Fungal infections are of continuous concern, especially with regard to immunocompromised patients. In an effort to develop new potential antifungal agents, we have begun synthesizing a library of potential inhibitors of the fungal Methionine Synthase (MetSyn) enzyme. Key differences between the B12-independent fungal MetSyn enzyme and the B12-dependent mammalian form can allow for an antifungal drug to 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 pterin and deazaguanine-based molecules as these mimic folate, an essential substrate for MetSyn function. We have begun testing these new molecules for activity in a fungal growth assay, as well as a fluorescent assay for monitoring MetSyn activity.

**Abstract**

Fungal infections are of continuous concern, especially with regard to immunocompromised patients. In an effort to develop new potential antifungal agents, we have begun synthesizing a library of potential inhibitors of the fungal Methionine Synthase (MetSyn) enzyme. Key differences between the B12-independent fungal MetSyn enzyme and the B12-dependent mammalian form can allow for an antifungal drug to 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 pterin and deazaguanine-based molecules as these mimic folate, an essential substrate for MetSyn function. We have begun testing these new molecules for activity in a fungal growth assay, as well as a fluorescent assay for monitoring MetSyn activity.

**Background**

In a representative synthesis of an inhibitor, a deazaguanine mimic is made, allowing the coupling of a furan linker’s amine to the deazaguanine, creating an amide (Figure 4). Synthesis of the protected amino acid tail begins with a protected asparagine, followed by the Hoffman rearrangement, and the addition of CBZ and tert-butyl ester (Figure 5).

**Routes to 7-CMP**

Multiple routes of synthesis to the folate mimic 7-carboxymethyl pterin (7-CMP) were conceived of and tested. Path A produced slightly better yields, where Path B allowed for quick synthesis and easy purification. This led to Path B to be our method of choice for 7-CMP synthesis (Figure 3).

**Representative Synthesis**

In a representative synthesis of an inhibitor, a deazaguanine mimic is made, allowing the coupling of a furan linker’s amine to the deazaguanine, creating an amide (Figure 4). Synthesis of the protected amino acid tail begins with a protected asparagine, followed by the Hoffman rearrangement, and the addition of CBZ and tert-butyl ester (Figure 5).

**Inhibitors and Screening**

The linker is then coupled to the amino acid tail, and deprotected, affording a potential inhibitor (Figure 6). A library of molecules has been prepared (Figure 7) and tested on various fungi and bacteria via Kirby-Bauer test, showing inhibitors H, N, and A with large zones of inhibition in comparison to the positive control (Figure 8).

**Screening Cont.**

As MetSyn converts homocysteine to methionine, MetSyn activity can be detected by homocysteine concentration. The MeasureIT-thiol quantification assay gives a fluorescent response in proportion to Hcy concentration. High fluorescence shows large concentration of Hcy, allowing the method to be adapted to show enzyme activity. High fluorescence compared to control shows inhibition of the enzyme when mixed with our novel inhibitors, while low fluorescence shows consumed Hcy, showing poor inhibition.

**References**


**Acknowledgements**

We would like to thank Dr. Pruet for his guidance and direction on this project, as well as Dr. Clark for his assistance in the protein purification of methionine synthase, and Dr. Nunnally for her aid in Kirby-Bauer tests. This work has been funded by the Indiana Academy of Sciences Senior Research Grant (J.P.), EPIC scholarship (A.B.), and Eli Lilly (Z.B.).