Hi my name is Noah Moriarty and I am a senior Biochemistry major and I have been working with Dr. Pruet on this project` for about a year now. And my name is Jessica Villegas. I am a junior biochemistry major on the pre-med track and we have been working with Dr. Pruet on the design and synthesis of potential antifungal agents

Fungal infections are a common public health concern, for example C. Albicans is one of the leading causes of infections in hospitals. So what exactly is a fungal infection? well they are overgrowths of fungi in the body which are often difficult to treat. The most common fungal infections are athlete's foot, ringworm, and UTI's. Some infections are more serious, like endocarditis and meningitis. This is especially true for immunocompromised patients, like those who suffer from diabetes or are receiving cancer treatment. The amount of immunocompromised patients in hospitals creates a perfect storm effect and leads to the mortality rate of C. albicans to be 40%. There are antifungal drugs that exist to treat fungal infections. and they work by disrupting the stability of the fungal cell membrane. However, they do have their drawbacks that can lead to toxicity and allergic reaction. Another issue is that fungal infections are growing drug resistance so there is always a need to develop new antifungal drugs that can function by a different pathway

A potential candidate that we've found for a new inhibitor that doesn't target the cell wall would be Methionine Synthase. MetSyn is the enzyme that produces the essential amino acid, Methionine. Methionine is important for many life processes and if an organism is unable to make methionine it will surely die. MetSyn produces methionine using folate and homocysteine whose binding pockets are in close proximity in the fungal enzyme but are far apart in the human enzyme. This enzymatic difference can be exploited to produce a single molecule that could bind both pockets of fungal methionine synthase but could not possibly bind both pockets of human methionine synthase as shown is figure 3. The general design of such an inhibitor will have a folate mimic, some structure to link between the pockets and a homocysteine mimic. This general design is shown in figure 4 where this molecule would selectively bind fungal metsyn leaving human metsyn alone. This molecule would behave as a competitive inhibitor binding in competition with the natural substrates and lowering the enzymes activity.

Our modeling software, auto dock, lets us virtually fit our inhibitors into our proteins active sites. we look to see if our inhibitor can reach both the HCys and folate pockets at the same time. Here in figure 5 we have four examples of our folate mimics in the folate pocket, some type of linker and an amino acid tails fitting right into the HCys pocket. These virtual docking let us confirm that one molecule can indeed fit in both pockets. Our protein, methionine synthase, can exist in two conformations: Open and closed. It is most often in the "open" form however after binding the natural substrates it conforms to closed. A good inhibitor should bind to the open enzyme but should also still fit in closed. By fitting in the closed, it ensures that the natural substrate can not bind. folate=green, HCys=purple, molecule=yellow . In fig 6, our inhibitor H binds in the open

conformation and still fits in the closed conformation. Several of our compounds have our folate mimc in the folate binding pocket, the amino acid tail fitting in the HCys binding pocket and some type of linker connecting the two, just like our inhibitor H. Based on what we have seen we can theoretically say that we can create one molecule to be a competitive inhibitor.

So from the modeling we can see that these inhibitor molecules theoretically bind competitively with the natural substrates and based on our best models we have to work to synthesize these potential inhibitors. For example, starting with pyrimidine over a series of 3 steps we have 8MG, a folate mimic. Append that to the linker. With the folate mimic attached to a linker, we can now do fischer esterification and attach the amino acid tail. This synthesizes our full inhibitor u. While exploring alternate synthesis, we can start by building the linker first with pyridine and then attaching the amino acid tail over two steps then using methods published in our lab, dbu amidation can be done to attach a pterin folate mimic to the linker-amino acid tail.

Following the same guidelines of synthesis, an entire library of potential inhibitors can be created. Though they all have subtle differences that make them unique and give them different activities, they all follow the general design of a folate mimic, linker, and amino acid tail.

And now that we have a full library of potential inhibitors, we need to test their activity. The first method of doing this is a fluorescence assay that indirectly tracks enzymatic activity using the concentration of its substrate homocysteine. The fluorescence is due to an added reagent that makes homocysteine fluoresce. In a typical assay, there are negative control of buffer, positive control of just homocysteine, and a standard enzyme reaction containing the enzyme and its substrates folate and homocysteine. Next a series of inhibitors will be tested with the enzyme and its substrates. Wells with high fluorescence, mean high concentration of homocysteine, which translate to low enzyme activity. Successful inhibitors will show higher fluorescence than the control enzyme reaction as it blocks the enzyme from consuming Homocysteine. Shown graphically in figure 11. The bars represent fluorescence intensity where the positive control has the most fluorescence, the enzyme is significantly lower and inhibitors bring it up varying degrees. From these tests, inhibitors like H,P, and N are able to effectively bind to and inhibit the enzyme.

We also use fungal growth assays, formally known as kirby bauer tests, to examine the bioactivity of our compounds. We do this by exposing discs that are concentrated by our inhibitors, to bacteria and fungi. Successful inhibitors will prevent fungal and bacteria growth which can be shown by zones of inhibition. For example, inhibitors H,P, N, S, and T in figure 12 show promising signs of inhibition against two fungal strains, C. Albicans, S. cerevisiae, as well as two bacterial strains, B. cereus and S. aureus. When the results from the fungal growth assay aligns with those from the enzyme assay, we can say that our compound will successfully inhibit the methionine synthase enzyme and stop pathogenic organisms from growing.

To conclude, we were able to model and then synthesize a library of potential antifungal compounds that can target fungal methionine synthase. In the future, we wish to test the mechanism for which we are inhibiting and killing g the fungi in our growth assay and also wish to test the selectivity of our inhibitor molecule against the human methionine synthase enzyme versus the fungal met syn enzyme to make sure we are doing what we want. Lastly we want to expand on our current library and synthesize as many of these inhibitors as possible doing that all based on our molecular modeling results.

Here are our references.

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Thank you. Thank you.