



COLLEGE OF PHARMACY  
**MEDICINAL CHEMISTRY**  
UNIVERSITY OF MICHIGAN

The Department of Medicinal Chemistry is pleased to announce the

**Ph.D. Dissertation Defense Seminar of**



**Christine Cuthbertson**

**Medicinal Chemistry, Ph.D. Candidate  
(Mentor: Dr. Nouri Neamati)**

*“Inhibition of Nucleotide and One-Carbon Metabolism  
for the Treatment of Cancer”*

**Friday December 4, 2020 at 3:00 p.m.**

**Zoom Meeting:**

**<https://umich-health.zoom.us/j/96695481212>**

**Meeting ID: 966 9548 1212**



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**Public Oral Examination**  
**For the Degree of Doctor of Philosophy**

**Christine Cuthbertson**

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**Committee Members**

Dr. Nouri Neamati (Chair)

Dr. Amanda L. Garner

Dr. Jolanta Grembecka

Dr. Shaomeng Wang

# Abstract

Metabolic reprogramming in cancer was first described in the early to mid-1900s and later labeled as a hallmark. Rapidly proliferating cells require sufficient concentrations of nucleotides and other biomass precursor molecules to sustain growth. Nucleotide biosynthesis and one-carbon metabolism (ICM) inhibitors were among the first targeted cancer chemotherapies and remain as some of the most successful (e.g. 5-fluorouracil and methotrexate). Despite mounting evidence, the mitochondrial enzymes dihydroorotate dehydrogenase (DHODH), serine hydroxymethyltransferase (SHMT2), and methylenetetrahydrofolate dehydrogenase (MTHFD2) have yet to be successfully clinically targeted for the treatment of cancer.

DHODH is a vital enzyme in the *de novo* pyrimidine biosynthesis pathway. However, the DHODH inhibitor brequinar (BREQ) failed all cancer clinical trials in solid tumors. Therefore, we sought to address a potential avenue to improve the efficacy of BREQ by employing a combination strategy to simultaneously inhibit nucleotide salvage. BREQ was synergistic with the equilibrative nucleoside transporter (ENT) inhibitor dipyridamole, but not the concentrative nucleoside transporter inhibitor phlorizin. This synergy carried over to ENT1/2 inhibition, but not ENT4. Our previously described brequinar analog **41** was also synergistic with dipyridamole as were the FDA-approved DHODH inhibitors leflunomide and teriflunomide but the latter required much higher concentrations than BREQ. Therefore, combination of BREQ with ENT inhibitors presents a potential anticancer strategy in select tumors.

SHMT2 and MTHFD2 participate in the folate metabolism arm of ICM and are emerging anticancer targets. Both are overexpressed in several cancer types and correlate with poor prognosis and other clinicopathological parameters. Significant progress towards the development of inhibitors against these enzymes has been made in a relatively short amount of time, but there is still much to be understood about their involvement in cancer progression. Thus, we performed extensive characterization of genetically engineered cell lines via transcriptomic profiling and bioinformatics. The data show changes in genes and gene sets related to hypoxia, MYC, and mTOR, all of which are well-established ICM-related pathways. Prior research connected SHMT2 and MTHFD2 to RNA metabolism, and we built upon that work by identifying links to alternative splicing and non-coding RNA processing. Glycosylation was also a strong theme for SHMT2. Additionally, we observed changes in expression of Ephrin-related genes supporting previous work that connected ICM to Ephrin signaling and differentiation. Moreover, we disclosed inhibitors that showed correlations to gene expression changes in response to modulation of SHMT2 and MTHFD2 expression which may pose as synthetically lethal agents.

Finally, we developed a pharmacophore model that led to the discovery of a novel class of MTHFD2 inhibitors. Virtual screening of our in-house database identified **CBN-1** which significantly stabilizes MTHFD2 to thermal denaturation and inhibits its enzymatic activity. Mechanistic studies revealed that **CBN-1** covalently binds MTHFD2 but does not react with general antioxidants. **CBN-1** analogs possess antiproliferative and antimigratory activity with **CBN-1** being the most potent in the series. **CBN-1** showed significant selectivity to cells cultured in galactose medium indicating impaired mitochondrial function and potential inhibition of OXPHOS. Structure-activity relationship studies with other analogs did not produce a superior compound to **CBN-1**, thus further optimization is required to improve the potency of our hit compound. Our hit **CBN-1** is the first covalent MTHFD2 inhibitor reported.

Collectively, this dissertation provides further evidence to pursue SHMT2 and MTHFD2 inhibitors for the treatment of cancer and a potential strategy to improve the efficacy of DHODH inhibitors in the clinic.

## Publications

**Cuthbertson, C.R.;** Guo, H.; Kyani, A.; Madak, J.T.; Arabzada, Z.; Neamati, N. The DHODH Inhibitor Brequinar is Synergistic with ENT1/2 Inhibitors. *ACS Pharmacol. Transl. Sci.* **2020**, *in press*

Madak, J.T.; Bankhead III, A.; **Cuthbertson, C.R.**; Showalter, H.D.; Neamati, N. Revisiting the Role of Dihydroorotate Dehydrogenase as a Therapeutic Target for Cancer. *Pharmacol. Ther.* **2019**, *195*, 111-131.

Madak, J.T.; **Cuthbertson, C.R.**; Miyata, Y.; Tamura, S.; Petrunak, E.M.; Stuckey, J.A.; Han, Y.; He, M.; Sun, D.; Showalter, H.D.; Neamati, N. Design, Synthesis, and Biological Evaluation of 4-Quinoline Carboxylic Acids as Inhibitors of Dihydroorotate Dehydrogenase. *J. Med. Chem.* **2018**, *61*, 5162-5186.

Madak, J.T.; **Cuthbertson, C.R.**; Chen, W.; Showalter, H.D.; Neamati, N. Design, Synthesis, and Characterization of Brequinar Conjugates as Probes to Study DHODH Inhibition. *Chem. Eur. J.* **2017**, *23* (56), 13875-13878.

## Manuscripts in Preparation

**Cuthbertson, C.R.;** Arabzada, Z.; Bankhead III, A.; Neamati, N. A Roadmap to Selectively Targeting SHMT2 and MTHFD2 in Cancer Cell Metabolism.

**Cuthbertson, C.R.;** Xu, S.; Bankhead III, A.; Samanta, S.; Tamura, S.; Kyani, A.; Arabzada, Z.; Ljungman, M.; Neamati, N. Transcriptomic Profiling of Genetically Engineered Cell Lines Augmenting One-Carbon Metabolism.

## Select Presentations

- 2020 *Pharmacophore-Based Discovery of a Novel MTHFD2 Inhibitor.* College of Pharmacy Research Forum, 2020, University of Michigan
- 2019 *Novel 4-Quinoline Carboxylic Acids as Inhibitors of Dihydroorotate Dehydrogenase.* College of Pharmacy Research Forum, 2019, University of Michigan
- 2018 *Design, Synthesis, and Characterization of Brequinar Conjugates as Probes to Study DHODH Inhibition.* Harnessing Immune Metabolism to Treat Cancer and Other Diseases Symposium, 2018, University of Michigan
- 2016 *Targeting Mitochondrial Serine Hydroxymethyltransferase (SHMT2) in Lung Cancer.* Cancer Metabolism Symposium, 2016, University of Michigan

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## Future Plans

Christine is seeking employment in student development and program management in the STEM field.