

## Design and synthesis of an A2-folate conjugate with a reductively labile disulfide bond

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### Goal/Purpose

Through the completion of this project, I hope to synthesize a potential chemotherapeutic drug that selectively enters cancerous cells and is activated by light. Furthermore, I will develop a link between the drug and folic acid that will be easily broken after entering a cell.

### Importance of Project

The targeted and triggered drug delivery system we are researching involves linking a folate group to a pyridinium bis-retinoid compound, which is synthesized from all-trans retinal and an amine. These retinoid compounds form cytotoxic photo-oxidation products upon exposure to blue light, but are not cytotoxic in their unoxidized form. The folate group was chosen as a linking group due to the increased expression of folate receptors on many types of cancer cells. Attaching folate to the retinoid compound will lead to a higher cellular concentration of the drug and a subsequently higher concentration of cytotoxic photo-oxidation products.

Folate-conjugates enter folate-receptor positive cells via receptor-mediated endocytosis<sup>1</sup>, whereupon the endocytosed vesicle joins with the endosome. Dissociation of the folate and conjugate moieties may occur because of increased acidity or reduction of a disulfide bond, which then allows the drug to act on the cell.

Many of these A2 compounds have been shown to conjugate to folic acid, albeit through the formation of an amide bond. However, amide bonds are not particularly labile, rendering them ineffective as potential drugs. This project will allow for the investigation of synthesizing an A2 compound that is conjugated to folic acid through a reductively cleavable disulfide bond.

By performing the research described previously, we hope to show that the folate-bound pyridinium bis-retinoid products selectively kill breast cancer cells while causing minimal effects on noncancerous cells.

### Main Proposal Body

The proposed A2 compound will be synthesized from all-trans retinal and cysteamine (Figure 1) in a biomimetic fashion, and subsequently purified using silica gel chromatography. The product will be characterized using MS+, HPLC and <sup>1</sup>H-NMR.

A2-cysteamine-folate will be synthesized according to the route used by Leamon, et. al., in the synthesis of EC145.<sup>2</sup> Standard fluorenylmethyloxycarbonyl-based solid phase peptide synthesis (Fmoc SPPS) on a Wang-resin polymeric support will be used to construct a folic acid moiety with 1-3 amino acid residues attached to the gamma-carboxylic acid of folic acid. The thiol groups of A2-cysteamine and cysteine will then be oxidized to yield a cleavable disulfide linker. The A2-cysteamine-folate compound will be purified using column chromatography, centripetal chromatography, or semi-preparative HPLC to determine which method results in the highest product yield.

While I develop a purification method for the proposed folate-conjugate, I will continue to test the cytotoxicity of various A2 compounds, namely A2-Histamine and A2-Methionine, in MDA-MB-435 cells. In addition, if I am able to purify the folate compound reasonably quickly, I will also test its effectiveness. The assays will be run according to the MTS/PMS cell proliferation assay protocol.<sup>3</sup> Cells are seeded in

24-well plates, with target concentrations of approximately 10000-20000 cells/well. Three plates are seeded for each assay. Two plates are selected for irradiation and contain different drug concentrations. The third plate serves as an irradiation control, containing the same drug concentrations as the other two plates while not being exposed to light. Enough wells are seeded per plate so that three groups of wells exist per plate: 1) cells incubated with drug, 2) cells incubated with DMSO at the same concentration as the drug and 3) cells incubated with media only to serve as a positive control. After incubating for 6 hours, the A2-compound to be tested is added to select wells. The range of drug concentrations to be tested is between 10  $\mu$ M and 1  $\mu$ M. The cells are incubated for an additional 24 hours, at which time selected wells are irradiated using a Mille Luce™ Fiber Optic Illuminator M1000 (Edmund Optics) light source and a dichroic blue filter (Edmund Optics) that allows the passage of 400-500 nm light. Irradiation times will vary between 5 and 15 minutes to optimize drug activation while minimizing death due to heat from the light source. Following irradiation, the MTT solution is added to allow for the formation of the formazan product. Quantitative results are obtained by measuring the absorbance of the wells at 490 nm on an ELISA microplate reader.

### Anticipated Academic Outcome

I anticipate using the results as a portion of my Honors thesis and also hope to present my preliminary findings at the College of Physical and Mathematical Science's Spring Research Conference. I also plan on going to medical school, and believe that this research will provide a solid background for future research projects in Dr. Vollmer-Snarr's lab and any medical research in which I may participate. Finally, some of our current findings are being prepared for publication, and the findings of this project could be included in these publications.

### Qualifications

Dr. Vollmer-Snarr is a member of Brigham Young University's Cancer Research Center. She has completed Ph.D. and post-doctoral training in organic synthesis and cancer research. She has also previously mentored several undergraduate students and graduate students involved in cancer research, most recently serving as my mentor for a Cancer Research Fellowship this past summer. In addition, I have worked in Dr. Vollmer-Snarr's lab for two years. During this time, I have worked on both the synthesis and biological evaluation of similar compounds to A2-cysteamine, having recently purified another A2 compound. I have also taken classes such as organic chemistry (lecture and lab), molecular biology, biochemistry (lecture and lab) and cell biology.

### Project Timetable

The first part of the project to be completed is the purification of the A2-cysteamine compound and its biological evaluation. After purification, the compound will be allowed to react with folic acid. The folic acid conjugate must then be purified prior to its testing on cancer cells.

### Fit With BYU's Mission [optional]

This project fits the aims of a BYU education because of its relation to cancer research. This research is intellectually stimulating because it forces the researcher to confront problems and constantly modify an approach to obtain results. Patience and perseverance are necessary in research and are developed with each project, thus building the character of the researcher. Finally, as one investigates the complexities of biology and chemistry, he or she appreciates life, its frailty, and the underlying processes of both creation and death. These aspects have already led me to be committed to a life of learning and service.

### Scholarly Sources

1. Rothberg, K. G.; Ying, Y.; Kolhouse, J. F.; Kamen, B. A.; Anderson, R. G. The glycopospholipid-linked folate receptor internalizes folate without entering the clathrin-coated pit endocytic pathway. *J. Cell Biol.* **1990**, 110, 637-649.

2. Vlahov, I.R.; Santhapuram, K. R.; Kleindl, P. J., Howard, S. J., Stanford, K. M.; Leamon, C. P. Design and regioselective synthesis of a new generation of targeted chemotherapeutics. Part 1: EC145, a folic acid conjugate of desacetylvincristine monohydrazone. *Bioorg. & Med. Chem. Letters* **2006**, 16, 5093-5096.

3. Promega Inc. Technical Bulletin - CellTiter 96® Non-Radioactive Cell Proliferation Assay. **2004**.