In Vitro Determination of Potency of Small Molecule Inhibitors of Arp2/3 Complex

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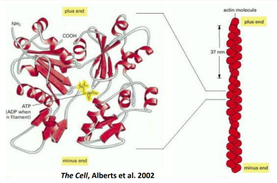
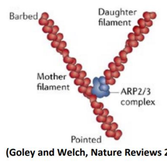
1. Introduction and Motivation

Actin is a key protein building block of actin microfilaments, which are constructed and deconstructed in response to cellular signaling pathways to regulate cellular processes such as motility, division, and endocytosis.[1] Arp2/3 Complex is a 7-subunit protein complex that is in involved in cellular construction of branched actin networks, functioning by attaching to the side of a pre-existing actin filament and nucleating a daughter branch.[2]

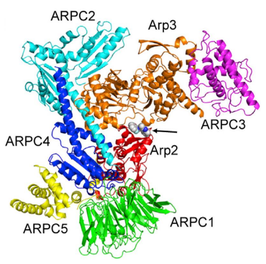


The Cell, Alberts et al. 2002



(Goley and Welch, Nature Reviews 2006)

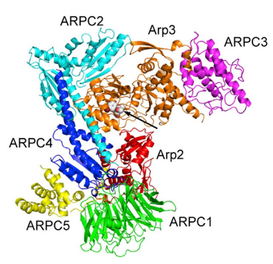
A



Binding Site of CK-666

A bulk actin polymerization assay is used as the key method to determine the potency of inhibitor candidates. Results of structure-activity relationships will be used to evaluate how actin inhibition may play a role in anticancer applications[3] and in general actin research.

B

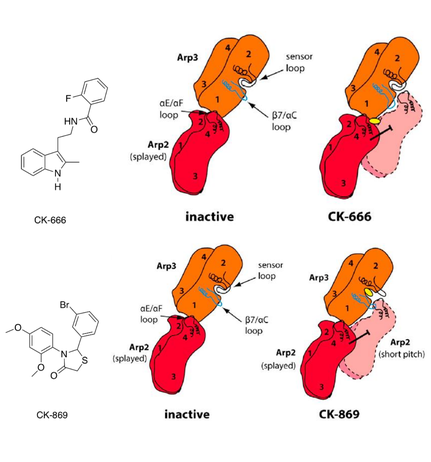


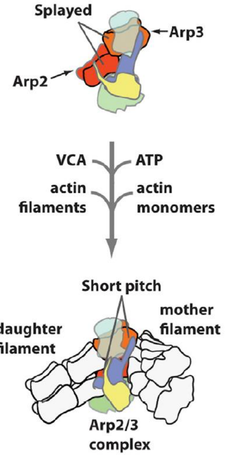
Binding Site of CK-869

Section 1 References: [1] Pollard, T.; Blanchoin, L.; Mullins, R. *Annu. Rev. Biophys. Biomol. Struct.* **2000**, *29*, 545-576.
[2] Goley, E. D.; Welch, M. D. *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 713-726.
[3] Yamaguchi H.; Lorenz, M.; Condeelis, J. *et al. J. Cell Biol.* **2005**, *168*, 441-452.
Images A and B: Hetrick, B.; Han, M. S.; Helgeson, L. A.; Nolen, B. J. *Chem. Biol.* **2013**, *20*, 701.

2. Mode of Inhibition

Arp2/3 inhibitor scaffolds were identified using high-throughput screening.[4] The CK-666 inhibitor scaffold stabilizes the inactive conformation of the Arp2/3 complex while the CK-869 inhibitor scaffold destabilizes the active conformation.[5]

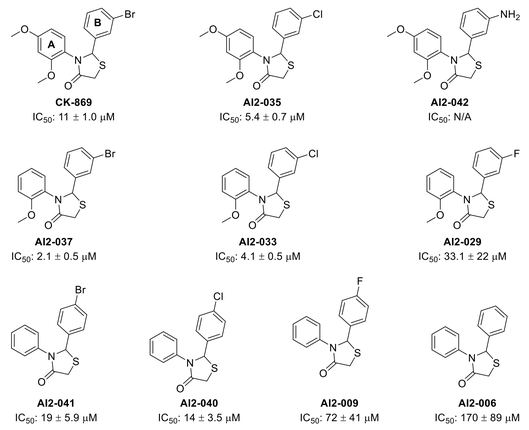




Section 2 References: [4] Nolen, B. J.; Tomasevic, N.; Russell, A.; Pierce, D. W.; Jia, Z.; Mccormick, C. D.; Hartman, J.; Sakowicz, R.; Pollard, T. D. *Nature* **2009**, *460*, 1031–1034.
[5] Hetrick, B.; Han, M. S., Helgeson, L. A.; Nolen, B. J. *Chem. Biol.* **2013**, *20*, 701; Hetrick, B.

3. CK-869 Inhibitor Scaffold and IC_{50} Results

Currently known inhibitors CK-666 and CK-869 must be used in undesirably high concentrations to achieve complete suppression of Arp2/3 complex *in vivo*.[6] The key goals of this project are to intelligently design, synthesize, and test the potency of a library of derivatives of each inhibitor class. Computational docking between proposed inhibitors and a crystal structure of Arp2/3 complex guided synthesis efforts that produced the following derivatives of CK-869 and CK-666, which were then studied using an *in vitro* actin polymerization assay to determine their potency.



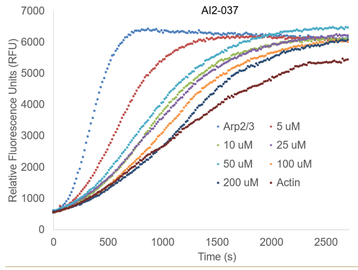
-These molecules are **4-thiazolidinones**

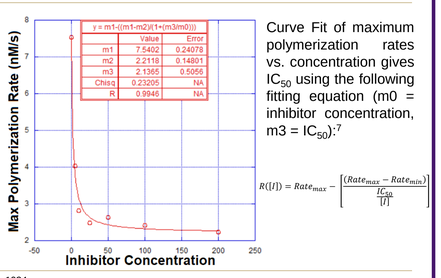
-The most favorable modification to the **A** ring was found to be removal of **one** methoxy substituent

-The *meta* rather than *para* substituent on the **B** ring was favorable

-Bromine was generally the best substituent on the **B** ring

-**AI2-037** showed a ~5 fold increase in inhibitory potency



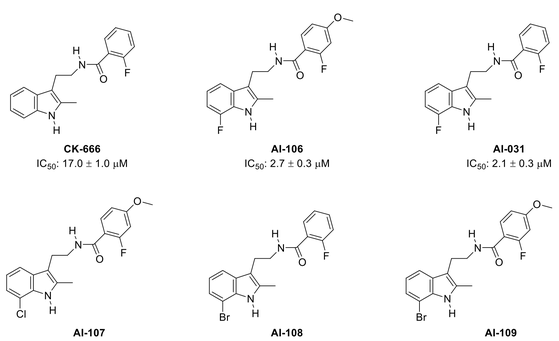


Curve Fit of maximum polymerization rates vs. concentration gives IC_{50} using the following fitting equation (m0 = inhibitor concentration, m3 = IC_{50}):[7]

$$R([I]) = Rate_{max} - \left[\frac{(Rate_{max} - Rate_{min})}{\frac{IC_{50}}{[I]}}\right]$$

Section 3 References: [6] Nolen, B. J.; Pollard, T. D. *et al. Nature* **2009**, *460*, 1031–1034.
[7] Baggett, A. W.; Cournia, Z.; Liu, S.-Y.; Nolen, B. J. *et al. ChemMedChem* **2012**, *7*, 1286-1294.

4. CK-666 Inhibitor Scaffold and IC_{50} Results

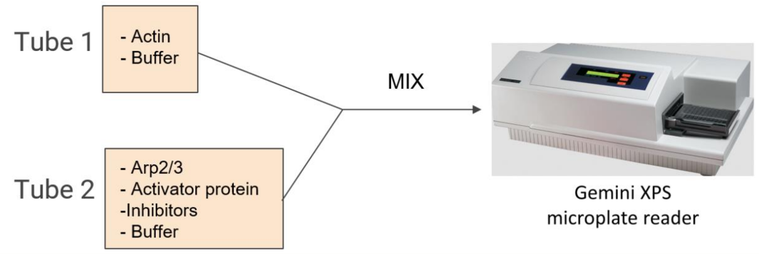


-These molecules are **acyl tryptamines**

-Modification at the **7 position** of the indole ring is generally favorable

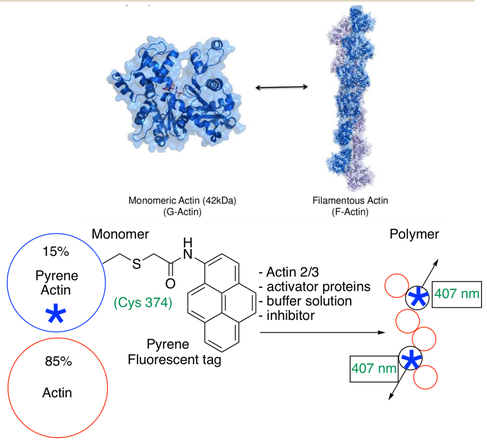
-Modification of the **para position** of the fluorobenzene ring with a methoxy group slightly negatively affects potency

5. Bulk Polymerization Assay



8 rxns run in parallel

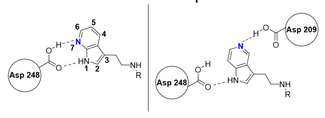
Control	1	Arp2/3, no inhibitor
Sample	2	5 uM
Sample	3	10 uM
Sample	4	25 uM
Sample	5	50 uM
Sample	6	100 uM
Sample	7	200 uM
Control	8	No arp2/3 or inhibitor



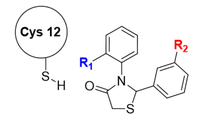
Section 5 References: Kouyama, T.; Mihashi, K. *Eur. J. Biochem.* **1981**, *114*, 33-38.

6. Future Directions

There are Aspartic acid residues near the site where the CK-666 scaffold binds. We plan to alter the indole ring by adding a nitrogen at position 4 or 7 to increase the number of hydrogen bonds between the inhibitor and Arp2/3



There is a Cysteine with a sulfur group near the site where the CK-869 scaffold binds. We plan to alter the R_1 group to increase the binding strength between the inhibitor and Arp2/3.



7. Acknowledgements and Funding

- Thanks to the Linfield College Student-Faculty Collaborative Research Grant
- Thanks to Conner Balzer in the Nolen Lab at the University of Oregon and other Nolen Lab members for assisting with assays
- Synthesis: Members of the Levent Cavas group at DEU