Synthesis of Small Molecule Derivatives of CK-666 as Potential Inhibitors of the Arp2/3 Complex

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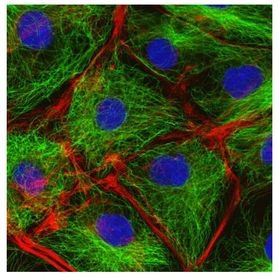
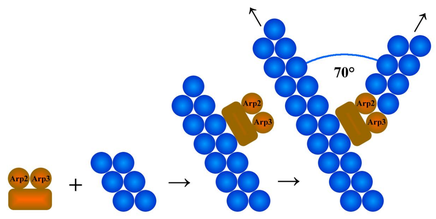
1. Introduction and Motivation

Actin related protein (Arp2/3) complex plays important roles in movement, endocytosis, and cell division. Constructing and deconstructing of actin mediates cellular motility.[1] The Arp2/3 protein contributes to movement by creating branches. Arp2/3 can get disturbed by viral and bacterial pathogens, and metastasis of cancer cells is linked to Arp 2/3 activity.[2] As a result, potent inhibitors that can block or prevent Arp2/3 to nucleate daughter strands of actin will be helpful as a basic research tool. Also they potentially can be used against cancers or diseases that use Arp 2/3 to survive.



Cytoskeleton: green-microtubules red-actin, blue-nucleus

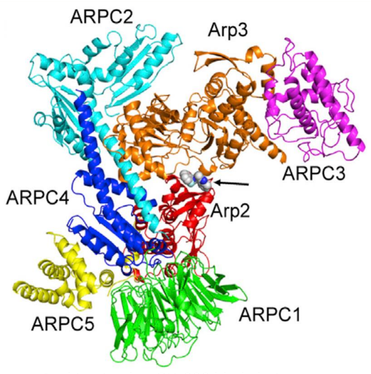


Arp2/3 attaches to the side of a preexisting filament of actin and templates formation of a daughter branch that grows out at a precise 70° angle

Section 1 References:
[1] Pollard, T.; Blanchoin, L.; Mullins, R. *Annu. Rev. Biophys. Biomol. Struct.* **2000**, *29*, 545-576.
[2] Zhang, C.; Hai, L. *et al. Oncotarget* **2017**, *8*, 33353-33364.
[3] (Image 1) British Society for Cell Biology https://bscb.org/learning-resources/softcell-e-learning/cytoskeleton-the-movers-and-shapers-in-the-cell.
[4] (Image 2) https://en.wikipedia.org/wiki/Arp2/3_complex#/media/File:Arp23_side_branching_model.png

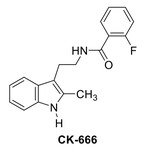
2. Small Molecule Inhibitor CK-666

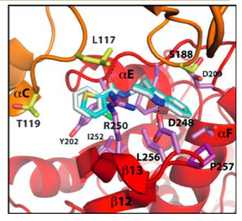
The known small molecule inhibitor (CK-666) has been identified through high throughput screening,[5] and characterized by X-ray crystallography.[6] It is highly desirable to develop more potent derivatives of this inhibitor class. Ideally our goal is to increase the potency towards Arp2/3 complex by three orders of magnitude.

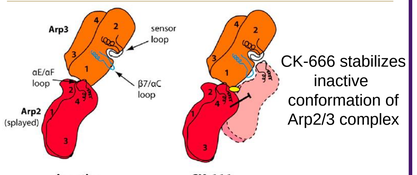


Acyl tryptamine CK-666 binds between subunits 2 and 3 of Arp2/3 complex

Zoomed-in view of binding pocket



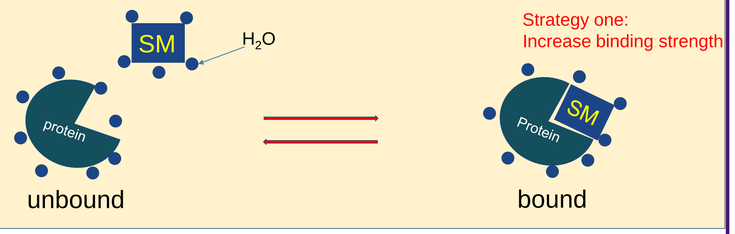


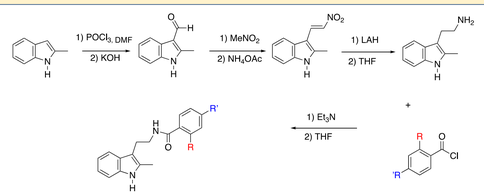


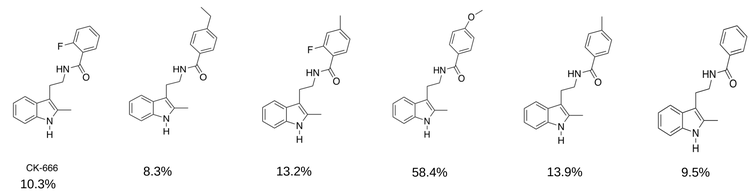
CK-666 stabilizes inactive conformation of Arp2/3 complex

Section 2 References:
[5] Nolen, B.; Tomasevic, N.; Russell, A.; Pierce, D.; Jia, Z.; McCormick, C.; Hartman, J.; Sakowicz, R.; Pollard, T. *Nature* **2009**, *460*, 1031-1034.
[6] Baggett, A.; Cournia, Z.; Han, M.; Patargias, G.; Glass, A.; Liu, S.; Nolen, B. *ChemMedChem* **2012**, *7*, 1286-1294.
[7] (Images) Hetrick, B.; Han, M. S.; Helgeson, L. A.; Nolen, B. J. *Chem. Biol.* **2013**, *20*, 701.

3. Acyl Tryptamine Strategy and Synthesis

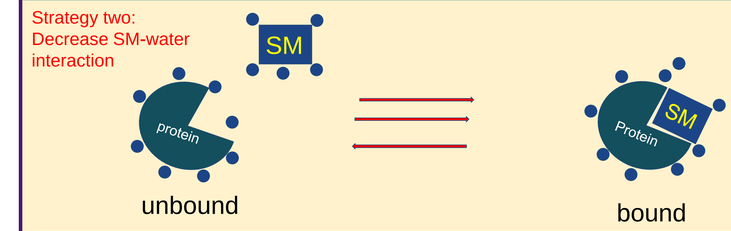


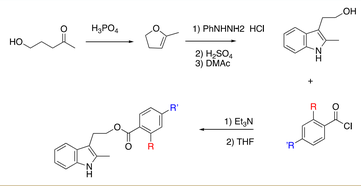


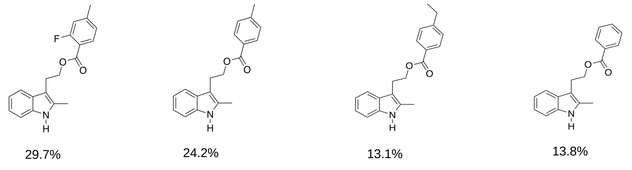


Section 3 References:
[8] Harada, H.; Hirokawa, Y.; Suzuki, K.; Hiyama, Y.; Oue, M.; Kawashima, H.; Yoshida, N.; Furutani, Y.; Kato, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1301-1305.

4. Ester-Linked Inhibitor Strategy and Synthesis

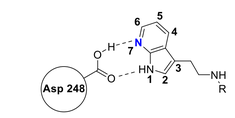




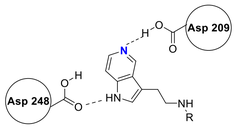


Section 4 References:
[9] Humphrey, G.R.; Kuethe, J. T. *Chem. Rev*. **2006**, 106, 2875-2911.

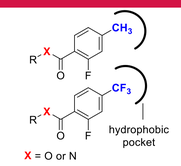
5. New Synthesis Targets and Methods



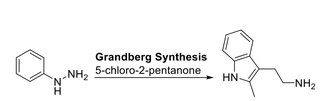
7-Azaindole series: strengthen hydrogen bonding between molecule and Asp 248



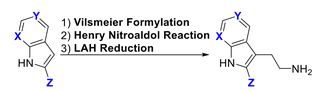
5-Azaindole series: create hydrogen bonding between molecule and Asp 209



Add hydrophobic groups to fluorobenzene ring



The Grandberg Synthesis[10] can produce 2-methyltryptamine in one step and will allow us to avoid the low-yielding reduction

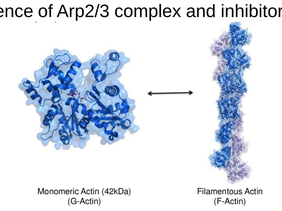


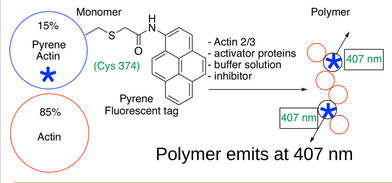
The 5-azaindole and 7-azaindole materials can be accessed through our initial 3-step route[11]

Section 5 References:
[10] Slade, J.; Parker, D.; Girgis, M.; Wu, R.; Joseph, S.; Repic, O. *Org. Process Res. Dev.* **2007**, *11*, 721-725.
[11] Popowycz, F.; Routier, S.; Joseph, B.; Merour, J.-Y. *Tetrahedron* **2006**, *63*, 1031-1064.

6. Next Step: *In Vitro* Assays to Determine Potency

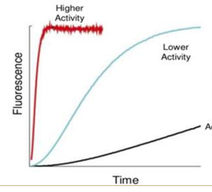
We study the potency of our inhibitor candidates by measuring the rate of polymerization of actin in the presence of Arp2/3 complex and inhibitors[12]





Polymer emits at 407 nm

Gemini XPS Microplate Reader



Red: positive control
Black: negative control
Blue: with inhibitor

Section 6 References
[12] Kouyama, T.; Mihashi, K. *Eur. J. Biochem.* **1981**, *114*, 33-38.

7. Acknowledgements and Funding

- Protein purification, hosting for biochemical assays: Conner Balzer and other members of the Nolen Lab
- Computational Docking of Inhibitor Candidates: Dr. Zoe Cournia, Bioacademy of Athens
- Linfield College Student-Faculty Collaborative Research Grant