Progress Towards a Library of Potential Fungicides

Anna Bockman
Valparaiso University, anna.bockman@valpo.edu

Jeffrey Pruet
Valparaiso University

Justin Van Sickle

Follow this and additional works at: https://scholar.valpo.edu/fires

Recommended Citation

This Poster Presentation is brought to you for free and open access by the Office of Sponsored and Undergraduate Research at ValpoScholar. It has been accepted for inclusion in Fall Interdisciplinary Research Symposium by an authorized administrator of ValpoScholar. For more information, please contact a ValpoScholar staff member at scholar@valpo.edu.
Progress Towards a Library of Potential Fungicides

Justin Van Sickle; Anna Boekman; and Dr. Jeffrey Pruett
Valparaiso University

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. 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. As there is an increase of drug resistant fungi, there is a need for new types of antifungal 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 new antifungal treatment.

The structure of MetSyn used by fungi is different than that used by mammals.3 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 Methionine (Fig. 3).

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.

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

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).1

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.

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

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.

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

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.

Inhibitor Candidates

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-methyltetrahydrofolate-diglutamate (Fig. 12), as this would most selectively target fungal MetSyn.

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

Future Work

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 lowered.

References


Acknowledgements

• Indiana Academy Senior Research Grant
• EPIC Scholarship
• Dr. Pruett
• Dr. Nunnally
• VU Chemistry Department