Synthetic Routes to a Library of Novel Methionine Synthase Inhibitors

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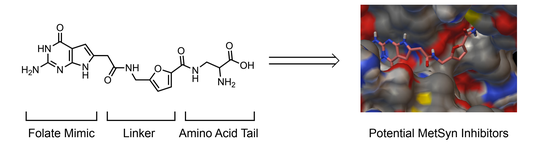
Abstract



Fungal infections are of continuous concern, especially with regard to immunocompromised patients. In an effort to develop new potential anti-fungal agents, we have begun synthesizing a library of potential inhibitors of the fungal Methionine Synthase (MetSyn) enzyme. Key differences between the B_{12}-independant fungal MetSyn enzyme and the B_{12}-dependant 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

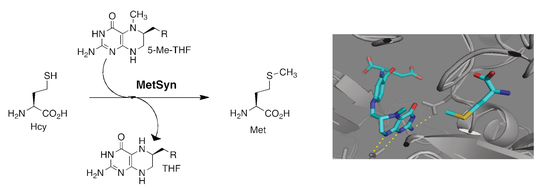


Figure 1. (Left) transformation of homocysteine to methionine by MetSyn (Right) binding of methylated folate and methionine in MetSyn

Fungal infections are a common public health concern in regards to immuno-compromised patients who have a much higher mortality rate due to their condition.[1] Methionine Synthase (MetSyn), is an enzyme which converts homocysteine to methionine using a methylated folate molecule (**Figure 1**). Due to the close binding of homocysteine and folate in the fungal enzyme[2,3], molecules can be synthesized to selectively inhibit the fungal enzyme, providing a safer anti-fungal treatment (**Figure 2**).

Figure 2. Cartoon of molecule binding to both binding sites of the fungal MetSyn vs. failing to bind in the human enzyme

Routes to 7-CMP

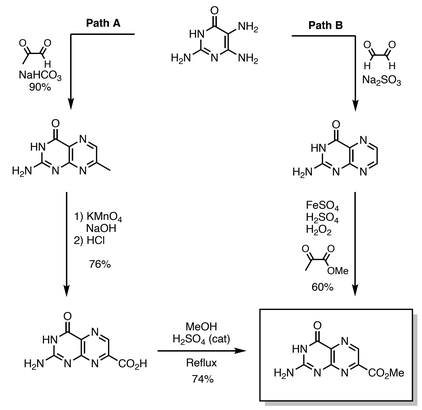


Figure 3. Various synthetic routes to 7-carboxymethyl pterin

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

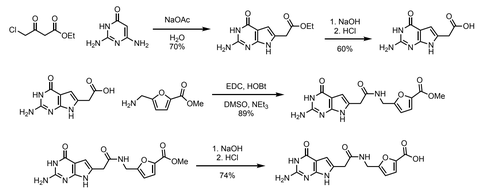


Figure 4. Synthesis of deazaguanine and with linker

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 *t*-butyl ester (**Figure 5**).

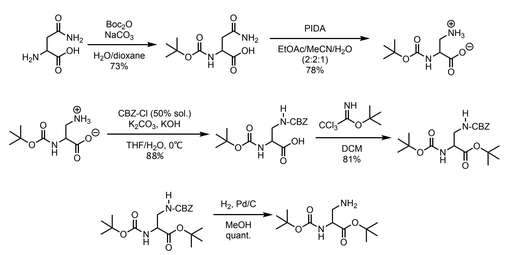


Figure 5. Synthesis of protected amino acid tail

Inhibitors and Screening

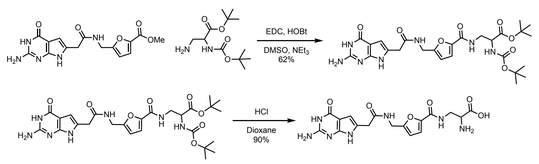


Figure 6. Coupling of amino acid tail to linker

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**).

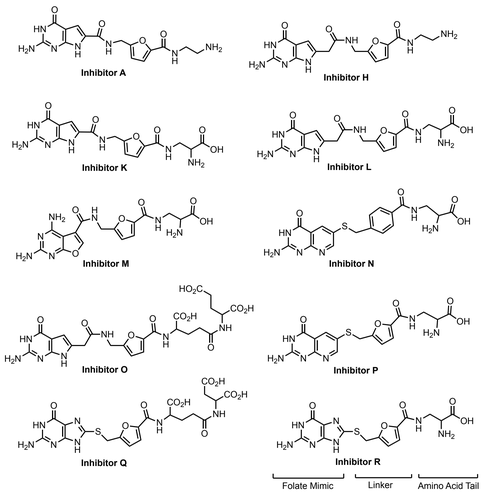


Figure 7. Current library of inhibitors, and inhibitor design

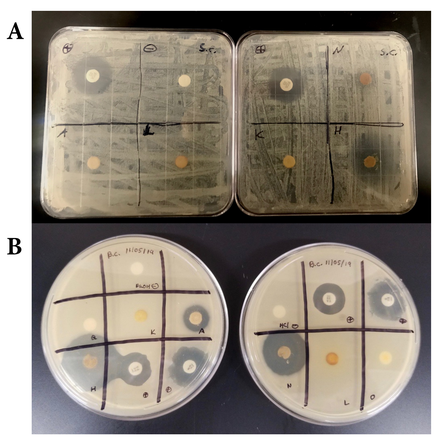


Figure 8. Kirby-Bauer tests against (**A**) S.cerevisiae, (**B**) and B. cereus

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.

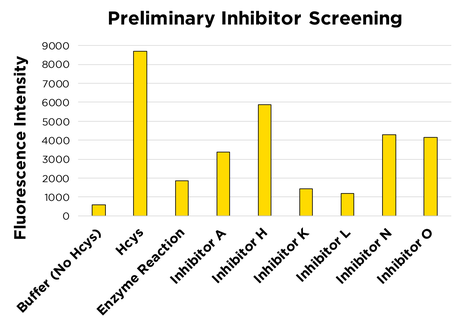


Figure 9. MetSyn inhibition assay, showing comparitive levels of inhibition by synthesized molecules

In conclusion, our inhibitors show promise in regards to inhibiting the enzyme methionine synthase. More work is being done in testing the enzymes directly on fungi and bacteria, as well as cancer cell lines. Folate pathway inhibitors have also been shown to be potent anti-cancer drugs, such as methotrexate, so ours may show activity as well.

References

1. Pfaller, M. A.; Diekema, D. J. (2007). Epidemiology of Invasive Candidiasis: A Persistent Public Health Problem. *Clinical Microbiology Reviews*. **20** (1): 133–63.
2. Suliman, H.S.; Appling, D.R.; Robertus, J.D. (2007). The gene for cobalamin-independent methionine synthase is essential in Candida albicans: A potential antifungal target. *Arch Biochem Biophys*. **467**, 218-226.
3. Ubhi, D.; Kago, G.; Monzingo, A. F.; Robertus J. D. (2014). Structural Analysis of a Fungal Methionine Synthase with Substrates and Inhibitors. *J. Mol. Bio*. **426**, 1839-1847.

Acknowledgements

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