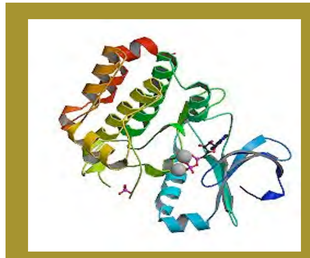

Identifying Effective Small Molecule Inhibitors of Aurora A Kinase

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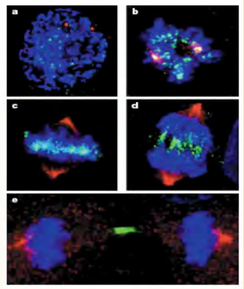
Introduction

Background on Aurora A Kinase

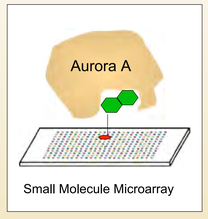
Aurora A kinase, a protein commonly found to be over expressed in human breast, ovarian, colon, prostate, neuroblastoma and cervical cancer cells, is believed to play an important role in cancer development. Although its exact mechanism is still unclear, it is known to signal cell centrosomes to separate into centromeres, an act that results in the stimulation of mitotic spindle assembly, chromosome segregation and cytokinesis. In this way, Aurora A kinase helps promote cell division.



Centrosome duplication, the formation of bipolar mitotic spindles, and chromosome alignment on the mitotic spindle are instigated by Aurora A activity

Previous Work

Compounds that bound to Aurora A were identified using the Broad's library of Diversity Oriented Synthesis (DOS) small molecules and small molecule arrays. After a process of selection, 15 of these "hits" were chosen for further testing.



Aurora A Kinase is run over a microarray of DOS molecules. A flourescent tag signals any interactions.

Our Specific Project's Goal

This project aims to identify effective small-molecule inhibitors of Aurora A that could someday be developed into pharmaceutical drugs for cancer patients, either alone or in combination with known drugs.

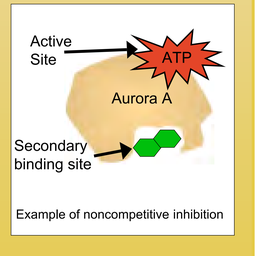
Experimental Design:

Measuring IC50 Values

"IC50" denotes the concentration of an inhibitor at which 50% of a protein, in this case Aurora A Kinase, is inhibited. It is a good indicator of the concentration at which each compound would be used in patients. This information is critical for identifying potential drugs, because large dosages often lead to harmful toxicity levels in patients. With this in mind, we looked for a compound with a relatively low IC50 Value.

ATP Competitive Assays

In some cases multiple molecules, including small molecule inhibitors and ATP, bind to a protein at the same protein active site. Such molecules are said to be "competitive" with one another: they cannot both bind to the same protein at once. Our concerns where that, in cells, ATP and the inhibitors might "fight" for the same active site in Aurora, preventing the inhibitors from binding to and inhibiting the kinase. Because in live cells the concentration of ATP may vary, any potential inhibitor must pass ATP competitive tests before further consideration as a drug.



Example of noncompetitive inhibition

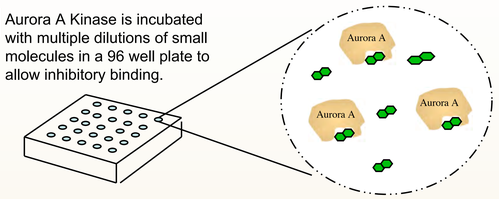
Synergy Experiments

Even if the potential inhibitors are not effective enough to be used as drugs by themselves, they will be tested to see if they have synergistic behavior with VX680, a pharmaceutical compound already in clinical trials. If they complement VX680's already potent inhibitory power, an effective cancer treatment could be developed.

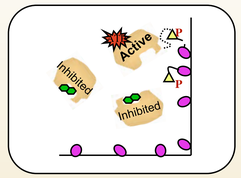
Materials and Methods

Determining IC50 Values

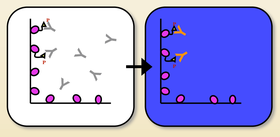
1 Aurora A Kinase is incubated with multiple dilutions of small molecules in a 96 well plate to allow inhibitory binding.



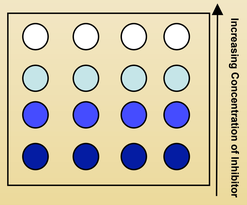
2 The kinase/small molecule solution is transferred to wells coated in a target of Aurora A Kinase. Any Aurora A that remains active phosphorylates this substrate using ATP.



3 The Aurora A is washed away. Antibodies that specifically recognize the phosphorylated target are added. Antibody binding is measured using a colorimetric assay.

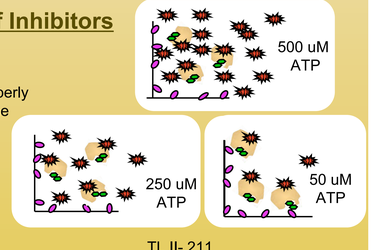


4 A spectrometer is used to read the different light intensities on the 96 well plate. Readings are graphed against concentration values of inhibitors yielding percentages of Aurora A activation and IC50's.



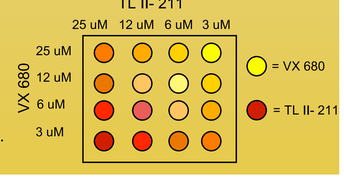
Testing for ATP Competitiveness of Inhibitors

To determine whether the inhibitors would function properly at high ATP levels, we ran the experiment at varying ATP concentrations.



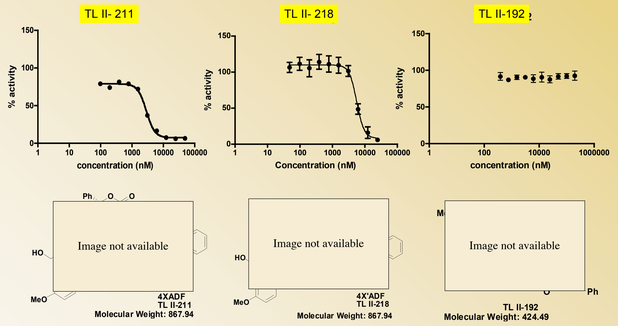
Identifying Points of Synergy

The most effective inhibitor,TL II-211, was run through the assay along with varying dilutions of VX 680. Their combined inhibition was compared to their individual IC50's in search of improvements.



Results

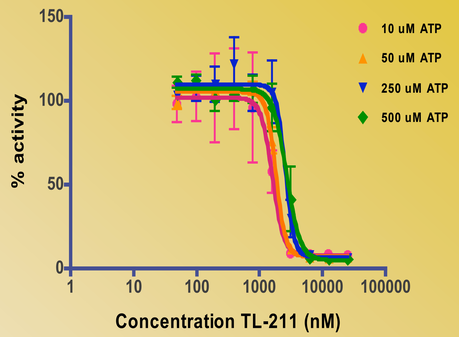
Inhibition curves of best and worst Aurora A inhibitors



Of the 15 compounds, TLII-211 and TLII-218 had the most potent IC50 values.

TLII-192 is a good example of a compound without an inhibition curve.

TL II-211 inhibitor effectiveness at varying ATP concentrations



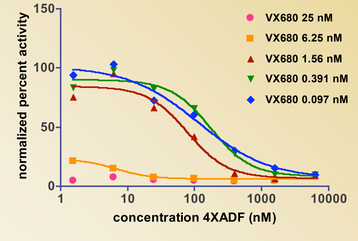
The relatively small changes in TLII-211's IC50 values at varying concentrations of ATP suggest that the inhibitor is not competitive with ATP.

Table of IC50 values from all compounds tested

Compoud	Starting Concentration (μM)	IC-50 (μM)
TL II-211	25	3.05 ± 0.84
TL II-218	25	5.10 ± 1.01
TL II-243B	100	11.04 ± 0.65
TL II-243A	100	15.21 ± 2.03
TL II-215	100	15.69 ± 3.69
TL II-245	100	18.52 ± 3.63
TL II-214	100	22.12 ± 3.85
TL II-224	200	45.00 ± 16.14
TL II-222	200	112.92 ± 70.21
TL II-191	200	Does not inhibit
TL II-192	200	Does not inhibit
TL II-244	25	Does not inhibit
TL II-206	200	Very Wide
TL II-223	100	Very Wide
VX680	0.025	0.00036 ± 0.0001

A total of ten IC50's were determined. The remaining five compounds demonstrated a lack of inhibition even at high concentrations.

Results of TL II-211 synergy testing with clinical candidate VX680



These data suggest that the point of synergy may lay at some concentration value of VX680 between 6.25 and 1.56 nM.

Conclusions and Future Directions

We were able to identify novel Aurora A kinase inhibitors. One compound, TL II- 211, was found not to be competitive with ATP.

Future Experiments would include:

- Determining point of synergy with VX680
- Testing cell viability of cancer cells with mutant Aurora A in the presence of inhibitors
- Kinase profiling to determine specificity for Aurora A

Acknowledgments

I'd like to thank:

- My mentors Katie Doud and Angela Koehler for having me this summer and patiently teaching me the ways of the lab.
- Megan Rokop, Allison Martino, and Kate MacSwain for making my internship possible
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