Tu Ho – Research Statement

I am a Ph.D. candidate in the Yoshimoto lab at the University of Texas at San Antonio, where I develop multi-step synthetic routes to access biologically relevant small molecules. My work focuses on building complex steroidal scaffolds and exploring new methods to modify their structures for therapeutic and analytical applications. I enjoy the process of designing synthetic strategies, troubleshooting challenging transformations, and optimizing routes to construct novel molecules. The following projects highlight my training in complex synthesis, heterocycle construction, and method development.

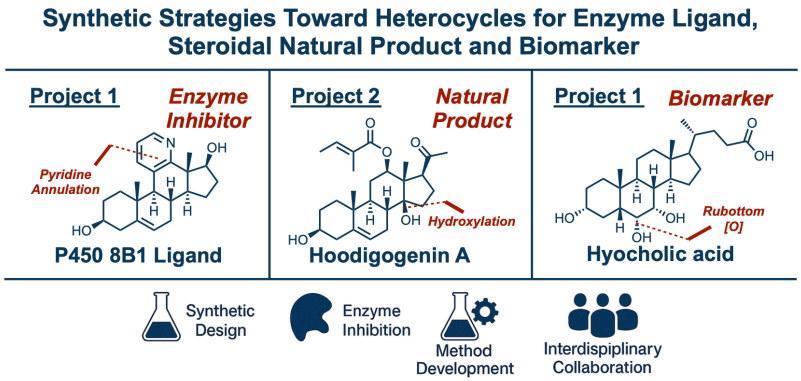

Figure 1. Summary of Ph.D. research: synthesis of heterocyclic steroid analogs (left), C14β hydroxylation for hoodigogenin A (center), and preparation of a hyocholic acid standard for isomer differentiation via derivatization and LC-MS (right).

Project 1: Syntheses of C11-C12 Pyridine Containing Steroid Analogs as P450 8B1 Inhibitors

Previous studies showed that mice lacking the *CYP8B1* gene, which encodes for P450 8B1 expression, resisted weight gain when fed a high-fat diet. Therefore, inhibiting cytochrome P450 8B1 activity has been suggested to be a potential treatment for diabetes and obesity. The established strategy for synthesizing a P450 inhibitor is to introduce a pyridine ring at the hydroxylation site on the substrate, which is at C12- position for P450 8B1. Based on this approach, my project focuses on the synthesis of a C12-pyridine-containing steroid as a potential P450 8B1 inhibitor. Previous efforts to synthesize a P450 8B1 inhibitor used a Suzuki-cross coupling of a 12-vinyl iodide derivative with pyridine-3-boronic acid. However, the resulting compound did not fit into the active site of P450 8B1 as demonstrated through protein crystallography. In this project, I synthesized a "shorter" analog with a fused-pyridine-containing steroid (Figure 2), which can effectively bind to P450 8B1's active site. From commercially available dehydroepiandrosterone (DHEA, **1**), I prepared 12-keto intermediate **2**, which underwent a pyridine annulation at C11-C12, followed by functional group manipulations to yield compound **2**. This pyridine-containing analog successfully bind to the active site of P450 8B1, as experimentally confirmed by our collaborator, Prof. Emily Scott, a protein crystallography expert, at the University of Michigan. Other analogs was synthesized include the addition of dimethylpyrazole and imidazole through Aza-Michael addition. Future directions involve the synthesis of steroid analogs that have more optimal and selective binding affinities towards P450 8B1 to treat diabetes and obesity.

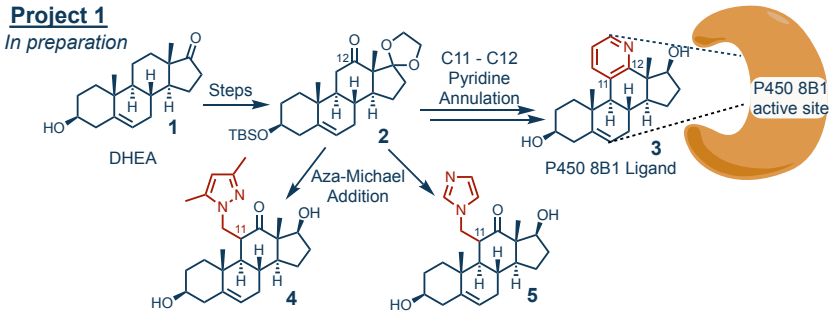

Figure 2. Synthesis of P450 8B1 ligands (**3,4,5**) from DHEA (**1**).

Project 2: Synthesis of Hoodigogenin A through a Copper-Catalyzed C14-Hydroxylation

The 14β-hydroxy group plays important role in biological activity of many steroidal natural products, including the anticancer natural product bufadienolide. Hoodigogenin A (Figure 3, **10**) is a plant-derived pregnane with a 14β-hydroxy group, possessing appetite suppression properties; however, its mode of action remains unclear. Therefore, the successful synthesis of hoodigogenin A from an inexpensive steroid like dehydroepiandrosterone (DHEA, Figure 3, **1**) could enable structure activity relationship (SAR) studies to identify the important chemical features that confer the biological activity of hoodigogenin A. However, introducing a 14β-hydroxy group has been challenging due to low yielding reaction conditions with poor stereoselectivity. Prior syntheses of

hoodigogenin A either incorporated the hydroxyl group indirectly—leading to product mixtures—or used starting materials like digoxin, which already contain the 14β-OH group, limiting opportunities for analog development for SAR studies.

As a result, I developed a synthetic route to prepare hoodigogenin A from DHEA (**8** to **1**), featuring a new C14β-hydroxylation of the steroid backbone. Specifically, the C5-alkene of DHEA (**1**) was masked as the 6-methoxy-3,5-cyclosteroid (**6**) to enable subsequent regioselective and chemo-selective synthetic transformations. The D-ring was converted to the 15-en-17-one intermediate as the key precursor to hydroxylate the C14 position. To obtain the 14β- hydroxy (**9**), the 15-en-17-one intermediate (**6**) was either converted to the silyl dienol ether (**7**), which would react with singlet oxygen or mCPBA to incorporate the oxygen at C14 or directly oxidized with a copper catalyst system. By modifying the side chain at the C17-position and esterifying the 12β-hydroxy group of (**8**) with tiglic acid, I success-fully synthesized hoodigogenin A (**1**). Future work will take advantage of this synthetic route to access structural analogs of hoodi-gogenin A for SAR studies. Furthermore, the hydroxylation methods at the 14β- position will be applied to synthesize other steroid anticancer natural products such as bufadienolides and cardenolides.

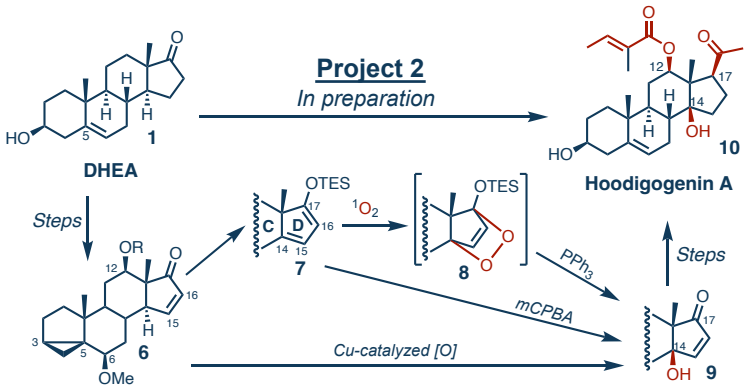

Figure 3. Synthesis of Hoodigogenin A (**10**) from DHEA (**1**).

Project 3: Synthesis of Hyocholic Acid and the Derivatization of Its C6-C7 Vicinal Diol with $NaIO_4$

Hyocholic acid (HCA) and cholic acid (CA) (Figure 4, **14** and **12**) are two bile acids that are constitutional isomers but with opposing biological properties. HCA (**14**) is abundant in species resistant to diabetes while elevated CA (**12**) levels is associated with obesity. Even though CA is commercially available and affordable, there are limited sources of HCA and it is expensive. Furthermore, there are not many syntheses of HCA that have been reported, limiting the study of this bile acid as it is related to cardiovascular diseases. Previously reported HCA syntheses involved an α-bromination of a C7-ketone intermediate followed by a substitution reaction with NaOH to yield the 6α-hydroxy group. However, inconsistent NMR data between these studies have raised questions about the stereochemistry and purity of the synthesized HCA. Therefore, this project sought to clarify these ambiguities by: (i) developing a stereoselective synthetic route to prepare HCA (**14**) from chenodeoxycholic acid (CDCA, **11**); and (ii) establishing a derivatization method to distinguish HCA from CA.

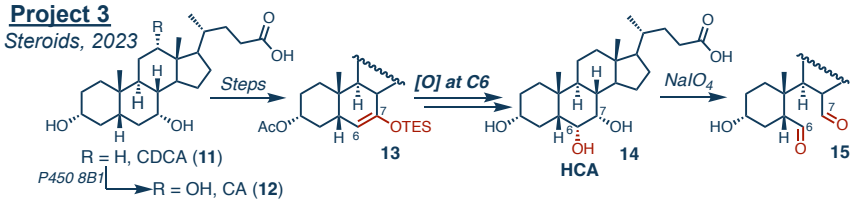

Figure 4. Synthesis of hyocholic acid (**14**) from chenodeoxycholic acid (**11**).

To address the above questions, I synthesized HCA from CDCA (**14** from **11**) via a Rubottom oxidation, forming a silyl enol ether intermediate **13** followed by mCPBA oxidation to stereoselectively install the 6α-hydroxy group, which was confirmed by X-ray crystallography. Additionally, I developed a new method to distinguish an isomer mixture of HCA (**14**) and CA (**12**) using $NaIO_4$ and mass spectrometry. Specifically, only the C6, C7-cis-vicinal diol of HCA was cleaved upon treatment with $NaIO_4$ to yield compound **15**, while CA, which lacks a vicinal diol, remained intact. Moreover, ox bile, which is widely sold as a dietary supplement for digestion, contains an unknown mixture of bile acids. Through derivatization with $NaIO_4$, I detected HCA and CA derivatives (conjugated to amino acids) in ox bile tablets. This work was published in *Steroids* in 2023.[1]

In summary, my doctoral work centers on developing new synthetic methods and multi-step routes to access biologically relevant molecules, including a C14-hydroxylation strategy for steroid natural products and the synthesis of heterocyclic steroid analogs for biological studies—experience well-aligned with the Frankowski Lab's focus on innovative small molecule design.

1. **Ho, T. M.**; Arman, H. D.; Yoshimoto, F. K. *Steroids, 2023*, 197, 109260.