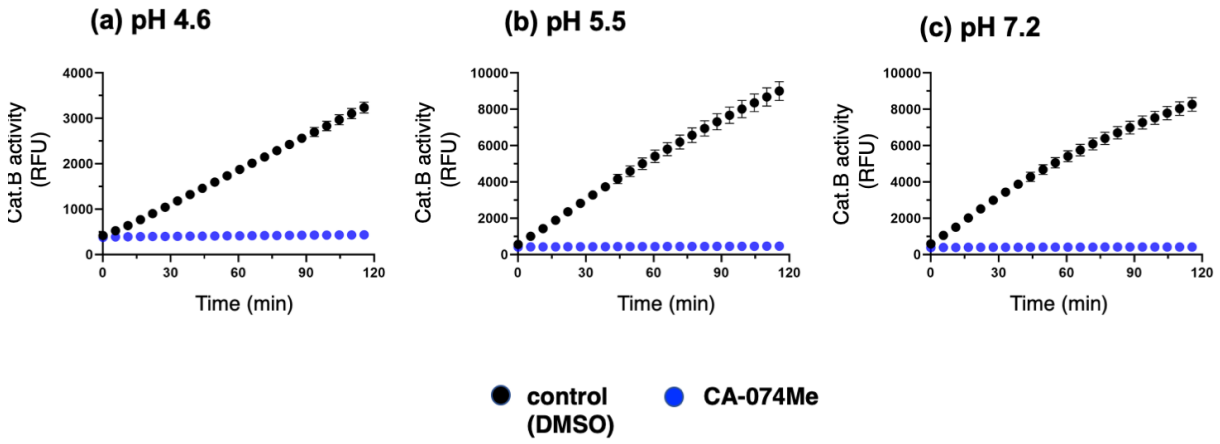


Figure S2. Kinetic analysis of CA-074 inhibition of cathepsin B at acidic to neutral pH conditions. Cathepsin B activity was monitored in time-course assays at different concentrations of CA-074, conducted at pH 4.6 (panel a), pH 5.5 (panel b), and pH 7.2 (panel c). Values for k_{obs} were calculated by Prism using the equation $Y=Y0*e^{(-kobs*X)}$ where X is time and Y/Y0 is activity (measured as slope of RFU vs time) with inhibitor relative to activity with no inhibitor. The k_{obs} values were used to calculate k_{inact} and K_I values by plotting k_{obs} vs inhibitor concentration (shown in Figure 3).

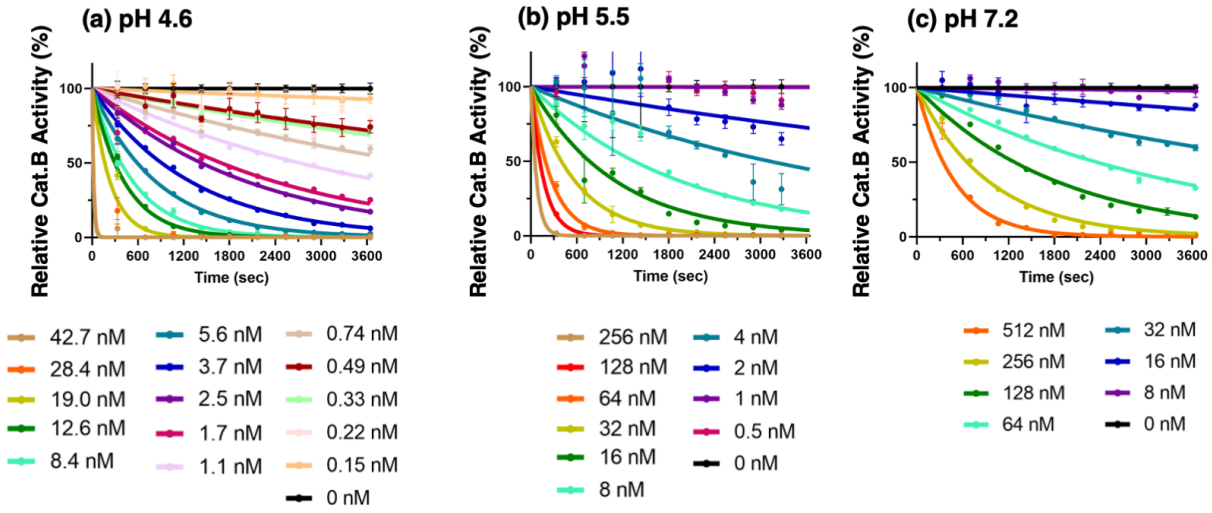


Figure S3. Kinetic analysis of CA-074Me inhibition of cathepsin B at acidic to neutral pH conditions.

(a) to (c). CA-074Me and cathepsin B in kinetic studies for k_{obs} values. Cathepsin B activity was monitored in time-course assays at different concentrations of CA-074, conducted at pH 4.6 (panel a), pH 5.5 (panel b), and pH 7.2 (panel c). Values for k_{obs} were calculated by the equation $Y=Y0*e^{(-kobs*X)}$ where X is time and Y/Y0 is activity with inhibitor relative to activity with no inhibitor. The k_{obs} values were used to calculate k_{inact} and K_I values by plotting k_{obs} vs inhibitor concentration (shown in d-f).

(d) to (f). CA-074Me inhibition of cathepsin B kinetics for K_I, k_{inact}, and k_{inact}/K_I values. Kinetic analyses of CA-074Me inhibition of cathepsin B was conducted at pH 4.6 (panel d), pH 5.5 (panel e), and pH 7.2 (panel f) to determine K_I, k_{inact}, and k_{inact}/K_I values, conducted as described in the methods.

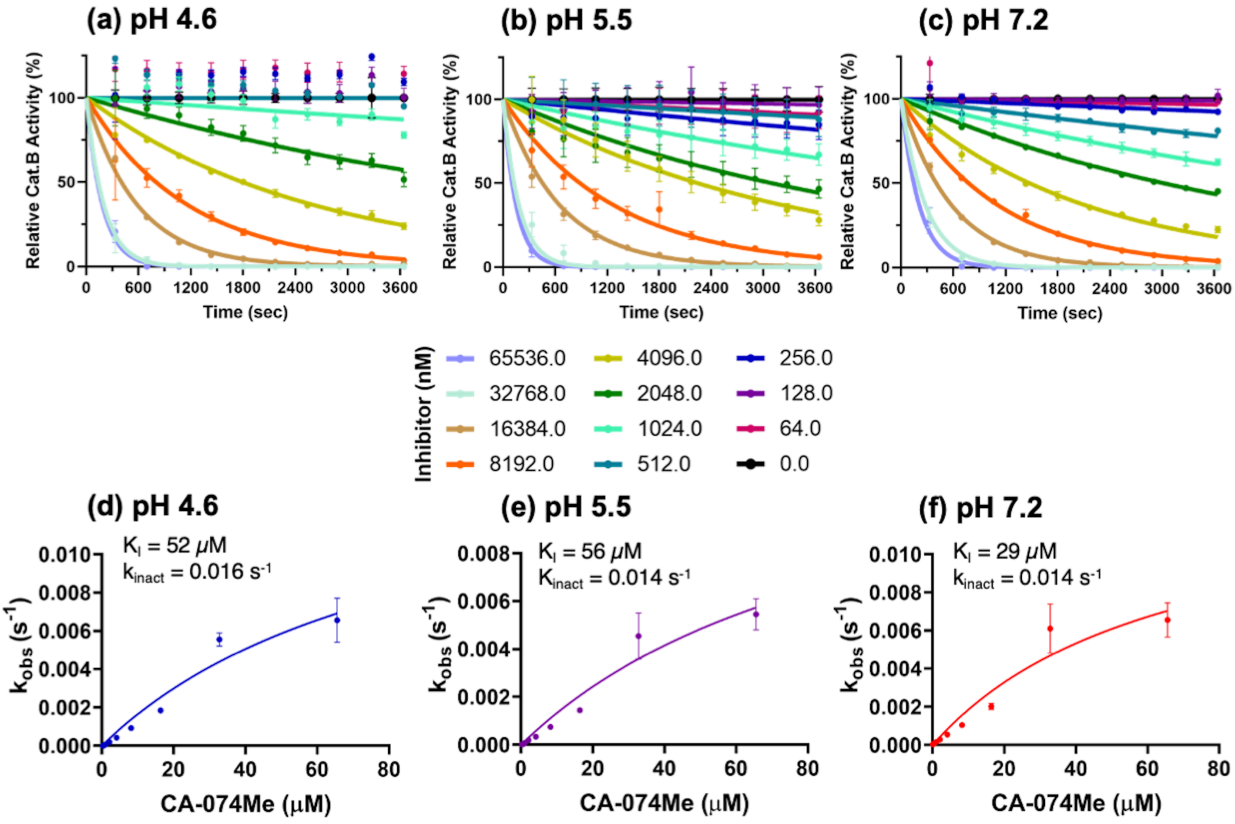


Figure S4. Binding energy of CA-074 bound to cathepsin B at pH 4.6.

Components comprising the binding energy of CA-074 binding to cathepsin B at pH 4.6 were assessed by the MOE docking software. Energies of the binding interactions of the inhibitor with the enzyme (specific residues are indicated) are shown, combined with the total binding energy indicated as -58.3 kcal/mol.

Ligand Interactions Report

Mon Oct 12 12:58:04 2020 (MOE 2019.01)

1QDQ: HYDROLASE / 1QDQ: HYDROLASE

Inhibitor		Enzyme				Interaction	Distance	E (kcal/mol)
N1	11	O	GLY	198	(A)	H-donor	2.84	-2.0
O2	17	O	HOH	399	(A)	H-donor	2.61	-1.6
C6	19	O	GLY	198	(A)	H-donor	3.37	-0.5
O1	14	N	GLY	74	(A)	H-acceptor	3.04	-2.3
O2	17	O	HOH	282	(A)	H-acceptor	2.86	-1.2
O3	22	NE2	GLN	23	(A)	H-acceptor	2.90	-1.4
O3	22	N	CYS	29	(A)	H-acceptor	3.26	-1.9
O4	28	NE1	TRP	221	(A)	H-acceptor	2.89	-4.2
O	46	O	HOH	388	(A)	H-acceptor	2.62	-1.1
O	46	O	HOH	395	(A)	H-acceptor	2.65	-3.3
OT	56	ND1	HIS	110	(A)	H-acceptor	2.70	-11.2
OT	56	NE2	HIS	111	(A)	H-acceptor	2.73	-10.9
O	46	NE2	HIS	111	(A)	Ionic	3.19	-3.3
OT	56	ND1	HIS	110	(A)	Ionic	2.70	-6.8
OT	56	NE2	HIS	111	(A)	Ionic	2.73	-6.6

CA074:
Bound @ pH4.6

Total Interactions:
-58.3 kcal/mol

Nuc-E Distance: N/Å

Figure S5. Binding energy of CA-074 bound to cathepsin B at pH 7.2.

Components comprising the binding energy of CA-074 binding to cathepsin B at pH 7.2 were assessed by the MOE docking software. Energies of the binding interactions of the inhibitor with the enzyme (residues are indicated) are shown, combined with the total binding energy indicated as -43.0 kcal/mol.

Ligand Interactions Report

Mon Oct 12 13:33:09 2020 (MOE 2019.01)

1QDQ: HYDROLASE / 1QDQ: HYDROLASE

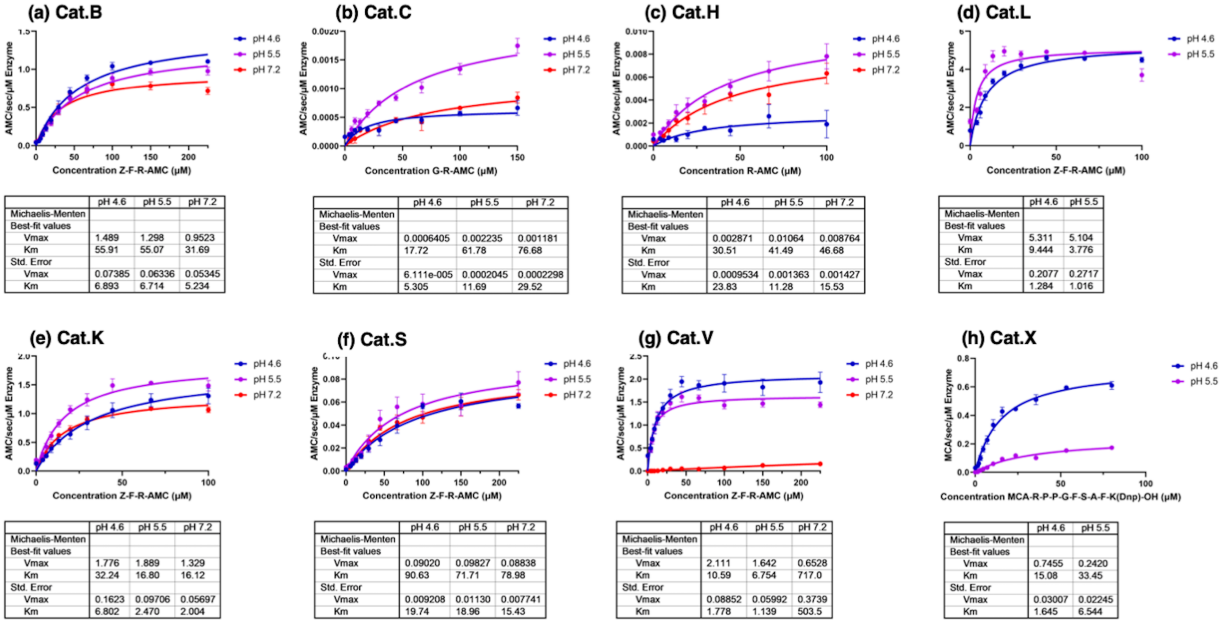
Inhibitor		Enzyme				Interaction	Distance	E (kcal/mol)
N1	11	O	GLY	198	(A)	H-donor	2.84	-2.0
O2	17	O	HOH	399	(A)	H-donor	2.61	-1.6
O1	14	N	GLY	74	(A)	H-acceptor	3.04	-2.3
O2	17	O	HOH	282	(A)	H-acceptor	2.87	-1.2
O3	22	NE2	GLN	23	(A)	H-acceptor	2.90	-1.3
O3	22	N	CYS	29	(A)	H-acceptor	3.26	-1.9
O4	28	NE1	TRP	221	(A)	H-acceptor	2.90	-4.4
O	46	O	HOH	388	(A)	H-acceptor	2.61	-1.1
O	46	O	HOH	395	(A)	H-acceptor	2.62	-3.1
OT	56	ND1	HIS	110	(A)	H-acceptor	2.69	-11.1
OT	56	NE2	HIS	111	(A)	H-acceptor	2.83	-6.1
OT	56	ND1	HIS	110	(A)	Ionic	2.69	-6.9

CA074:
Bound @ pH7.2

Total Interactions:
-43.0 kcal/mol

Nuc-E Distance: N/Å

Figure S6. K_m plots for cathepsin B and cysteine cathepsins at pH 4.6, 5.5, and 7.2.
K_m values were determined for cathepsin B (panel a) and the cysteine cathepsins (cathepsins C, H, L K, S, V, and X with each of their indicated substrates, shown in panels b, c, d, e, f, g, and h, respectively. Michaelis-Menten kinetics was used for K_m data analysis (conducted as described in the methods) using the equation $v_0=V_{max}*[S]/(K_m+[S]$ with curve-fitting using Prism 9. Cathepsin L and cathepsin X had no activity at pH 7.2 and, therefore, K_m was determined for pH 4.6 and 5.5.



	pH 4.6	pH 5.5	pH 7.2
Michaelis-Menten Best-fit values			
Vmax	1.489	1.298	0.9523
Km	55.91	55.07	31.69
Std. Error			
Vmax	0.07385	0.06336	0.05345
Km	6.893	6.714	5.234

	pH 4.6	pH 5.5	pH 7.2
Michaelis-Menten Best-fit values			
Vmax	0.0006405	0.002235	0.001181
Km	17.72	61.78	76.68
Std. Error			
Vmax	6.111e-005	0.0002045	0.0002298
Km	5.305	11.69	29.52

	pH 4.6	pH 5.5	pH 7.2
Michaelis-Menten Best-fit values			
Vmax	0.002871	0.01064	0.008764
Km	30.51	41.49	46.68
Std. Error			
Vmax	0.0009534	0.001363	0.001427
Km	23.83	11.28	15.53

	pH 4.6	pH 5.5
Michaelis-Menten Best-fit values		
Vmax	5.311	5.104
Km	9.444	3.776
Std. Error		
Vmax	0.2077	0.2717
Km	1.284	1.016

	pH 4.6	pH 5.5	pH 7.2
Michaelis-Menten Best-fit values			
Vmax	1.776	1.889	1.329
Km	32.24	16.80	16.12
Std. Error			
Vmax	0.1623	0.09706	0.05697
Km	6.802	2.470	2.004

	pH 4.6	pH 5.5	pH 7.2
Michaelis-Menten Best-fit values			
Vmax	0.09020	0.09827	0.08838
Km	90.63	71.71	78.98
Std. Error			
Vmax	0.009208	0.01130	0.007741
Km	19.74	18.96	15.43

	pH 4.6	pH 5.5	pH 7.2
Michaelis-Menten Best-fit values			
Vmax	2.111	1.642	0.6528
Km	10.59	6.754	717.0
Std. Error			
Vmax	0.08852	0.05992	0.3739
Km	1.778	1.139	503.5

	pH 4.6	pH 5.5
Michaelis-Menten Best-fit values		
Vmax	0.7455	0.2420
Km	15.08	33.45
Std. Error		
Vmax	0.03007	0.02245
Km	1.645	6.544