Progress Towards a Library of Potential Fungicides

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Abstract

Methionine Synthase (MetSyn) is an enzyme that uses folate and homocysteine to create the amino acid methionine, which is essential for all organisms. There are key differences between the fungal MetSyn enzyme and the mammalian (human) form, especially with regard to the proximity of the two binding sites for folate and homocysteine. Taking advantage of these differences, an antifungal drug could be developed to exclusively bind the fungal enzyme and inhibit fungal growth while leaving the host (patient) unaffected. We are currently exploring the synthesis of various molecules that mimic folate, an essential substrate for MetSyn function. We plan to screen these molecules against various fungal species as well as in an isolated system with the MetSyn enzyme itself.

Background

Fungal infections are a common public health concern especially in regards to immunocompromised patients who are at a greater risk of death in such cases.[1] As there is an increase of drug resistant fungi, there is a need for new types of anti-fungal drugs. Methionine Synthase (MetSyn), an enzyme which converts homocysteine (Hcy) to methionine using a substituted folate molecule (**Fig. 1**), could provide a potential pathway for safe antifungal treatment.

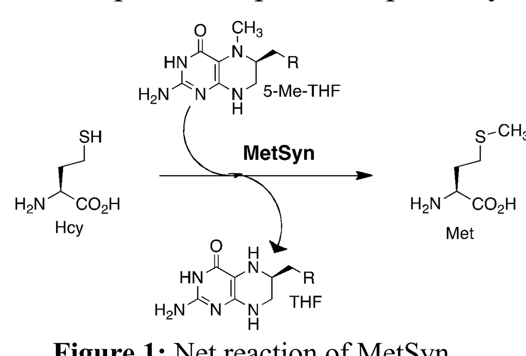

Figure 1: Net reaction of MetSyn

The structure of MetSyn used by fungi is different than that used by mammals.[2] In the fungal enzyme, folate and homocysteine bind to two pockets that are close together (**Fig. 2**). In humans, the folate and Hcy bind pockets are very far apart. This makes it possible to design synthetic compounds which could simultaneously bind the folate and homocysteine pockets in the fungal enzyme, while being unable to competitively bind the mammalian MetSyn form (**Fig. 3**).

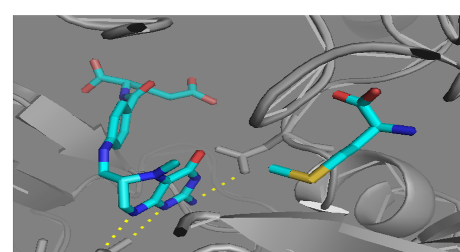

Figure 2: In fungal MetSyn, folate and Hcy bind in close proximity.

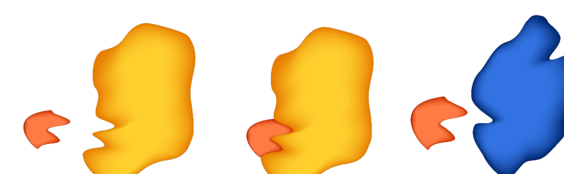

Figure 3: Cartoon model for a molecule binding fungal MetSyn, but it cannot fit inside human MetSyn.

Therefore, compounds designed to inhibit fungal MetSyn could serve as antifungal drugs which should not affect the biochemistry of the patient.

General Design of Molecules

A molecule specific for fungal MetSyn would need a folate mimic attached to a Hcy mimic by some short linker (**Fig. 4**). This should properly fit in the fungal enzyme, because the two binding pockets are so close together.

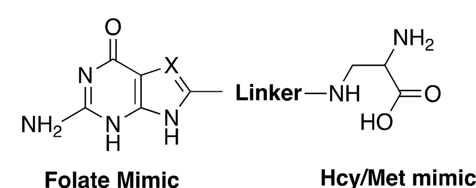

Figure 4: Generalized Inhibitor Molecule

Using the modeling program AutoDock, we can virtually screen potential molecules to confirm they can reach both binding sites (**Fig. 5**).

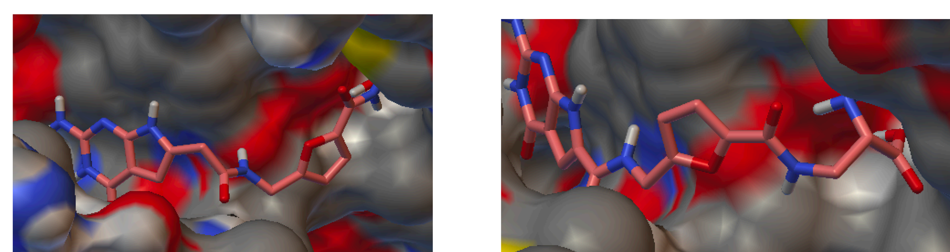

Figure 5: Computer Modeling of potential molecules binding within the enzyme binding pockets.

Representative Syntheses of Fungal Inhibitor Candidates

Synthesis of compounds which match our general design (**Fig. 4**) begins by attaching synthesizing a folate mimic and then adding a "linker" group (**Fig. 6**).[3]

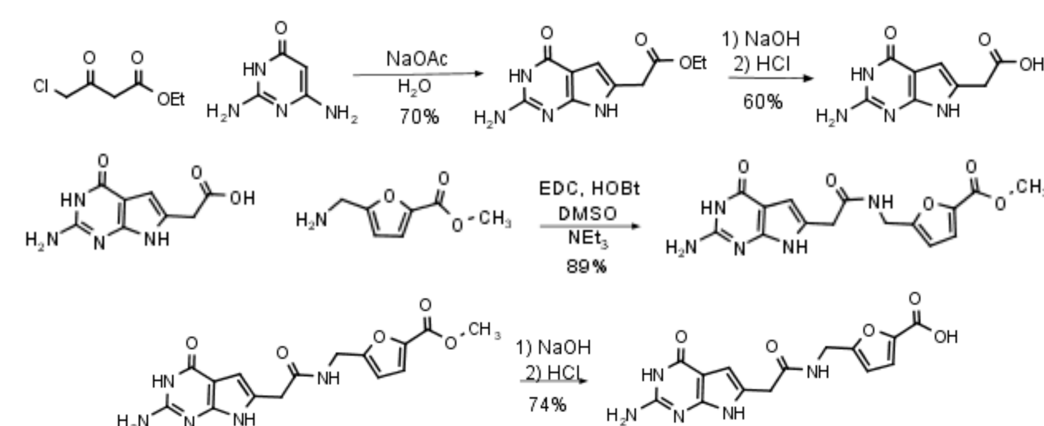

Figure 6: A deazaguanine was synthesized, which can mimic folate. This was then coupled to a furan linker

As an alternative to the synthesis above, 8-mercaptoguanine was made and alkylated with a version of the furan linker (**Fig. 7**). This has the benefit of being a shorter synthesis, while also introducing diversity in the structure.

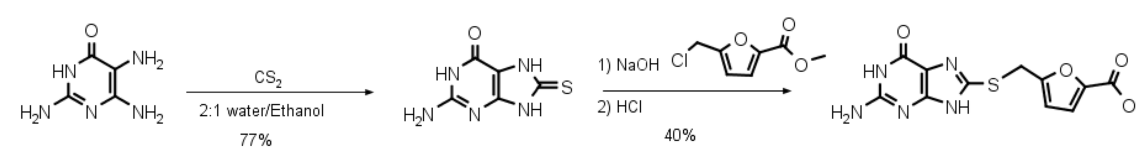

Figure 7: Alternative structure, which still contains a folate mimic and linker

These two molecules would then need to be attached to a Hcy mimic. The synthesis of this "amino acid tail" is done starting from asparagine (**Fig 8**).[4]

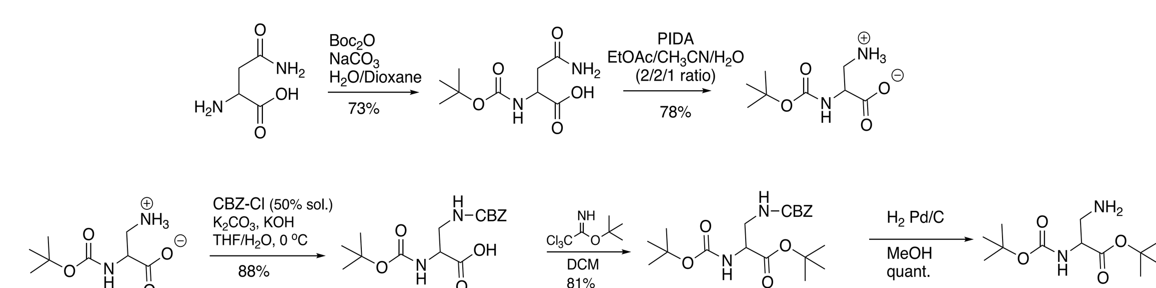

Figure 8: Synthesis of the protected amino acid tail to mimic Hcy

With the protected amino acid tail in hand, this was coupled to one of the folate mimics baring a linker (**Fig. 9**). The protecting groups ensured that only one possible amide bond could form in the coupling reaction.

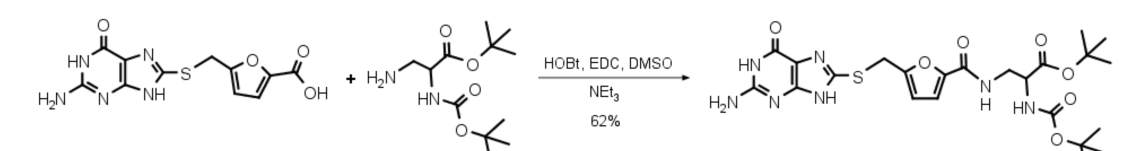

Figure 9: Amide bond coupling of protected amino acid tail to an extended folate mimic

To complete the synthesis of the designed inhibitor candidate, the protecting groups were removed under acidic conditions (**Fig. 10**).

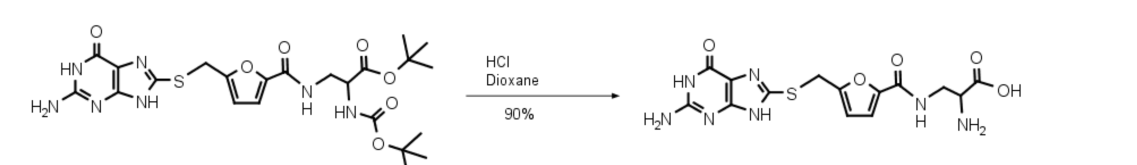

Figure 10: Deprotection step to provide the final product

Inhibitor Candidates

By using methods similar to those shown in **Figures 6-10**, a number of similar potential inhibitors have been synthesized (**Fig. 11**). This library of molecules provides a number of subtle structural differences. The hope is that these small changes will help identify a molecule that has the perfect "fit" in the enzyme.

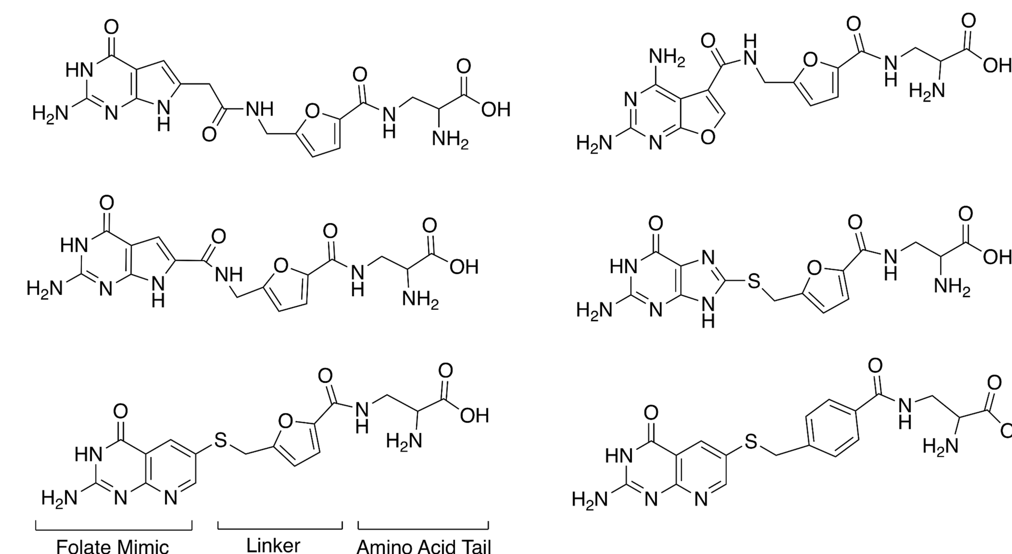

Figure 11: Current library of compounds to be tested

Alternative Folate-mimic Strategy

Another unique aspect about fungal MetSyn, compared to the mammalian enzyme, is that it requires a poly-glutamated folate substrate, while the mammalian enzyme can function with folate containing only one glutamate.[2] Therefore, we can also model our folate mimic to closely resemble 5-methyl-tetrahydrofolate-diGlutamate (**Fig. 12**), as this would more selectively target fungal MetSyn.

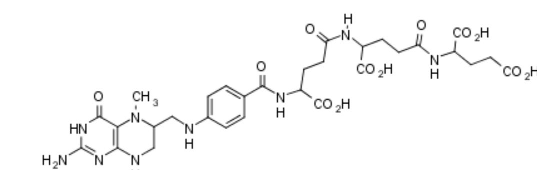

Figure 12: 5-Me-Tetrahydrofolate-diGlutamate; a natural substrate of fungal MetSyn

To make a molecule which more closely resembles this version of folate, a diglutamate must be synthesized (**Fig. 13**).

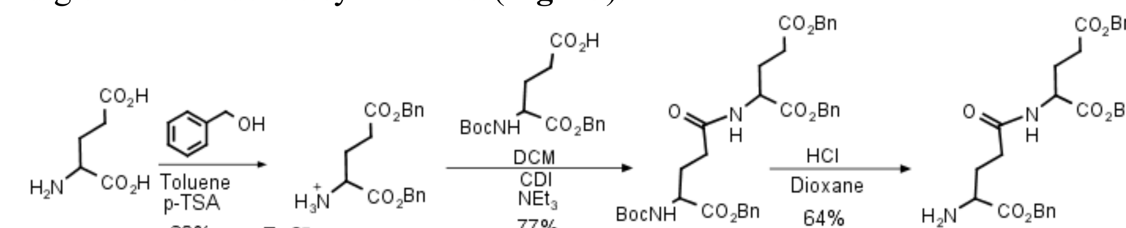

Figure 13: Synthesis of a di-glutamate, linked at the side-chain carboxylic acid

This was then linked onto one of our folate mimics (**Fig. 14**), and the benzyl esters removed (**Fig. 15**) to give the final product. .

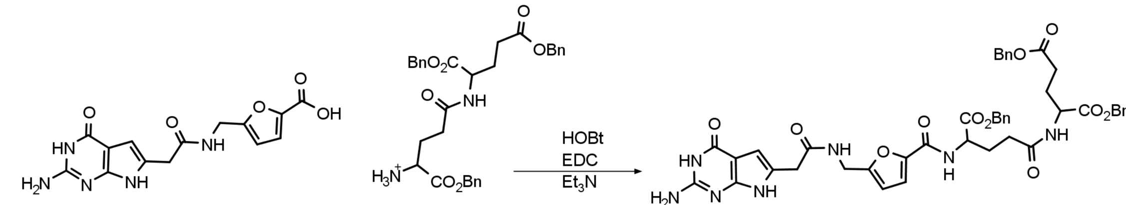

Figure 14: Synthesis of a di-glutamate, linked at the side-chain carboxylic acid

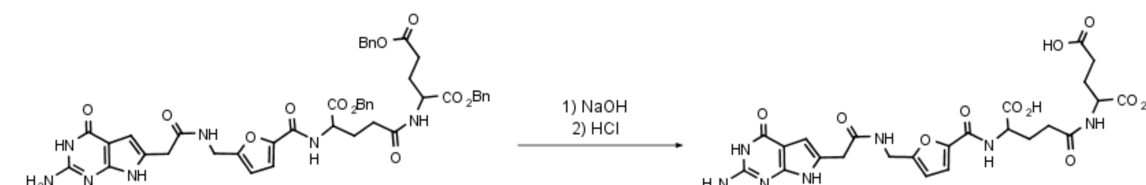

Figure 15: Removal of benzyl esters to provide a di-glutamated folate mimic

Future Work

As the MetSyn enzyme converts homocysteine to methionine (**Fig. 1**), a simple method for monitoring MetSyn activity would be optical detection of homocysteine concentration. The MeasureIT-thiol quantification assay gives a fluorescent response which can be used to generate a calibration curve of homocysteine levels (**Fig. 16**).[5]

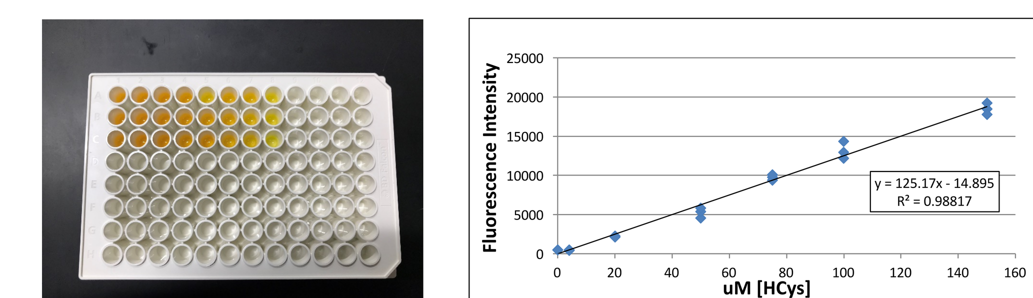

Figure 16: Fluorescent detection of Homocysteine concentration.

This was adapted to observe the MetSyn enzyme reaction (**Fig. 17**). As expected, the enzyme alone gives little fluorescence, but Hcy results in a high fluorescent response. When the enzyme in functioning, the Hcy is consumed, and the fluorescence is lower.

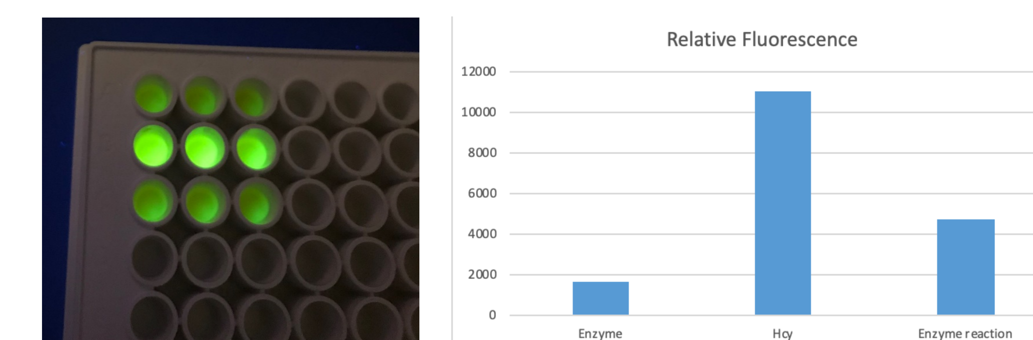

Figure 17: Monitoring MetSyn activity by fluorescent detection of Hcy levels

The next step would be to expose MetSyn to our compounds. A successful inhibitor would results in significant homocysteine left over, giving a "turn-on" fluorescence response to indicate inhibition.

We are also in the process of screening our compounds against microorganisms with the help of Dr. Nunnelly in the biology department.

References

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Acknowledgements

- Indiana Academy Senior Research Grant
- EPIC Scholarship
- Dr. Pruet
- Dr. Nunnelly
- VU Chemistry Department

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