Progress Towards Synthesis of Azaindole Derivatives of Arp2/3 Complex Inhibitor CK-666

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1. Introduction and Motivation

Actin is a globular multi-functional protein. It forms actin filaments in the cytoskeleton. Actin-related protein 2/3 complex (Arp 2/3) is a structural protein that is an actin nucleator and creates branching (Figures 1 & 2).[1] Arp 2/3 has 7 subunits. Actin microfilament networks involving Arp 2/3 have been linked to instances of cancer metastasis[2,3] in which Arp 2/3 mediates tumor cell migration.[4] There are two structural classes of inhibitors that have been discovered previously by the Pollard Lab at Yale via high throughput screening, CK-666 and CK-869.[5] We are working towards the isolation of small molecules that we hypothesize to have increased potency of inhibition of Arp 2/3 to provide a tool to further study basic actin polymerization mechanisms and to study against cancer cell lines.

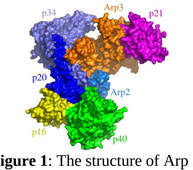


Figure 1: The structure of Arp 2/3

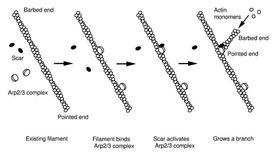


Figure 2: Method of Arp 2/3 complex function

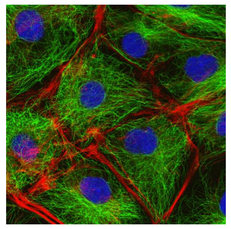


Figure 3: Cell Membrane

In 2005, the overexpression of Arp 2/3 in MTLn3 rat adenocarcinoma cells was discovered.[2] In 2013-2017 the inhibition of Arp 2/3 in mouse models was found to reduce the proliferation of these cancer cells.[3-4] One goal of our research is to find the link between Arp 2/3 and anticancer activity. We seek to do so via the synthesis of derivatives of CK-666 and CK-869, two classes of Arp 2/3 inhibitors.[5] The binding site of CK-666 is located between subunits 2 and 3 (Figure 3), and the binding site of CK-869 is located on subunit 3 (Figure 4).[5-6]

1. Pollard, T.; Blanchoin, L.; Mullins, R. *Annu. Rev. Biophys. Biomol. Struct.* **2000**, *29*, 545-576.*Mol. Biol.* **2006**, *7*, 713-726
2. Yamaguchi, H.; et al. *Cell Biol.* **2005**, *168*, 441-452
3. Liu, Z.; et. al. *Oncology Reports.* **2013**, *30*, 2127-2136
4. Zhang, Q; et al. *Oncotarget.* **2017**, *8*, 33353-33364
5. Nolen, B. J.; et al. *Nature* **2009**, *460* (7258), 1031–1034
6. Hetrick B.; et al. *Chem. Biol.* **2013**, *20*, 701.

2. Small Molecule Inhibitor CK-666 and Rationale

We are researching 2-methyl-7-azaindole as an analog of CK-666, which binds to and inhibits Arp2/3 Complex as shown in Figures 5 and 6. We sought synthesis methods that should provide good yield and purity of the small molecules.

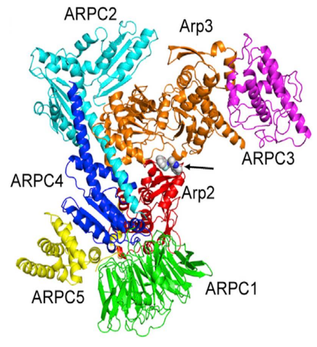


Figure 4: Binding Site of CK-666[5]

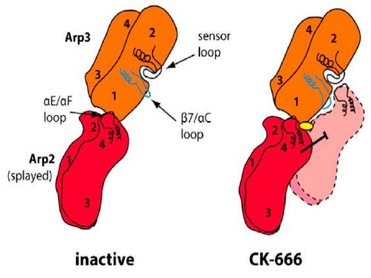


Figure 5: Proposed Inhibition Mechanism of CK-666[6]

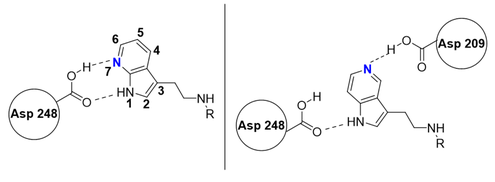


Figure 6: Potential Hydrogen Bond Formation

5. Nolen, B. J.; et al. *Nature* **2009**, *460* (7258), 1031–1034
6. Hetrick B.; et al. *Chem. Biol.* **2013**, *20*, 701.

3. Synthesis

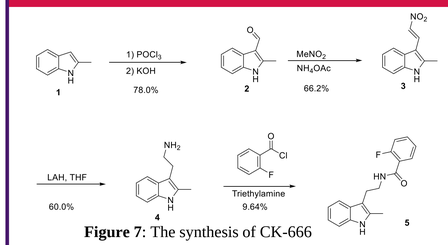


Figure 7: The synthesis of CK-666

We did the synthesis of CK-666 before we tried with 2-methyl-7-azaindole. First, the Vilsmeier reaction of **1** yielded **2** in 78% yield. Then, Nitroaldol reaction of **2** yielded **3** in 66.2% yield. After that, LAH reduction of **3** followed by acid chloride reaction of **4** yielded **5** in 9.64% combined yield. Each reaction was successful and had a good percent yield except the final step.

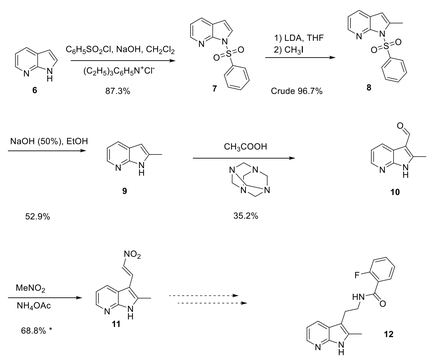


Figure 8: The synthesis completed over summer with initial yields for completed steps

The synthesis towards **12** that we did so far includes 1) Protecting/Directing group installation, 2) Methylation, 3) Hydrolysis to recover free N-H, 4) Duff reaction, and 5) Nitroaldol/Henry reaction. Based on the synthesis of CK-666, the Henry reaction should be successful and give us a good percent yield, but it did not work well with **10**.

7. Srivastava, T.; et al. *Tetrahedron* **2002**, *58* (38), 7619–7624.
8. Jing Wu,; et al. *European Journal of Medicinal Chemistry* **2014**, *80*, 340-351
9. Desarbre, E.; et al. *Tetrahedron* **1997**, *53*(10), 3638–3643.
10. Verbiscar, A. J. *Journal of Medicinal Chemistry* **1972**, *15*(2), 149–152.
11. Tung, Y.S.; et al. *Journal of Medicinal Chemistry* **2011**, *54* (8), S14-S14.

4. Analysis NMR and TLC

The coupling constant of the peaks corresponding to $\mathbf{H_g}$ and $\mathbf{H_f}$ is 17 Hz which supports that those two doublets represent the trans-hydrogens on the alkene. The observed orange/yellow color of the compound is consistent with extended π-conjugation predicted in the structure of **11**. Based on NMR and TLC analyses, we do believe we made the desired compound **11** and a currently unidentified compound **U**. The mixture of compound **11** and unidentified compound **U** also has a trace of starting material **10** (<1%).

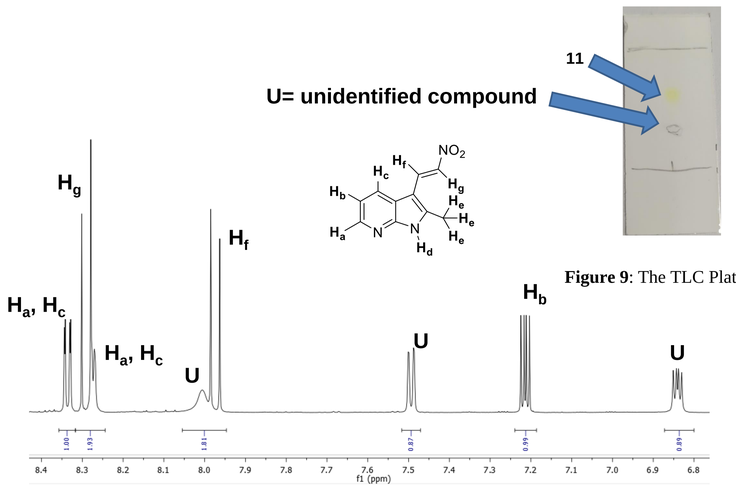
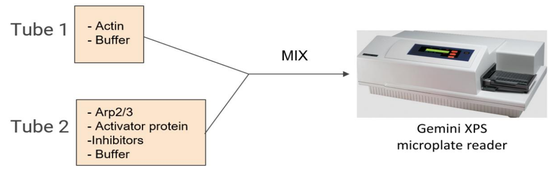


Figure 9: The TLC Plate of **11**

Figure 10: The NMR of Desired Compound **11** & Unknown Compound **U**

5. Planned Bulk Polymerization Assay



We will run a bulk polymerization assay after we get compound **12**.

Figure 11: Gemini XPS microplate reader used for biochemical assays and outline of assay components

We will collect the data from bulk polymerization assay to get the graph of inhibitor potency like Figure 12.

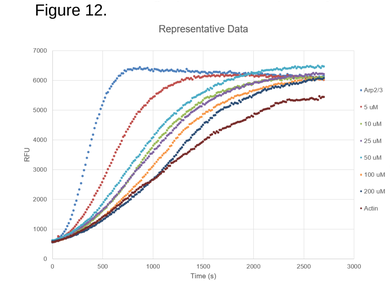


Figure 12: Inhibitor Potency (-> IC_{50})

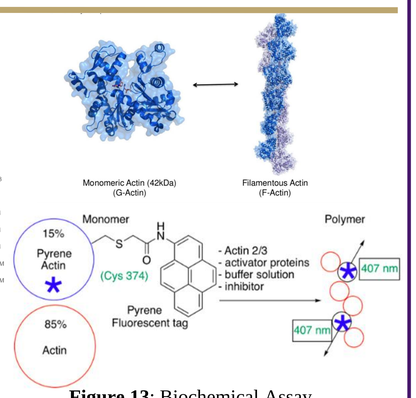


Figure 13: Biochemical Assay

6. Conclusion and Future Directions

Based on the TLC analysis and NMR the next step will be using column chromatography to purify the mixture of compound **11** and **U**. We will determine the identity of **U** by using High Res. Mass Spectrometry in addition to NMR and IR. We will run next two reactions (LAH reduction and acid chloride reaction) to form the final product **12**. We will obtain full characterization (IR, ^{1}H NMR, ^{13}C NMR, HRMS) for all new compounds. Then we will run the bulk polymerization assay to determine the potency of **12**. to be able to compare our product with CK-666 to see which one inhibits Arp 2/3 most strongly. Finally, this potency data will be used to refine our collaborator's computational docking model of derivatives of CK-666 with Arp2/3 and help us target even more potent inhibitors.

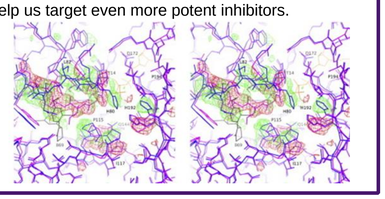


Figure 14: Docking model of CK-666

7. Funding and Acknowledgements

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