Selection of Cyclic-Peptide Inhibitors Targeting Aurora Kinase A: Problems and Solutions

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Supplementary Material

S1. Aurora A Selected Sequences

After six rounds of panning, clones were sequenced. All sequences from Aurora A selections are included in Table S1.

S2. Streptavidin Selected Sequences

After four rounds of panning, clones were sequenced. All sequences from Streptavidin selections are included in Table S2.

Table S1 Selected Sequences From Aurora A Selections

C	D	G	V	P	W	H	C	G	G
C	D	G	T	P	R	R	C	G	G
C	G	H	P	Q	V	P	C	G	G
C	H	P	Q	G	D	T	C	G	G
C	H	P	Q	G	P	R	C	G	G
C	V	N	G	V	P	Y	C	G	G
C	G	F	T	D	W	K	C	G	G
C	H	P	Y	G	D	T	C	G	G
C	P	M	Y	G	R	N	C	G	G
C	V	R	G	V	P	Y	C	G	G
C	V	G	V	L	P	W	C	G	G
C	E	W	Y	L	P	F	C	G	G
C	V	P	W	W	T	D	C	G	G
C	D	F	L	H	W	D	C	G	G
C	D	L	W	G	L	R	C	G	G
C	D	W	L	S	M	N	C	G	G
C	E	F	W	L	G	R	C	G	G
C	G	F	R	T	S	W	C	G	G
C	L	M	P	W	S	G	C	G	G
C	L	R	M	W	G	S	C	G	G
C	M	M	G	L	P	F	C	G	G
C	Q	W	P	P	Q	W	C	G	G
C	R	Y	P	W	M	G	C	G	G
C	S	L	W	L	P	H	C	G	G
C	T	F	N	Y	L	G	C	G	G
C	T	F	S	L	K	G	C	G	G
C	T	G	R	F	W	P	C	G	G
C	Q	R	T	W	W	G	C	G	G
C	T	M	G	G	Y	M	C	G	G
C	P	R	F	L	P	W	C	G	G
C	S	L	F	S	P	Y	C	G	G
C	S	L	Y	S	I	S	C	G	G
C	S	N	K	W	L	P	C	G	G
C	T	R	P	W	W	L	C	G	G
C	V	V	P	W	V	P	C	G	G
C	D	K	W	L	M	P	C	G	G
C	T	S	W	P	Y	G	C	G	G
C	I	L	P	G	V	W	C	G	G
C	M	R	Q	W	G	Y	C	G	G

Table S2 Selected Sequences From Streptavidin Coated Magnetic Beads Selection

C	H	P	Q	G	D	T	C	G	G
C	G	K	K	F	S	P	C	G	G
C	R	T	Q	I	A	S	C	G	G
C	H	P	Q	N	G	R	C	G	G
C	G	H	P	Q	V	P	C	G	G
C	L	V	E	T	S	L	C	G	G
C	I	S	F	M	C	D	C	G	G
C	Y	C	S	V	S	F	C	G	G
C	I	Q	F	F	W	R	C	G	G

S3. Determination of the HABA-Streptavidin Dissociation Constant

The HABA-Streptavidin dissociation constant was determined through titrating increasing amounts of HABA into a fixed concentration of Streptavidin (25 μL) in PBS and measuring absorbance values at 500 nm. Absorbance values of HABA alone at these same increasing concentrations were also measured and subtracted as background from the raw values of the HABA-Streptavidin complex absorbance measurements. The concentration of the HABA-Streptavidin complex was calculated from these background-subtracted measurements using ε = 35,000 L Mol^{-1} cm^{-1} as the molar extinction coefficient, and the resulting data was plotted against HABA concentration to yield the curve in Figure S1. The points were fitted to equation (1),

$$[LR] = \frac{[R_t][L]}{K_d + [L]} \qquad (1)$$

where $[R_t]$ is the concentration of HABA binding sites (equal to the Streptavidin concentration), [L] is the concentration of ligand (HABA), K_d is the dissociation constant and [LR] is the concentration of the ligand /receptor complex (HABA bound to Streptavidin). This allowed the experimental determination of

106.3 μM as the K_d, which is in good agreement with the published value of 100 μM (Green paper; Avidin and Streptavidin).

S4. HABA Displacement Assay of Aurora A Selected Peptides

Since half of the selected sequences from the initial Aurora A selection contained the streptavidin binding HPQ motif, all major convergent sequences were tested for possible streptavidin binding including: (CHPQGDTC)G, (CGHPQVPC)G, (CDGVPWHC)G, and (CDGTPRRC)G at two concentrations shown in Figure S1.

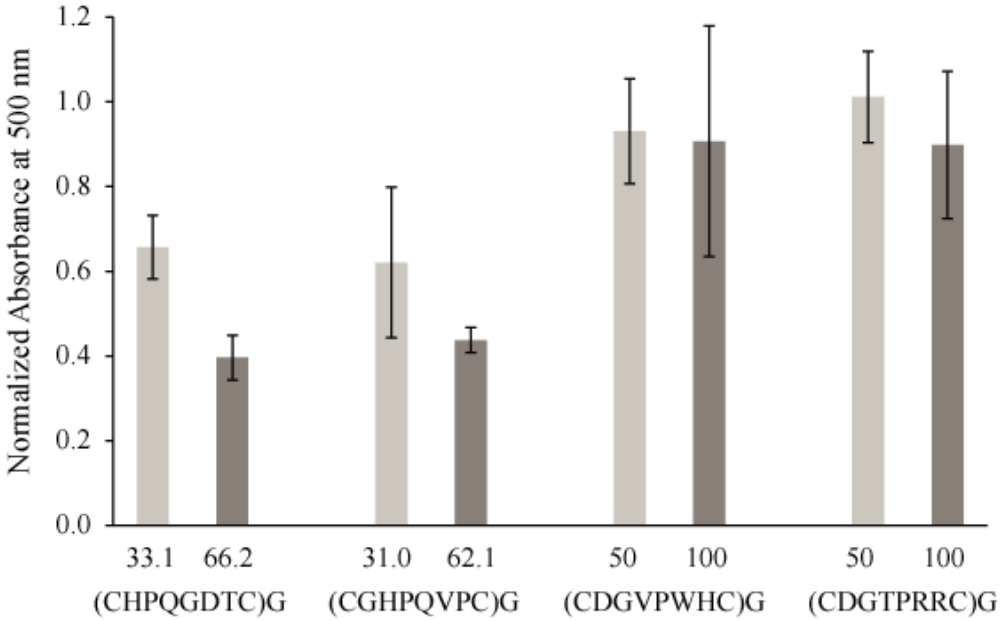


Figure S1. HABA Displacement Assay of Aurora A Selccted Peptides. The four selected peptides were evaluated for streptavidin binding, and only the HPQ containing peptides appreciably bind streptavidin as shown.

S5. Kinetic Analysis of Aurora A Selected Peptide

Kinetic analysis of the selected peptide, (CTRPWWLC)G, fit to different modes of inhibition are shown in Figure S2 with fit analysis. As can be seen, noncompetitive inhibition fit best to the kinetic data.

$$v = \frac{V\,\max}{1+\left(\frac{Km}{[S]}\right)\left(1+\frac{I}{Ki}\right)}$$

Equation S2. Competitive Inhibition

$$v = \frac{V\,\max}{\left(1+\frac{I}{Ki}+\frac{Km}{[S]}\right)}$$

Equation S3. Uncompetitive Inhibition

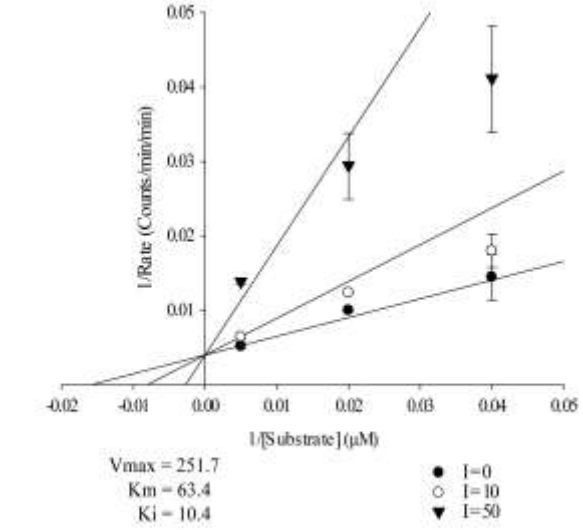
$$v = \frac{V\,\max}{\left(1+\frac{I}{Ki}\right)\left(1+\frac{Km}{[S]}\right)}$$

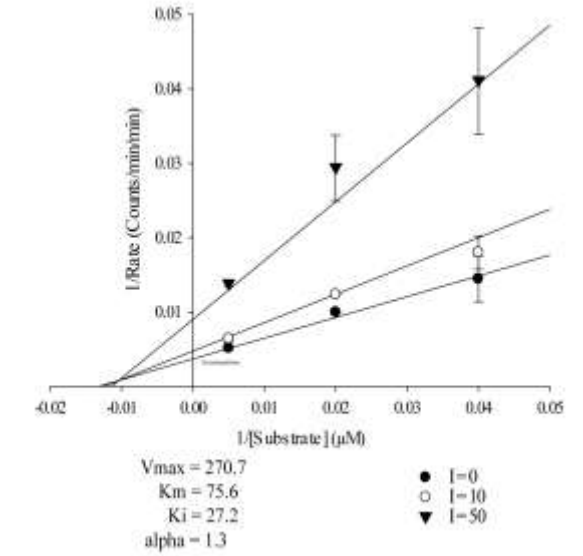
Equation S4. Noncompetitive Inhibition

$$v = \frac{V\,\max}{\left(\left(\frac{Km}{[S]}\right)\left(1+\frac{I}{Ki}\right)+\left(1+\frac{I}{\alpha Ki}\right)\right)}$$

Equation S5. Mixed Inhibition

Where:
v = rate (Counts/min/min)
Km = substrate concentration at half maximal rate
Vmax = maximal rate
S = substrate (kemptide, LRRASLG)
Ki = inhibitor dissociation constant
α = constant





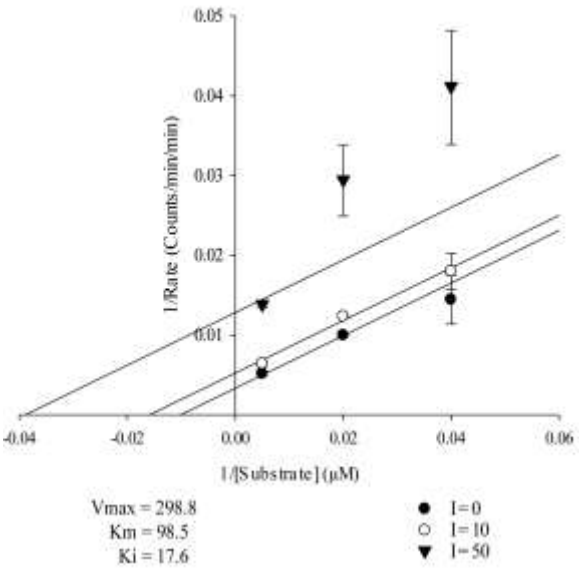


Figure S2. Kinetic Analysis of Aurora A Selected Peptide Data Fit to Inhibition Models. The Goodness of Fit statistical analysis is shown for each model.

Competitive Inhibition Fit (Top Fit)
Goodness of Fit
Degrees of Freedom = 15
AICc = 100.474
Sy.x = 13.124
R^2 = 0.948
Sum of Squares = 2,583.431

Mixed Inhibition Fit (Middle Fit)
Goodness of Fit
Degrees of Freedom = 14
AICc = 94.5
Sy.x = 10.319
R^2 = 0.970
Sum of Squares = 1,490.761

Uncompetitive Inhibition Fit (Bottom Fit)
Goodness of Fit
Degrees of Freedom = 15
AICc = 100.859
Sy.x = 13.265
R^2 = 0.947
Sum of Squares = 2,639.347

Noncompetitive Inhibition Fit
(Main Article)
Goodness of Fit
Degrees of Freedom = 15
AICc = 90.721
Sy.x = 10.009
R^2 = 0.970
Sum of Squares = 1,502.695

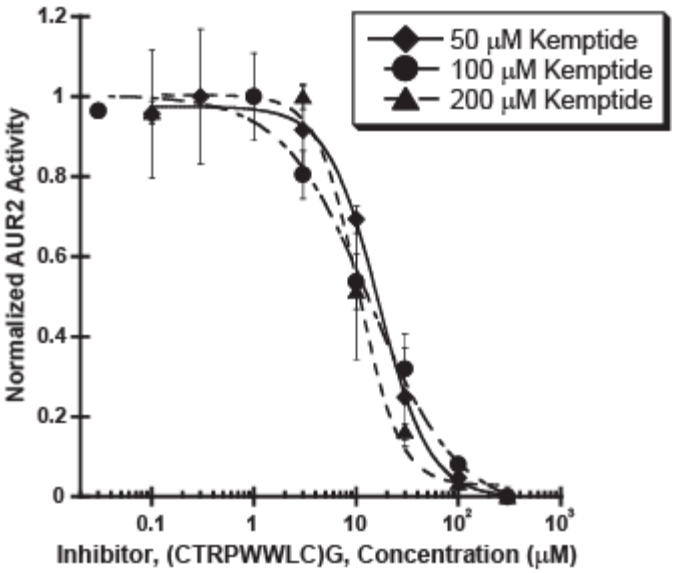


Figure S3. Aurora Kinase A Assay of Selected Peptide, (CTRPWWLC)G, at Three Concentrations of Kemptide. IC50 curves for the selected peptide does not change appreciably at 50, 100 and 200 μM 100 kemptide, which supports a noncompetitive mode of inhibition for the selected peptide, (CTRPWWLC)G.

S6. HPLC Traces of Synthesized Compounds.

The traces of synthesized selected peptides are shown in Figure S2. All peptides were purified by reverse phase HPLC using a linear gradient of acetonitrile and water with 0.1 % TFA. Specific purification details are in the subheading of each traces.

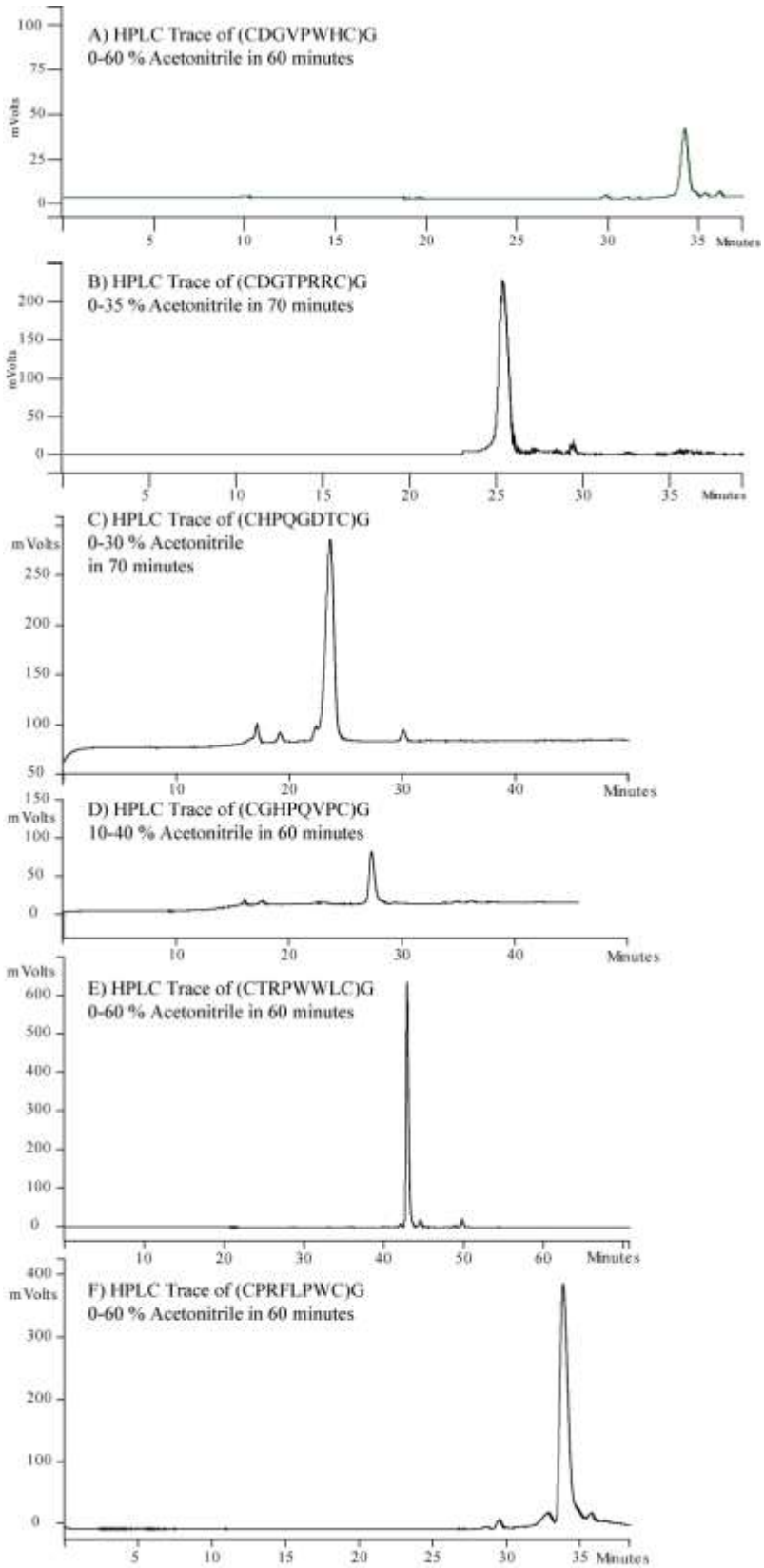


Figure S4. Selected Aurora A Peptides HPLC Traces.

S7. Mass Spectrometry Data of Synthesized Selected Peptides

Table S3 Mass Spectrometry (ESI) Data for All Peptides

Peptide	Expected m/z	Found m/z
(CDGVPWHC)G	970.0	970.2
(CDGTPRRC)G	961.0	961.5
(CHPQGDTC)G	913.9	914.3
(CGHPQVPC)G	894.0	894.3
(CTRPWWLC)G	1118.3	1118.5
(CPRFLPWC)G	1075.3	1075.4