Modeling Guided Synthesis of Potential Fatty Acid Synthase Inhibitors for the Treatment of Diffuse Large B-Cell Lymphoma

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Thesis directed by

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Department Chair and Professor of Chemistry and Chemical Biology
Dedication

I would like to dedicate this work to my brother, mother, and father who have supported me through the last five years at Northeastern. Thank you for providing an unending amount of love and encouragement. I know it was not easy to let me attend a university on the complete opposite side of the country, but I am extremely grateful for the chance you gave me to experience a different environment from Southern California. Mom, thank you for the daily phone calls and face time chats that enabled me to still feel close to home and gave me the strength I needed to get through this rigorous program. I do not know any other mother who does as much or cares as much about her children as you. I am extremely grateful for your ability and willingness to drop everything and come to Boston when I am in need. Dad, thank you for making me believe I could be successful so far away from home and for your constant humor. Riley, thank you for always picking up the phone when I needed someone to talk to, and for all the times you listened to me vent about school and research. I love all of you and am so grateful to have you in my life.
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Finally, thank you to my supportive family, without you I would not have been able to thrive so far away from home.
Abstract

Although there has been a significant increase in the long-term survival rate of patients with diffuse large B-cell lymphoma (DLBCL), there is still an unmet need for treating those who are not responsive to current therapeutics. It is well known that cells are capable of storing excess glucose as triglycerides, through the conversion of citrate to palmitate by fatty acid synthase (FAS). The up-regulation of FAS across all DLBCL cell types makes it an appropriate target for inhibitor design. Cerulenin has been shown to cause apoptosis in DLBCL cells through fatty acid synthase inhibition, but a low IC50 of 1 μg/mL prevents it from being more widely used. There is a need for more potent inhibitors of FAS for the treatment of DLBCL. Molecular modeling has been shown to be a useful technique for inhibitor design and could be used to develop more potent analogs of cerulenin. While cerulenin has been shown in complex with bacterial FAS, it has never been shown in complex with human FAS since human FAS structures have shown poor resolution (>3 Å). A crystal structure for the KS-MAT di-domain of human FAS has been solved, but is only an apo-structure. This project involves modeling guided synthesis of cerulenin derivatives which are capable of inhibiting the human FAS KS domain.
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<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>TMS</td>
<td>tetramethylsilane</td>
</tr>
<tr>
<td>$^1$HNMR</td>
<td>proton nuclear magnetic resonance</td>
</tr>
<tr>
<td>$^{13}$CNMR</td>
<td>carbon nuclear magnetic resonance</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>UV</td>
<td>ultra violet</td>
</tr>
<tr>
<td>FAS</td>
<td>fatty acid synthase</td>
</tr>
<tr>
<td>KS</td>
<td>ketoacyl synthase</td>
</tr>
<tr>
<td>MAT</td>
<td>malonylacyl transferase</td>
</tr>
<tr>
<td>YASARA</td>
<td>yet another scientific artificial reality application</td>
</tr>
<tr>
<td>PDB</td>
<td>protein data bank</td>
</tr>
<tr>
<td>YAMBER3</td>
<td>assisted model building with energy refinement</td>
</tr>
<tr>
<td>PROCHECK</td>
<td>program to check the stereochemical quality of protein structures</td>
</tr>
<tr>
<td>RMSD</td>
<td>root mean square deviation</td>
</tr>
<tr>
<td>POOL</td>
<td>partial order optimal likelihood</td>
</tr>
<tr>
<td>THEMATICAS</td>
<td>theoretical microscopic titration curves</td>
</tr>
<tr>
<td>CHARM19</td>
<td>chemical hazard assessment and risk management</td>
</tr>
<tr>
<td>NHL</td>
<td>non-Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>DLBCL</td>
<td>diffuse large B-cell lymphoma</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>----------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td>ClogP</td>
<td>calculated partition coefficient</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxy Ribonucleic Acid</td>
</tr>
<tr>
<td>BCL</td>
<td>B-Cell Lymphoma</td>
</tr>
<tr>
<td>CCND2</td>
<td>Cyclin D2 (coding gene)</td>
</tr>
<tr>
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<td>Fibronectin 1</td>
</tr>
<tr>
<td>LMO2</td>
<td>LIM domain only 2</td>
</tr>
<tr>
<td>SCYA3</td>
<td>small inducible cytokine A3</td>
</tr>
<tr>
<td>SWOG</td>
<td>Southwest Oncology Group</td>
</tr>
<tr>
<td>CHOP</td>
<td>Cyclophosphamide, Hydroxydaunorubicin, Oncovin, Prednisone</td>
</tr>
<tr>
<td>ECOG</td>
<td>Eastern Cooperative Oncology Group</td>
</tr>
<tr>
<td>R-CHOP</td>
<td>Rituximab-CHOP</td>
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<td>PKC-β</td>
<td>Protein Kinase B</td>
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<tr>
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<td>Spleen Tyrosine Kinase 1</td>
</tr>
<tr>
<td>m-TOR</td>
<td>Mammalian Target of Rapamycin</td>
</tr>
<tr>
<td>FASN</td>
<td>Fatty Acid Synthase (gene)</td>
</tr>
<tr>
<td>CPT-1</td>
<td>Carnitine Palmitoyl Transferase 1</td>
</tr>
<tr>
<td>PI3K</td>
<td>Phosphoinositide 3-kinase</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen-activated Protein Kinase</td>
</tr>
<tr>
<td>ACP</td>
<td>Acyl Carrier Protein</td>
</tr>
<tr>
<td>KS</td>
<td>β-ketoacyl ACP synthase</td>
</tr>
<tr>
<td>MAT</td>
<td>Malonyl/Acetyl ACP Transferase</td>
</tr>
<tr>
<td>KR</td>
<td>β-ketoacyl ACP reductase</td>
</tr>
<tr>
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<td>Full Name</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>DH</td>
<td>β-ketoacyl ACP dehydrogenase</td>
</tr>
<tr>
<td>ER</td>
<td>Enoyl-ACP reductase</td>
</tr>
<tr>
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<td>Thioesterase</td>
</tr>
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<td>CoA</td>
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<tr>
<td>GLIDE</td>
<td>ligand docking program</td>
</tr>
<tr>
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<td>Acetic Acid</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>TEA</td>
<td>Tri-ethyl amine</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Ethyl Acetate</td>
</tr>
<tr>
<td>CDCl3</td>
<td>Deuterated Chloroform</td>
</tr>
<tr>
<td>PP</td>
<td>parts per million</td>
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GENERAL EXPERIMENTAL INFORMATION

All reaction were performed in glassware or 2 mL vials. Glassware for dry reactions was dried for at least 12 hr at 60° or flame dried under argon before use. Dry reactions were performed under anhydrous, inert argon atmosphere with freshly distilled dry solvents. Dichloromethane was distilled from calcium hydride immediate prior to use. All reagents were purchased from Fisher Scientific or Sigma Aldrich without further purification. NMR spectra were obtained from a Varian Mercury 400 (400 MHz) spectrometer and reported in ppm downfield relative to TMS peak. ¹H NMR data is reported by multiplicity, coupling constant, followed by number of protons. Reaction monitoring by TLC was performed using SiliaPlate TLC Aluminium Backed TLC F-254 (Silicycle Inc.) and visualized using a 254nm/366nm UV lamp or appropriate stain. Flash chromatography was performed using SiliaFlash P60 (230-40 mesh) silica gel (Silicycle).

Homology Modeling: ¹¹

The protein structure of human FAS KS-MAT didomain (PDBID 3HHD) was used for homology modeling of KS domain active site residues. Homology modeling of the KS catalytic domain was performed using the YASARA suite of programs.¹¹ The crystal structure (PDB ID 3HHD) was refined by energy minimization for 500 picoseconds using an explicit solvent molecular dynamics simulation with a YAMBER3 force field in YASARA.¹¹ Refinement of the structure involves a short steepest descent minimization for the removal of the largest intermolecular and intramolecular clashes. A second steepest descent minimization is then performed with potential energy terms as well as a simulated annealing procedure. Model quality was verified by PROCHECK and MolProbity and was found to be of sufficiently good quality.
Compound Docking$^{12,13,14}$:

The model KS domain chain A structures were prepared using the Maestro 9.1 protein preparation wizard (Schodinger, LLC, 2010, New York, NY) prior to docking.$^{12,13,14}$ Bond orders were then assigned and the orientation of hydroxyl groups, amide groups of the side chains of Asn and Gln, and the charge state of histidine residues were optimized. A restrained minimization of the protein structure was performed using the default constraint of 0.3 Å RMSD and the OPLS 2001 force field.

LigPlot$^{15}$:

PDB file prepared by YASARA and Glide modeling of cerulenin to human FAS KS domain chain A.

POOL Prediction$^{17}$:

POOL prediction input file for human FAS KS-MAT domain (3HHD) was pre-processed by isolation and minimization of chain A using YASARA suite of programs.$^{11}$ THEMATICS calculations were performed and the electrical potential computed by a Poisson-Boltzmann procedure.$^{16}$ The Poisson-Boltzmann procedure is based on values from CHARMM19 (a standard forcefield) of atomic charges and generated molecular surface generated by the atomic radii assigned to atoms in the input structure. Only standard amino acids can be used in the THEMATICS calculation, and will delete all heteroatoms.$^{16}$
CHAPTER 1: Molecular Modeling of Cerulenin Derivatives

1.0.0 Introduction
1.0.1 Diffuse Large B-Cell Lymphoma

In the United States, Non-Hodgkin lymphoma (NHL) accounts for four percent of all malignancies and is the most common hematologic tumor type.\(^1\) The occurrence of NHL has almost doubled in the last twenty years due to longer life expectancies as well as new diagnostic procedures.\(^1\) Diffuse large B-cell lymphoma (DLBCL) is an aggressive subtype of NHL, and accounts for around thirty percent of annual lymphoma cases.\(^1\) DLBCL typically presents itself in the lymph nodes, while more complicated types can be seen in other areas.\(^1\) A DLBCL tumor exists as an accumulation of large B cells, with the majority of cases (\(~80\%)\) represented by tumors comprised of germinal center centroblasts.\(^2\) Immunoblastic type DLBCL accounts for around ten percent of all cases.\(^1\) Chromosomal abnormalities of the 3q27 locus and the BCL-6 gene are also linked to DLBCL. Although some biomarkers and genes have been identified, there is a lack of therapies to target them.

A Leukemia and Lymphoma Molecular Profiling Project was developed in 2002, and used DNA microarrays to determine subgroups within DLBCL.\(^1\) The two major subgroups identified from this study were germinal center and activated B-cell DLBCL.\(^1\) The study also found that patients that were categorized as having the germinal center subtype had an increased five-year survival rate over the others.\(^1\) Further studies focused on other predictors of survival between the two subtypes.
1.0.2 Current Therapies

Miller et al. reported results from a SWOG study in 1998 that involved the treatment of patients with DLBCL with CHOP and radiation.\(^1\) CHOP chemotherapy involves the compounds Doxorubicin, Cyclophosphamide, Prednisone, and Vincristine. This therapy has been the standard of treatment for DLBCL since published by United States Intergroup in 1992. Although there was an increase in the five-year survival rate of this combination treatment, a high recurrence rate was seen ten years after treatment. Another study, by ECOG, used CHOP in addition to radiation and showed similar results, in that five-year survival rates were better but still showed high recurrence of disease long-term.\(^1\) Both of these studies pointed towards a need for a treatment with better long term results, as well as the realization that early stages of DLBCL must have a different molecular identity than late stage disease. The SWOG study also realized that risk factors played a major role in the survival of DLBCL patients.\(^1\) Risk factors include patients who are older than sixty years of age, performance status greater than or equal to two, stage two patients, and those with high lactate dehydrogenase levels.\(^1\) Patients who have one or three or more of these risk factors were reported to have five-year survival rates of ninety-four, seventy-one, and fifty percent respectively. Elderly patients are usually treated through the use of palliative care, where alleviation of symptoms stems from radiotherapy. Single-agent treatments of these patients involve alkylating agent, vincristine, anthracycline, and cytarabins.\(^1\) Long term survival rates in these patients is only around thirty percent. Rituximab was approved by the FDA in 1997 and patented in 1998. This was considered a game changer in the treatment of DLBCL because patients experienced better long-term outcomes.

Rituximab is a monoclonal anti-CD20 antibody and when combined with CHOP therapies showed a better overall four-year survival of ninety-two percent.\(^1\) It was also reported that
patients in stage one or stage two (non-bulky disease) could be successfully treated with a Rituximab-CHOP combination therapy. These patients did not have to undergo any radiation in addition to chemotherapy. R-CHOP remains a standard for treatment in those who do not have bulky disease. Although the introduction of Rituximab led to upwards of ninety percent response rates in low-risk DLBCL patients, the five-year survival rate for all patients was at best fifty percent.¹

This has lead to the development of new therapies for patients who do not respond to R-CHOP. Bortezomib was created to inhibit the proteasome and is being tested in patients that have mediastinal DLBCL.¹ A maintenance therapy, using Enzastaurin, has also been developed to inhibit PKC-beta for treatment of patients in the high risk categories.¹ Lenalidomide has also been used to treat DLBCL and has shown a twenty-five percent response rate in refractory patients.¹ Antibody-drug conjugates, like Bevacizumab, have also been created to help in the long term outcome of the disease.¹ Researchers have also found potential inhibition targets of Syk, histone deacetylases, bel-2, m-TOR, and FASN.¹

Dasnamoorthy et al. reported that germinal center DLBCL cells express genes involved with mTOR and fatty acid synthesis.² For non-germinal center DLBCL, it was reported that P13K and MAPK were detected.² However, fatty acid synthase as well as CPT1 were seen across all DLBCL cell lines.³ It was also reported that cerulenin was able to inhibit and induce apoptosis of DLBCL cell lines with an IC₅₀ value of C₅₀ of 1μg/mL.³ Design and synthesis of cerulenin derivatives could therefore be potentially better inhibitors of FAS and provide a better treatment for all cell lines of DLBCL.
1.0.3 Fatty acid synthase inhibition

Fatty acid synthase allows for the cyclic cycle of saturated fatty acid synthesis to occur through the use of acetyl coenzyme A. Two types of fatty acid synthases are observed across species; FAS I and FAS II. Higher order eukaryotes and fungi utilize FAS I, while FAS II occurs in plants and bacteria.\textsuperscript{6,7} Although FAS I exists in both Eukaryotes and fungi, the quaternary structure of the enzyme vastly differs between the two species.\textsuperscript{6} Fungi type I FAS exists as an \( \alpha_6\beta_6 \) heterododecamer of 2.6-MDa.\textsuperscript{7} This structure is much larger than the 270-kDa homodimeric chains from mammalian FAS.\textsuperscript{7}

Each chain of mammalian FAS consists of six catalytic domains, each responsible for different activities needed for fatty acid synthesis. The six domains of FASN include the thioesterase (TE), enoyl-ACP reductase (ER), \( \beta \)-hydroxyacyl ACP dehydratase (DH), \( \beta \)-ketoacyl ACP reductase (KR), malonyl/acetyl ACP transferase (MAT), and \( \beta \)-ketoacyl ACP synthase (KS) domains.\textsuperscript{8} Fatty acid synthesis is initiated by the acetylation of the active site cysteine of the KS domain by the acyl carrier protein (ACP).\textsuperscript{8} MAT then acts as a catalyst to add a malonyl CoA unit back onto ACP.\textsuperscript{8} KS uses an acetyl group to de-carboxylate malonyl CoA through a Claisen condensation reaction.\textsuperscript{8} This leaves an 3-oxo-acyl compound that is reduced by KR, dehydrated by DH, and then reduced again by ER.\textsuperscript{8} The product is a saturated acyl compound that enters back into the elongation cycle until the chain is around 16-18 carbons long. The TE unit then releases the saturated fatty acid from FAS.\textsuperscript{8} The activity of FAS is low in most human tissues because of the modern diet being rich in fatty acids, and further synthesis is not needed.\textsuperscript{7} Cancer cells, however, have been shown to up-regulate the expression of FASN and the activity level of the enzyme correlates with tumor malignancy.\textsuperscript{7} FASN is over expressed in many cancer cell lines, while present in extremely low levels in healthy tissues. Since fatty acid synthesis is
essential for tumor growth and survival, fatty acid synthase represents a potential target for the development of new anti-tumor therapies.

Figure 1: A representation of the cyclic fatty acid synthesis cycle.\textsuperscript{7,8,9}

Glucose that cannot be utilized within the cell is exported out of the mitochondria as citrate.\textsuperscript{8} Citrate is then converted to malonyl-CoA through acetyl Co-A.\textsuperscript{8} Malonyl-CoA is then used by
fatty acid synthase to produce palmitate. Palmitate goes on to form palmitoyl-CoA, which enters the mitochondria to be oxidized. Inhibition of fatty acid synthase leads to an accumulation of malonyl-CoA and causes the inhibition of the rate-limiting enzyme, carnitine palmitoyltransferase-1 (CPT-1). When CPT-1 is inhibited fatty acid oxidation is halted and triglycerides cannot be stored. Failure to store triglycerides in DLBCL cells induces apoptosis.

Current compounds that target the enoyl reductase step of the cycle are isoniazid and triclosan. Known ketoacyl synthase inhibitors include cerulenin, C75, thiolactomycin, and platensimycin. Cerulenin was determined a non-competitive inhibitor of FASN in the 1960s and was initially synthesized from Cephalosporum caerulens. Cerulenin activity stems from covalent modification of active site cysteine and its ability to occupy the long acyl-binding pocket of the KS domain and targets Cys-His-His KS enzymes. Binding occurs through nucleophilic attack by a cysteine residue from KS to open the epoxide ring of cerulenin. FASN becomes inactivated due to the elongation chain undergoing a condensation reaction with malonyl and acetyl CoA residues. This compound has many functions such as antimicrobial/antitumor activity and decrease food intake/body weight in mice. Due to the ability of cerulenin to inhibit the FAS pathway, design of cerulenin could result in better treatment of DLBCL.
1.0.4 Modeling

To date cerulenin has not been able to be modeled to human FAS. High-resolution crystal structures aid in the design of inhibitors, but in the past have only existed for FAS II from bacteria and plants. All bacterial FAS II units have been identified and modeling has been performed with cerulenin/various other FAS inhibitors. Although this might show a general trend of binding energies between proposed ligands, it does not accurately represent human FAS binding. Cerulenin has also been modeled to fungal FAS I, which is the same enzyme type as human FAS. However, these enzymes possess vastly different quaternary structures and further highlights a need for modeling directly to human FAS. Crystal structures of mammalian FAS were determined in 2006 and 2008, but presented less than optimal resolutions of 4.30 and 3.22 angstroms respectively. A 2010 project by Pappenburger et al. produced a crystal structure of the MAT-KS di-domain of human fatty acid synthase.

The reported crystal structure from PDB ID 3HHD highlighted four interacting chains of the KS-MAT di-domain. A hydrogen-binding network of the human KS domain was identified in producing an acyl-binding tunnel. The residues of this tunnel involved in hydrogen bonding include polar Arg224 and Glu333 bidentate charged pair. Hydrogen binding also occurs between Glu333 and Tyr222. The active site of the KS domain involved in binding is Cys161, Phe200, Tyr222, Arg224, His293, His331, Glu333, and Phe395. A cavity filled with polar residues has been hypothesized as a potential site for binding inhibitors and made up of Asp76, Gln78, Glu115, Asn189, Val190, Leu192Thr196, Arg224, and Glu333. It was also reported that Gln136 and Met 132 from chain D were also important residues for defining the active site of human FAS.
Figure 2: Structure of human fatty acid synthase KS-MAT Didomain.\textsuperscript{7}

Representation of chain A (magenta), chain B (red), chain C (green), and chain D (aqua). The didomain is shown by β-ketoacyl synthase (KS) domain linked to the malonyl-ACP transferase (MAT) domain by a linker domain (LD). Trajtenberg \textit{et al.} presented a ligand bound structure of a point-mutated FAS, FabF, from \textit{Bacillus subtilis}. This paper reported that cerulenin is first stabilized in the FAS active site by non-covalent interactions, which allow the active site cysteine to be properly oriented next to cerulenin.\textsuperscript{10} \textit{B. subtilis} Cys 163 then performs a nucleophilic attack on cerulenin carbon three, resulting in opening the epoxide ring.\textsuperscript{10} An important feature of the human FAS active site is that it is larger than FAS from other species.\textsuperscript{10} The reported distance between the top of the active site, Gly113, and the active site Cys161 is 12Å.\textsuperscript{10} This feature is useful for designing cerulenin derivatives because there is significant room for modifications to the unsaturated tail end of the compound.
**Figure 3**: Highlighted overlay of human and *B. subtilis* fatty acid synthase active site residues.\(^7,10\)

(a) Overlay of *B. subtilis* (blue, chain A) and human (magenta, three letter code = chain A, one letter code=chain D) fatty acid synthase active site residues.\(^7,8\) The crystal structure from ILS7 represents the non-covalent interactions between cerulenin and *B. subtilis* active site residues. The figure depicts hydrogen bonding of the carbonyl oxygen of cerulenin carbon four with Cys163 and Phe398 bacterium residues. (b) Crystal structure from ILS8 depicts covalent modification of Cerulenin carbon three by the active site cysteine from *B. subtilis*. The epoxide ring is opened via this modification and results in hydrogen bonding of the free hydroxyl group with Phe399.
1.2.0 Results and Discussion

In order to begin modeling inhibitors against human fatty acid synthase, the 3HHD pdb file (KS-MAT didomain from human FAS chain A) first had to be minimized using YASARA.\textsuperscript{11} A POOL prediction was then performed using the optimized pdb file to predict key active site residues. The results from the POOL prediction correlated with the residues highlighted by Pappenberger \textit{et al.}\textsuperscript{17,18} Six of the seven chain A residues described in the 3HHD paper were identified in the top ten residues of the POOL prediction. These residues were His293, His331, Cys161, Tyr222, Glu333, and Arg 224. The sixth residue mentioned in the paper, Phe395, was still within the coefficient cutoff of 0.001.

Inclusion of chain D would make the protein too large and difficult to model. Thus, chain A was isolated for modeling, even though this was not ideal. The combination of POOL predicted and 3HHD mentioned residues were used to define the box used for Maestro 10.1 GLIDE docking. Key residues Gly113, Cys161, His293, and Gly394 defined the four corners of the grid box. Ligand prep of cerulenin was performed by saving the compound as a SMILES file in ChemDraw before importing to GLIDE.

\textbf{Table 1:} POOL Score for the prediction of important human FAS active site residues

<table>
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<th>Rank</th>
<th>POOL score</th>
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<th>Residue Number</th>
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<td>1.000</td>
<td>0.018</td>
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<td>293</td>
</tr>
<tr>
<td>2.000</td>
<td>0.004</td>
<td>HIS:A</td>
<td>331</td>
</tr>
<tr>
<td>3.000</td>
<td>0.003</td>
<td>CYS:A</td>
<td>161</td>
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<td>6.000</td>
<td>0.001</td>
<td>TYR:A</td>
<td>222</td>
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<tr>
<td>7.000</td>
<td>0.000</td>
<td>GLU:A</td>
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<td>10.000</td>
<td>0.000</td>
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<tr>
<td>24.000</td>
<td>0.000</td>
<td>PHE:A</td>
<td>395</td>
</tr>
</tbody>
</table>
Figure 4: LigPlot 2D modeling of cerulenin using the results from Autodock and Glide docking software

The use of both Glide and Autodock docking programs allowed for an accurate representation of human FAS bound to cerulenin. As visualized by the Ligplot 2D modeling, both software depicted hydrogen bonding between the two most important active site residues; Cys161 and His293. The modeling also revealed hydrophobic interactions (shown by red eyelash lines) between the atoms of cerulenin and the key active site residues.
In order to participate in covalent binding with Cys161, cerulenin first has to participate in non-covalent interactions with the active site. Hydrogen bonding with Cys161 must therefore occur for cerulenin derivatives to be effective FAS inhibitors. Presence of hydrogen bonding and docking score of cerulenin derivatives were determined by Glide docking. LogP values were
determined by ChemAxon software. The derivatives that formed the necessary hydrogen bond with Cys161 included all derivatives except the 2-methyl acid, the protected glycerol, and the glycine peptide derivatives. An effective inhibitor of hFAS can be determined by a docking score that is better than cerulenin, where the more negative the value is best. The derivatives with better docking scores than cerulenin include the acid, amide, n-methyl amide, n-propyl amide, and glycine peptide derivatives. Even though the peptide derivative presented with the best docking score, it did not form a hydrogen bond with Cys161, and would not be an effective inhibitor. Another important aspect for each derivative is their associated logP value, where P is the partition coefficient between octanol and water. A logP value <0.8 is associated with impermeability to the cell membrane. Appropriate logP values were found from the methyl ester, protected glycerol, and the n-propyl amide derivatives.

Figure 6: Glide docking 3D model of cerulenin to human FAS.

Hydrogen bonding observed between His293 and Cys161
Figure 7: Molecular modeling of 2-methyl acid derivative

(a) 2D ligplot of glide modeling: hydrogen bonding observed between His293, Thr295, and Ser112, no hydrogen bonding with Cys161, wrong binding pose and orientation within the binding pocket
(b) 3D glide modeling: no Cys161 hydrogen bonding, and incorrect binding pose
Figure 8: Molecular modeling of acid derivative.

(a) 2D ligplot of glide modeling, hydrogen bonding observed between His293, Gly294, and Cys161, correct binding pose and orientation observed within the binding pocket (b) 3D glide modeling, observed Cys161 hydrogen bonding, and correct binding pose
Figure 9: Molecular modeling of methylester derivative.

(a) 2D ligplot of glide modeling, hydrogen bonding observed between His293, Thr112, and Cys161, correct binding pose and orientation observed within the binding pocket (b) 3D glide modeling, observed Cys161 hydrogen bonding, and correct binding pose
Figure 10: Molecular modeling of protected glycerol derivative.

(a) 2D ligplot of glide modeling, hydrogen bonding observed between His293 and Thr295, no hydrogen bonding observed with Cys161, incorrect binding pose and orientation observed within the binding pocket (b) 3D glide modeling, no observed Cys161 hydrogen bonding, and incorrect binding pose
Figure 11: Molecular modeling of amide derivative.

(a) 2D ligplot of glide modeling, hydrogen bonding observed between His293, Gly394, Ser112, Gln333, and Cys161, correct binding pose and orientation observed within the binding pocket (b) 3D glide modeling, observed Cys161, Ser112, and His293 hydrogen bonding, and correct binding pose
Figure 12: Molecular modeling of amide derivative.

(a) 2D ligplot of glide modeling, hydrogen bonding observed between His293, Gly394, Ser112, Gln333, and Cys161, correct binding pose and orientation observed within the binding pocket (b) 3D glide modeling, observed Cys161, Ser112, and His293 hydrogen bonding, and correct binding pose
Figure 13: Molecular modeling of n-methyl amide derivative.

(a) 2D ligplot of glide modeling, hydrogen bonding observed between His293, Gly394, Ser112, Glu333, and Cys161, correct binding pose and orientation observed within the binding pocket (b) 3D glide modeling, observed Cys161, Ser112, and His293 hydrogen bonding, and correct binding pose
Figure 14: Molecular modeling of glycine peptide derivative.

(a) 2D ligplot of glide modeling, hydrogen bonding observed between His293, Gln13, and Thr295, no hydrogen bonding observed with Cys161, incorrect binding pose and orientation observed within the binding pocket (b) 3D glide modeling, no observed Cys161 hydrogen bonding, incorrect binding pose
CHAPTER 2: SYNTHESIS OF CERULENIN DERIVATIVES

2.0.0 Introduction

2.0.1 Olefin Cross Metathesis

The alkene (olefin) cross metathesis reaction involves a more mild approach for selectively building complex, substituted alkene compounds. Metathesis involves the changing of positions of substituents from different alkenes. The reaction takes place via an alkylidene exchange between alkenes to create two new alkene molecules. In 2005, the Nobel Prize in chemistry was awarded to Yves Chauvin, Robert Grubbs, and Richard Schrock for their contributions in developing this metathesis method.

Chauvin was the first to describe the metathesis mechanism, in 1971. The mechanism involved the use of a catalyst (metal methylene) to form an internal alkene from two terminal alkenes. Chauvin also described a catalytic cycle where a metal methylene formed a metallocyclobutane intermediate from the reaction with an alkene. The metal alkylidene formed by this reaction was able to catalyze the exchange of more alkenes. Chauvin proposed the necessity of the metal-alkylidene complex.

Schrock was the first to develop useful catalysts for metathesis reactions. The problems associated with early catalysts were instability and sensitivity to air and moisture. Schrock and his group at MIT were able to determine the importance of tungsten and molybdenum as active metals for catalyzing alkene cross metathesis. This group was later able to synthesize active catalysts without additives. Using this information, Grubbs was able to develop the first stable and selective metal catalyst for alkene cross metathesis.
The catalyst that Grubbs developed was a vinylidene type with a ruthenium core.\textsuperscript{21} Ruthenium was chosen because experiments found that ruthenium trichloride could polymerize alkenes in solvents as protic as water.\textsuperscript{21} Eventually, Grubbs and his colleagues were able to design commercially available ruthenium based catalysts (Grubbs 1\textsuperscript{st} and 2\textsuperscript{nd} generation) that could be used in metathesis reactions of sterically hindered alkenes.\textsuperscript{21}

Many types of alkene substrates are now able to undergo cross metathesis reactions due to the invention of Grubbs catalysts. Chatterjee et al. explained the importance of properly matching different types of alkenes to selectively yield a higher percentage of cross metathesis product. Four types of alkenes were reported, with type one alkenes being the most reactive.\textsuperscript{21} It was also found that metathesis between two different types of alkenes could result in enhanced selectivity of the desired product.\textsuperscript{21} Alkenes were ranked by their ability for homo-dimerization with more selective reactions occurring between a sterically hindered alkene and internal alkenes.\textsuperscript{21}

Grubbs cross metathesis was chosen for synthesizing cerulenin derivatives for a number of reasons. The first being that Grubbs cross metathesis provides mild conditions that should not affect the sensitive nature of the functional groups on cerulenin. Any acidic or basic reaction condition would either break open the unstable epoxide ring or attack the carbonyl carbons. Second, these reactions conditions should be selective for the cross metathesis between the proposed R groups (glycerol mono-acrylate and n-propyl acrylamide) and cerulenin. Third, the use of Grubbs-II catalyst over others would allow for a higher catalytic stability in both air and moisture.
2.1.0 Results and Discussion

2.1.1 Proof of Concept Reactions

Proof of concept reactions were conducted in order to optimize reaction conditions for the small scale synthesis of cerulenin derivatives. This was the appropriate means for optimizing reaction conditions due to the costly nature of cerulenin. First, the internal alkene octadec-9-en-1-yl acetate was synthesized by the reaction between oleyl alcohol and acetic acid to form octadec-9-en-1-yl; 70% yield.\textsuperscript{22} \textbf{1} underwent a Grubbs cross metathesis reaction with methyl acrylate to produce methyl (E)-11-acetoxyundec-2-enoate.\textsuperscript{22} The first cross metathesis reaction to form \textbf{2} was performed using a solventless system, Hoveyda Grubbs II (1mol\%), and an excess of methyl acrylate (5eq); 95% yield.\textsuperscript{22} The second cross metathesis reaction was performed with Grubbs II (5mol\%), dichloromethane, and an equal amount of methyl acrylate; 90% yield. Due to the insoluble nature of cerulenin the cross metathesis using Grubbs II and DCM was chosen for the synthesis of cerulenin derivatives.

\textbf{Scheme 1}. Reaction Conditions (a) AcOH, 120°, 17hr, 70%; (b) Methyl Acrylate (5eq), Hoveyda Grubbs II 5mol\%, 50°, neat, 95%;\textsuperscript{22} (c) Methyl Acrylate (1eq), Grubbs II, 5mol\%, DCM, reflux, 90%
2.1.2 Synthesis of Cerulenin Derivatives

DLBCL cells, as well as other cancer types, are known to overexpress mono-acyl glycerol lipase. Attaching a glycerol molecule to the unsaturated side of cerulenin could result in effective delivery of cerulenin to cells and across the cell membrane. Once inside the cell, mono-acyl glycerol would could cleave glycerol and leave the carboxylic acid derivative. The molecular modeling data depicted a better binding energy for the acid derivative when compared to cerulenin. Even though the calculated logP value was low for this derivative, it should not matter because it is transported into the cell by means of the glycerol derivative. This makes the glycerol derivative an appropriate pro-drug for a potential inhibitor of fatty acid synthase through its cleavage to a carboxylic acid derivative.

Scheme 2. Reaction Conditions (a) Acryloyl chloride, DCM, TEA, 0° to rt, 3hr, 95%; (b) MeOH, Amberlyst resin, microwave, 60°, 15min, 85%; (c) DCM, Grubbs II, 5mol%, reflux, 21hr

Scheme 2 depicts the synthesis of the glycerol derivative. This began with the nucleophilic addition of solketal to acryloyl chloride to form 4; 95% yield. The product then underwent microwave de-protection (via Amberlyst ion exchange resin) to form 5; 85% yield. Grubbs-II
catalyst aided the cross metathesis between cerulenin and 5 the desired product 6. Methyl (E)-11-acetoxyundec-2-enoate was identified by mass spectrometry but due to the small amount of material and functional group sensitivity, purification and isolation resulted in the degradation of the final product.

The n-propyl amide derivative was also proposed for synthesis because it presented the best docking score from molecular modeling. This molecule also possessed a logP value within the appropriate cutoff for cell membrane permeability. Reaction Scheme 3 highlights the nucleophilic acyl substitution reaction between n-propyl amine and acetic acid to form 7 (65% yield), followed by Grubbs II cross metathesis reaction between cerulenin and 7 to form the desired derivative. Reaction monitoring by TLC showed disappearance of both starting materials and appearance of two product spots. Analysis by mass spectrometry depicted degradation of the desired product by opening of the epoxide ring. There was also a considerable amount of homodimerization of the n-propyl amine starting material. Degraded product could not be quantified due to previously mentioned separation and isolation problems.

Scheme 3. Reaction Conditions (a) Acryloyl chloride, EtOAc, TEA, 0° to rt, 24hr, 65%; (b) DCM, Grubbs II, 5mol%, reflux, 21hr
2.2.0 Experimental Methods

\[
\begin{align*}
C_{17}H_{35}OH & \quad \rightarrow \quad C_{17}H_{35}C\equiv C \quad CH_2COO\quad \text{(E)-octadec-9-en-1-yl acetate (1)}
\end{align*}
\]

To a round bottom flask (250mL) was added oleyl alcohol (0.195mol, 60mL) and acetic acid (0.98mol, 56.2 mL) while stirred. The reaction was allowed to reflux overnight at 120°C. The reaction was then allowed to cool to room temperature and excess acetic acid was removed in vacuo. The mixture was washed with water, dried over magnesium sulfate and purified by column chromatography 20% Ethyl acetate/Hexanes (70%) yield. \[^1\]HNMR (400MHz, CDCl\textsubscript{3}) δ 5.35–5.33 (m, 2H), 4.06–4.03 (t, J = 7 Hz, 2H), 2.04–1.98 (m, 7H), 1.63–1.55 (m, 2H), 1.30 (s, 22H), 0.89–0.86 (t, J = 7 Hz, 3H) ppm. \[^13\]CNMR (400MHz, CDCl\textsubscript{3}) δ 130.21, 64.88, 32.13, 29.99, 29.95, 29.75, 29.63, 29.55, 29.46, 29.43, 28.83, 27.44, 27.40, 26.13, 22.91, 21.25, 14.34
methyl (E)-11-acetoxyundec-2-enoate (2)

To a glass microwave tube (10mL) was added octadec-9-enyl acetate (1 eq, 1.4mmol, 0.5mL) and methyl acrylate (5eq, 7mmol, 0.6mL) while stirring and heated to 50°C. To the reaction mixture was added Hoveyda Grubbs II (1mol%, 0.014mmol, 8.8mg) and left to react for 21hr. The reaction was quenched with ether and product was isolated via column chromatography in 20% Ether/Hexanes (95% yield). $^1$HNMR (400MHz, CDCl$_3$) δ 7.23-6.94 (m, 1H), 5.84-5.76 (d, $J$=16, 1H), 4.06-4.04 (t, $J$= , 2H), 3.69 (s, 3H), 2.20-2.15 (m, 2H), 2.02 (s, 3H), 1.59-1.55 (m, 2H), 1.46-1.42 (m, 2H), 1.29 (s, 8H) ppm.
methyl \((E)\)-11-acetoxyundec-2-enoate (3)

To a glass microwave tube (10mL) was added octadec-9-enyl acetate (1 eq, 1.4mmol, 0.5mL), methyl acrylate (1eq, 1mmol, 0.13mL) and DCM (2mL) while stirring and heated to 40°C. To the reaction mixture was added Grubbs II (5mol%, 0.014mmol, 8.8mg) and left to react for 21hr. The reaction was quenched with ether and product was isolated via column chromatography in 20% Ether/Hexanes (90% yield). \(^1\)HNMR (400MHz, CDCl\(_3\)) \(\delta\) 7.25-6.920 (m, 1H), 5.82-5.78 (d, \(J = 16\), 1H), 4.05-4.02 (t, \(J = \), 2H), 3.71 (s, 3H), 2.21-2.16 (m, 2H), 2.03 (s, 3H), 1.62-1.57 (m, 2H), 1.46-1.42 (m, 2H), 1.29 (s, 8H) ppm. \(^{13}\)CNMR (400MHz, CDCl\(_3\)) \(\delta\) 171.46, 167.41, 149.93, 121.08, 64.81, 51.60, 32.39, 29.46, 29.33 29.23, 28.79, 28.18, 26.08, 21.24
(2,2-dimethyl-1,3-dioxolan-4-yl)methyl acrylate (4)

To a round bottom flask (250mL) was added solketal (6g, 5.64mL, 0.045mol) and dichloromethane (80mL) and stirred at 0°C. To the reaction mixture was added TEA (7mL) and was left to react for five minutes. A solution containing dichloromethane (20mL) and acryloyl chloride (4.8mL) was then added slowly to the reaction mixture. The reaction was allowed to react for one hour at room temperature. Product was isolated by the addition of sodium bicarbonate, organic phase extracted, dried over magnesium sulfate, and product was concentrated under reduced pressure. The product (95% yield) was purified by column chromatography in 20% ethyl acetate/hexanes. $^1$HNMR (400MHz, CDCl$_3$) δ 6.58-6.42 (dd, 1H), 6.19-6.06 (m, 1H), 5.87-5.84 (d, 1H), 4.37-3.75 (m, 5H), 1.43 (s, 1H), 1.37 (s, 1H) ppm. $^{13}$CNMR (400MHz, CDCl$_3$) δ 165.98, 131.53, 128.13, 109.94, 73.72, 66.42, 64.88, 26.82, 25.51 ppm.
2,3-dihydroxypropyl acrylate 5

To a glass microwave tube (10mL) was added 4 (186mg, 1mmol), methanol (5mL), and Amberlyst 15 ion exchange resin (100mg). The reaction tube was placed inside the cavity of the microwave and irradiated at 60°C for 15min. The reaction was then allowed to cool to room temperature and then filtered using methanol. Product purified by column chromatography in 10% methanol/dichloromethane. The product (85% yield) was purified by column chromatography in 20% ethyl acetate/hexanes. $^1$HNMR (400MHz, CDCl$_3$) $\delta$ 6.44-6.32 (m, 1H), 6.10-6.02 (m, 1H), 5.78-5.75 (t, $J$=10.4Hz, 1H), 4.29-3.66 (m, 7H) ppm. $^{13}$CNMR (400MHz, DEPT, CDCl$_3$) $\delta$ approx. 77, 66, 62 ppm.
**2,3-dihydroxypropyl (2E,5E)-9-((2R,3S)-3-carbamoyloxiran-2-yl)-9-oxonona-2,5-dienoate**

To a reaction vial (4mL) was added 5 (45μmol, 8.45mg) and cerulenin (45μmol, 10mg) in dichloromethane (0.5 mL) while stirring at 40°C. To the reaction mixture was added Grubbs-II catalyst (2.25μmol, 1.9mg) in dichloromethane (0.5mL). The reaction was monitored by TLC and allowed to react overnight. ChemDraw prediction $^1$HNMR: 7.21 (2H), 6.89 (1H), 5.77 (2H), 5.49 (1H), 5.35 (1H), 4.41 (1H), 4.19 (1H), 4.16 (1H), 3.94 (1H), 3.70 (2H), 3.59 (1H), 3.53(1H), 2.80 (2H), 2.49 (2H), 2.24 (2H) ppm. Observed similar peaks from crude $^1$HNMR (400MHz, CDCl$_3$) δ 6.99, 5.48, 4.26-4.22, 3.62, 3.49, 2.76, 2.17 ppm. ChemDraw predictions shown below. Experimental $^1$HNMR supplied in supplementary materials.
To a round bottom flask (50mL) was added propyl amine (1mmol, 74.8μL) in acetonitrile (19.8mL) and triethyl amine (686μL). The reaction mixture was cooled to 0°C and was added acryloyl chloride (2.5 mmol, 198μL) dropwise. The mixture was allowed to react at room temperature and monitored by TLC. The reaction mixture was concentrated under reduced pressure and was product extracted with ethyl acetate. Product was washed with 1N HCl, sodium hydroxide, then brine and dried over magnesium sulfate, filtered, and evaporated. Product was purified by flash chromatography in 15% EtOAc/Hexanes to yield 4mg of product. $^1$HNMR (400MHz, CDCl$_3$) $\delta$ 6.68-6.62 (m, 1H), 6.46-6.40 (m, 1H), 5.81-5.78 (t, J=10.4Hz, 1H), 3.74-3.70 (m, 2H), 1.65-1.57 (m, 2H), 0.92 (t, 3H) ppm. $^{13}$CNMR (400MHz, DEPT, CDCl$_3$) $\delta$ 169.12, 130.87, 130.18, 46.45, 22.68, 11.48 ppm.
To a vial (4mL) was added n-propyl acrylamide (36μmol, 4mg) and cerulenin (1.2eq, 45μmol, 10mg) in dichloromethane (0.5mL). The reaction mixture was stirred, heated to 40°C, and was added Grubbs-II catalyst (2.25μmol, 1.9mg) in dichloromethane (0.5mL). The reaction was monitored by TLC and allowed to react overnight. ChemDraw prediction $^1$HNMR; 8.41 (1H), 7.21 (2H), 6.63 (1H), 6.18 (1H), 5.49 (1H), 5.35 (1H), 3.70 (2H), 3.17 (2H), 2.80 (2H), 2.49 (2H), 2.24 (2H), 1.52 (2H), 0.86 (3H) ppm. Observed similar peaks from crude $^1$HNMR (400MHz, CDCl$_3$) δ 6.67, 6.39-6.23, 5.36-5.30, 3.84-3.72, 3.18, 2.85, 2.76, 2.28-2.2, 1.66-1.51, 0.86-0.83 ppm. ChemDraw prediction shown below, and experimental $^1$HNMR supplied in supplementary materials.
CONCLUSIONS AND FUTURE WORK

Molecular Modeling techniques proved effective in determining potentially better inhibitors of the human KS domain of FAS. Cerulenin modeled to the KS active site revealed the potential for further modification of the unsaturated tail end of cerulenin due to the increased space in the human KS active site. It was also discovered that certain modifications to the tail end of cerulenin could result in a flip of position of the inhibitor in the active site. This change in binding pose made it impossible for some proposed derivatives (i.e. protected glycerol, 2-methyl acid and the glycine peptide derivative) to undergo essential hydrogen bonding with Cys161. It was shown that more hydrophobic modifications (methyl ester, and amide) resulted in improved docking scores. The n-propyl amide derivative displayed the best potential for improved fatty acid synthase inhibition due to its docking score, Cys161 hydrogen bonding, and logP value.

Proof of Grubbs-cross metathesis reactions, between an internal and highly substituted alkene were successful, failed to be isolated from the synthesis of cerulenin derivatives. TLC and mass spectrometry depicted the success of the cross metathesis reaction, but purification techniques resulted in the degradation of both products. Mass spectrometry data from the n-propyl amide derivative showed the opening of the epoxide group to form a hydroxyl. Future work would need to involve optimization of purification and isolation techniques for the proposed derivatives. Another potential improvement to the reaction conditions would be to synthesize a new catalyst that might prevent opening of the epoxide ring.

Once these derivatives are successfully purified, cell assays will be performed to identify their IC\textsubscript{50} values. The viability of DLBCL cells will first be checked by the MTT assay. Apoptosis of cells will then be identified by flow cytometric analysis of Annexin-V/propidium iodide. This
will be essential in determining whether the trend in docking score actually correlates with improved IC$_{50}$ values of the cerulenin derivatives. From this point, future derivatives can be designed and tested using the improved molecular modeling of derivatives docked to the human KS domain of hFAS. Optimization of reaction conditions and purification will also aid in the ability to synthesize future derivatives.
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APPENDIX: REPRESENTATIVE NMR DATA