Slide 1

Hello everyone, my name is Ruby Chen, and I work in Professor Martin Burke's lab at University of Illinois. Today, I am happy to share with you my project on understanding organic small molecule-mediated metal mobilization.

Slide 2

There are two common types of protein related diseases, the overexpression and underexpression of proteins. A common strategy to treat protein overexpression is to introduce a small molecule to inhibit the protein to suppress excess protein function, and this strategy has been applied to a variety of drugs, including anticancer drugs like sunitinib and carfilzomib. However, there are currently no effective strategies to treat the underexpression of proteins, leading to unmet medical needs regarding protein deficiency diseases such as Gaucher Disease, Maple syrup urine disease, and phenylketonuria.

Slide 3

A normal organism with no mutations would have several mechanisms to perform a certain function, and therefore when the functional capacity falls below normal physiology level for an organism missing a protein, it is possible to introduce a small molecule prosthetic to add upon the functional capacity arising from the backup mechanisms to restore normal physiology. Our lab has already shown that Amphotericin B, a well-known antifungal drug, is capable of forming ion channels for potassium transmembrane transportation and can rescue yeast that are potassium transporter deficient. In my project, we mainly focus on another small molecule natural product, hinokitiol, to investigate its capability of replacing a missing protein iron transmembrane transporter.

Slide 4

Iron is effectively transported across cell membranes with the aid of protein iron transporters, while an organism missing such a protein cannot effectively transport iron, leading to the buildup of iron on one side of the membrane and creating a chemical gradient. We seek to develop an optimized small molecule that can harness this gradient to chelate and mobilize iron into the cell, within different cell compartments, and out of the cell.

Slide 5

Hinokitiol was first identified to be capable of iron mobilization through a yeast diffusion rescue assay. In this experiment, we used yeast missing the iron transporter protein to observe if the addition of the small molecule can restore yeast growth. As shown in the top right figure, we see a ring of yeast growth restoration with the addition of hinokitiol in the center of the plate. In addition, we observed that hinokitiol does not restore yeast growth at high concentrations close to the center of the plate or at low concentrations further away from the center than the yeast growth ring. Using the same yeast model, our lab has reported that tropolone, a similarly structured natural product capable of chelating iron, does not mobilize iron as hinokitiol does. We therefore seek to understand the reason of this phenomenon to enable the rational design of an iron mobilization optimized derivative of hinokitiol.

Slide 6

We therefore developed a hypothesis on the correlation between iron mobilization activity and small molecule lipophilicity. Cell membranes are lipid bilayers, with the hydrophilic parts facing out and lipophilic parts in the middle, creating a lipophilic environment in between the bilayers as opposed to the aqueous environment outside of the membrane. A more polar molecule is more energetically favorable in aqueous solutions, and is less likely to enter the lipid bilayer, while lipid bilayer entry is a required step for transmembrane iron transportation. On the other hand, with optimal lipophilicity, the activation energy need for cell membrane entry is small, which allows transmembrane mobilization of the small molecule-iron complex.

Slide 7

To confirm our hypothesis and further establish the correlation between lipophilicity and iron mobilization activity, I synthesized a wide array of hinokitiol-like derivatives. The retrosynthetic plan to acquire the derivatives require bromotropolones as key building blocks, which can be subjected to a palladium catalyzed Suzuki coupling with boronic acids to readily yield desired derivatives. Building blocks 1, 2, and 3 are first synthesized. We simply methylate tropolone, which is naturally abundant and commercially available, and brominate the intermediate to acquire building block 1. To get to block 2, one of the alkenes in 1,3-hexadiene was first reacted with bromoform through a carbene like mechanism, and the leftover alkene was dihydroxylated by OsO_4, yielding the dibromo dihydroxyl bicyclic product. Swern oxidation and rearrangement under the basic swern conditions lead to the beta bromotropolone, and its methylation yields building block 2. A similar route but starting with 1,4-hexadiene would yield building block 3.

Slide 8

Using this strategy, I have synthesized more than 30 hinokitiol derivatives with different substituent constitution on alpha, beta, and gamma positions of the tropolone ring.

Slide 9

To quantitatively measure the iron transport capacity of these derivatives, we used a liposome assay where iron sources is put in the liposome, and the outside solution contains no iron. Hinokitiol derivatives were then added, and we measure how much iron gets transported outside of the liposomes 30 minutes after the addition. The results are shown as heat maps, where red shows no transportation and bright green indicates efficient transportation. In the case of hinokitiol addition, we see that transportation is not effective at either low concentrations or high concentrations but is effective at optimal concentrations. For derivatives with substituents at the same tropolone carbon, we observed that at shorter carbon chains, the concentration needed for the transportation to be efficient tends to be higher, and the performance of the derivatives increases as the linear carbon chains are increased in length until an optimal chain length, upon which the performance starts to drop. This same correlation can be observed with the alpha and gamma derivatives as well.

Slide 10

To evaluate the toxicity of these compounds, we performed minimum inhibitory concentration assays on wild type yeast. This assay allows us to find the highest dosage yeast can tolerate before

the concentration becomes too high and will kill the yeast. Another correlation with small molecule lipophilicity is observed here. Derivatives with shorter alkyl chains are less toxic at higher concentrations, while ones with longer chains can start to kill at smaller concentrations. The same is observed with both beta and gamma derivatives.

Slide 11

These results are consistent with our hypothesis, and also agree with the correlation between yeast growth restoration with the lipophilicities of various other iron chelators. We then hypothesized that with the addition of a certain amount of lipophilicity, derivatives of the less lipophilic chelators will be able to mobilize iron. To verify this, we chose deferiprone, which is an FDA approved iron chelator. As shown in the bottom left, in a hexanes/water system, deferiprone partitions into water while hinokitiol goes into hexanes, which visualizes the lipophilicity difference of the two compounds. We have previously shown that deferiprone does not promote iron uptake or transportation.

Slide 12

Through a simple reaction, we systematically added one carbon at a time to the deferiprone motif, and the same yeast minimum inhibitory concentration and liposome iron transportation assay were performed. For the minimum inhibitory assay, it was observed that the derivatives with shorter carbon chain lengths do not kill, while the longer chain derivatives start to kill at higher concentrations. The shorter chain derivatives show no transportation at all concentrations, but as the lipophilicity of the derivatives increase, some derivatives start to show transportation. Once past the optimal lipophilicity, the performances of the longest chain derivatives start to diminish again. This is consistent with the result we obtained testing hinokitiol.

Slide 13

A possible mechanism for the poor mobilization performance of the small molecules is that they form large aggregates at high concentrations, making it impossible for them to pass through the cell membrane. Our lab has previously obtained the crystal structure of the hinokitiol-iron complex, and the data showed that isopropyl groups are aligned together, likely due to Van der Waals interaction. Moreover, we have observed that the derivatives with branched substituents have poorer activity at high concentrations. Our current explanation for this phenomenon is that linear substituents can more freely rotate and are therefore less likely to end up in a perfectly aligned conformation that could favor aggregation. To further disturb the chemical space around the substituent, we have also synthesized deuterated derivatives. Commercially available deuterated alkyl halides were reacted with magnesium to yield Grignard reagents, which attacked trimethyl borate to yield deuterated alkyl boronic acids upon acid promoted hydrolysis. The derivatives were then made using Suzuki coupling reactions from the freshly prepared boronic acids and the bromotropolone building blocks. Preliminary results have shown that the deuterated derivative has better performance at high concentrations, and we will continue to investigate the biological properties of all the compounds.

Slide 14

To acquire definite evidence for all of the mechanism of actions we have previously proposed, we designed a solid-state NMR paramagnetic relaxation enhancement experiment to work out the location of hinokitiol and its derivatives relative to a lipid bilayer. To enable this, I synthesized ^{13}C labeled hinokitiol and its derivatives. The labeled molecules will then be complexed with gallium, a diamagnetic substitute for iron. The interaction of the ^{13}C nucleus with the radical labels will allow us to detect the location of the hinokitiol-Ga complex with respect to the radical labels and hence the lipid bilayer. With this chemical probe in hand, in addition to all the preliminary results showing a very likely correlation between lipophilicity and iron mobilization, we will be able to fully uncover the mechanism of this small-molecule mediated iron mobilization.

Slide 15

At the end of my presentation, I would like to thank Professor Burke and my two mentors Daniel and Andrew for supervising my research, the whole Burke group for their support, and thank you for listening.