Investigations into the Significance of Epidermal Fatty Acid Binding Protein (FABP5) in Breast Cancer Survival and Design of Novel FABP5 Inhibitors

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Abstract

In the United States, 1 in 8 women will develop invasive breast cancer in their lifetime. By the end of 2019 alone, it is expected that approximately 300,000 new cases of invasive and in situ breast cancer will be reported. Epidermal Fatty Acid Binding Protein (FABP5), predominantly targeted for the alleviation of chronic pain, has recently been implicated in the proliferation of breast cancer. Specifically, it has been demonstrated that over expression of FABP5 is directly correlated to upregulation of Vascular Endothelial Growth Factor Receptors (VEGFR), which regulate tumor metastasis in breast cancer. Experiments were conducted to investigate the effects of competitive FABP5 inhibitors SB-FI-26 and SB-FI-103 on the survival rate of breast cancer cells, through an MTT assay. Both inhibitors demonstrated decreased cell viability at 72 hours (SB-FI-26 IC_{50} = 11.55 μM), with SB-FI-103 exhibiting enhanced potency (SB-FI-103 IC_{50} = 7.886 μM). Additionally, 138 novel FABP5 inhibitors were computationally designed using the known FABP5 inhibitor, 3-(2(4-benzylpiperidin-1-yl)-2-oxoethyl)-1-methyl-1*H*-indole-2-carboxylate (SB-FI-31), as a scaffold. Following *in silico* analysis, three putative inhibitors were identified, demonstrating both sufficient solubility (cLogP $\lessapprox$ 5.0) and free binding energy (< -9.0 kcal/mol). Furthermore, all three compounds exhibited canonical interactions with Arg 129 and Tyr 131, strictly conserved residues across all members of the FABP family. Results of the established that inhibition of FABP5 is sufficient to prohibit cell growth in an MCF-7 breast cancer cell line, suggesting these inhibitors may serve as viable breast cancer therapeutics.

1. Introduction

1.1 The role of Fatty Acid Binding Proteins in pain alleviation

Fatty acids are integral to bodily systems due to their dual role as both energy sources and mediators of cell signaling [1]. In turn, Fatty Acid Binding Proteins (FABPs), intracellular carriers of fatty acids, are key components in metabolic regulation [2,3]. Specifically, FABPs are capable of making interactions with anandamide (AEA), an endogenous ligand for cannabinoid receptors [4]. In particular, AEA binds to and activates Cannabinoid Receptor 1 (CB1), which upon activation, will produce anti-nociceptive effects [5,6]. Subsequently, AEA has been found to play an extremely significant role in pain tolerance [7]. This is especially important, as present pain alleviation treatments are limited to Nonsteroidal Anti-inflammatory Drugs (NSAIDs) and opioids [8,9]. Both NSAIDs and opioids induce negative side effects and as a result, current research regarding pain alleviation has looked towards CB1 receptors and AEA.

One characteristic of endocannabinoids, like AEA, is that they are rapidly synthesized and then degraded [10]. The hydrolysis of AEA is executed by Fatty Acid Amide Amylase (FAAH), in which AEA is broken down into Arachidonic Acid and Ethanolamine (Fig. 1).

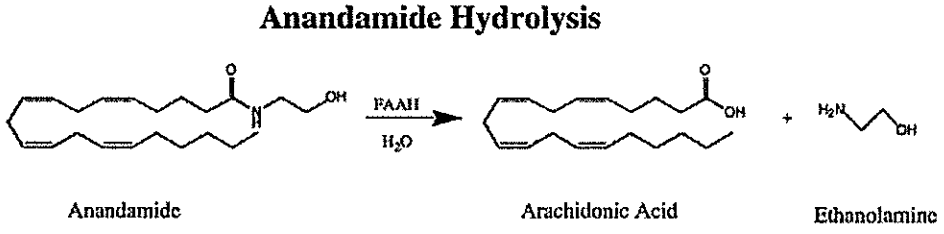


Fig. 1 Degradation of Anandamide into Arachidonic Acid and Ethanolamine The endocannabinoid Anandamide

Degradation of AEA will result in a decrease of activated CB1 receptors, and subsequently, a decrease in pain alleviation. Therefore, FAAH appeared to be a perfect inhibition target for pain alleviation, and many studies initially focused on producing compounds that could inhibit FAAH

[12]. However, in phase I clinical trials, patients who were treated with FAAH inhibiting compounds exhibited signs of extreme neurological damage, and in one case- brain death [13, 14]. Thus, pharmaceutical companies terminated the testing of FAAH inhibiting drugs.

Research then turned to inhibition of FABP. FABPs transport AEA to their site of degradation- FAAH, and inhibition of FABP activity would also result in a decrease in AEA hydrolysis (Fig. 2).

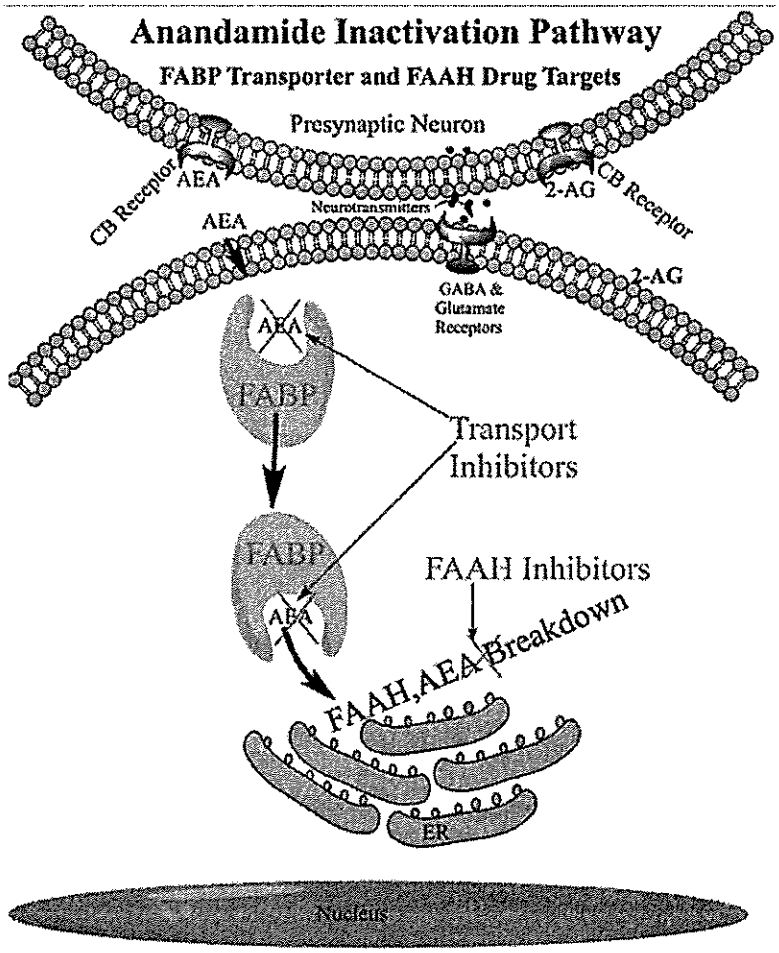


Fig. 2 Inactivation of Anandamide Anandamide is transported by FABP to FAAH to be degraded. Inhibition of FAAH was initially proposed to promote pain alleviation, however studies have shown that inhibition of FAAH often has fatal consequences. Therefore, studies turned to FABPs as a potential pain alleviation target. Image Source: Reference [11]

1.2 Epidermal FABP (FABP5)

FABPs are a family of proteins with members ranging from FABP1 to FABP12. Each member is expressed in different parts of an organism, and research has shown that inhibition of specific FABPs will have adverse effects [15]. For example, knockdowns of Heart-Type FABP (FABP3) in zebrafish have been demonstrated to have severe effects on cardiac development and mitochondrial functions [16].

For the purposes of pain alleviation, Epidermal FABP (FABP5) became the inhibition target of choice because studies have shown that inhibition of FABP5 is able to produce analgesic effects without adverse consequences [17].

1.3 FABP5 and Breast Cancer

Research surrounding FABP5 has been primarily in the context of pain alleviation, however recently, FABP5 has been demonstrated to play a profound role in the progression and metastasis of breast cancer [18,19,20].

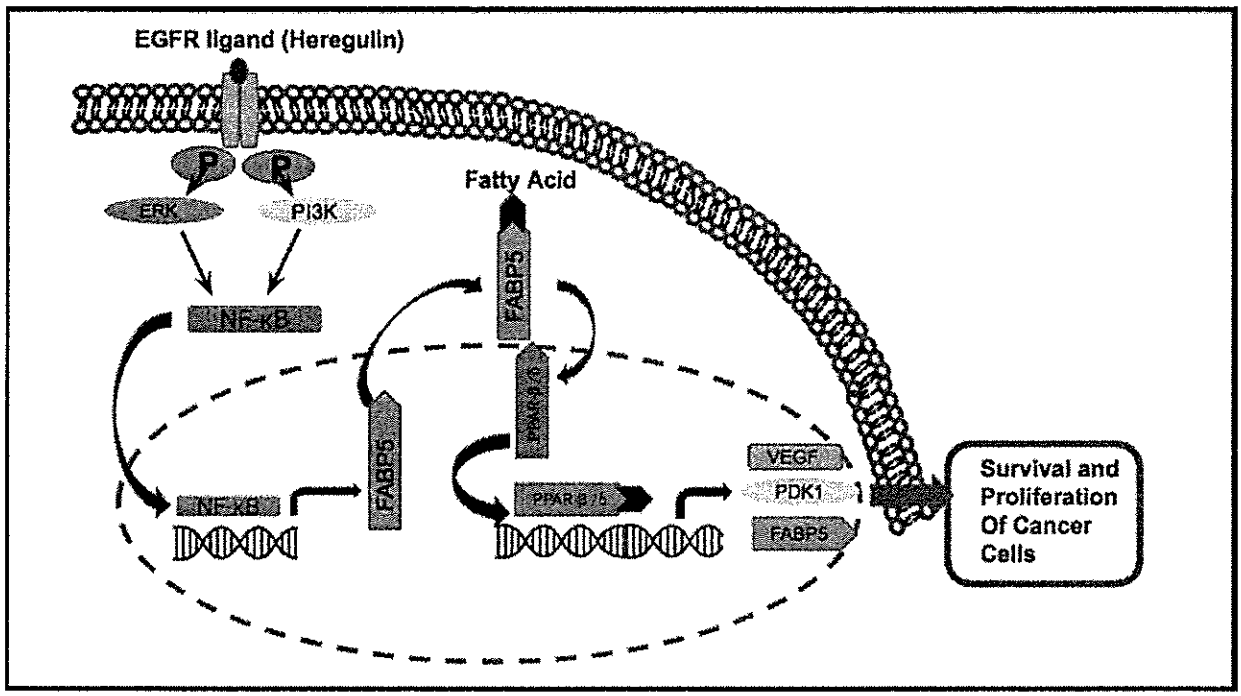


Fig. 3 Upregulation of FABP5 promotes cell proliferation in breast cancer It has been demonstrated that over expression of FABP5 is directly correlated to upregulation of Vascular Endothelial Growth Factor Receptors (VEGFR), which regulate tumor metastasis in breast cancer. FABP is responsible for transporting ligands to PPARβ/δ, effectively enhancing the transcription activity of the PPARβ/δ pathway. Image Source: Image was student generated from reference [21].

FABP5 leaves the nucleus and binds to fatty acids. Fatty acid bound FABP5 then translocates back into the nucleus and transfers the fatty acid to nuclear receptor peroxisome proliferator-activated receptor beta/gamma (PPARβ/δ), effectively inducing transcription of

genes associated with cell survival and proliferation. This FABP5/PPARδ pathway is essential to the proliferation of breast carcinoma cells by activated EGFR [22]. Additionally, since EGFR is overexpressed in breast cancer cells, the FABP5/PPARδ pathway will further exacerbate the breast cancer.

1.4 FABP5 inhibiting compounds

Through a virtual screening of over one million compounds, 48 compounds with the potential to inhibit FABP5 were found. These 48 compounds were tested for biological activity against FABP5 with a fluorescent displacement assay. Four of the 48 compounds were found to exhibit greater than 50% inhibition at 10 μM [23] (Fig. 4).

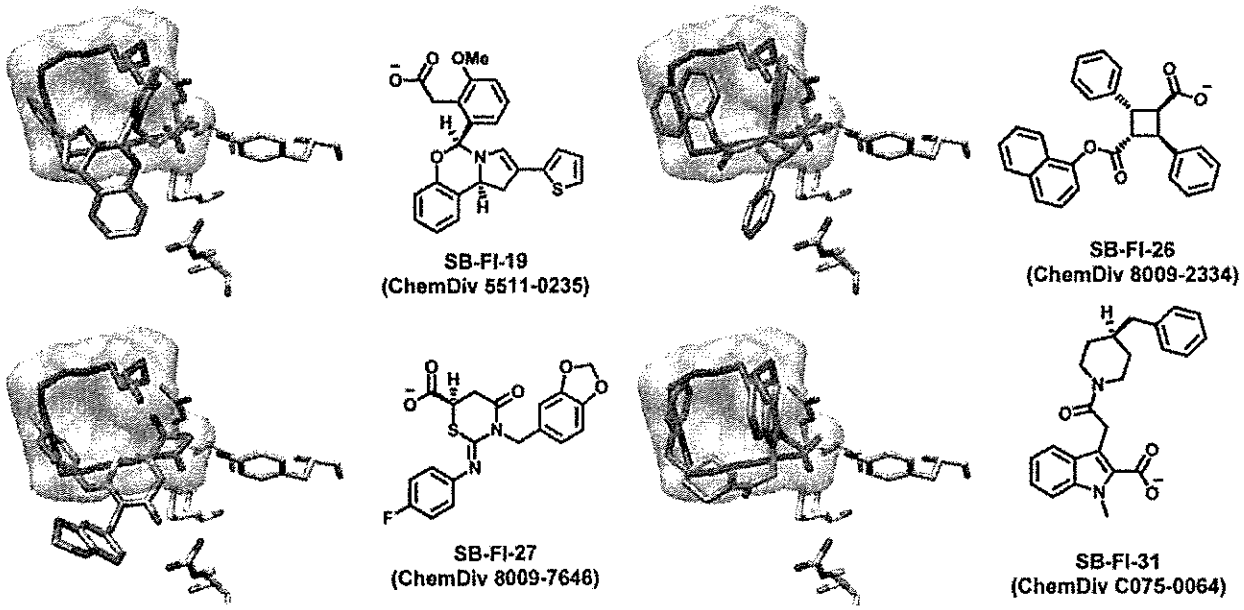


Fig. 4 Lead compound inhibitors of FABP5 Over 1 million compounds were screened for affinity to FABP5. Four "lead" compounds were found to exhibit an inhibition of 50% or more at 10 μM. All four of the compounds, SB-FI-19, SB-FI-27, SB-FI-26, and SB-FI-31 contain at least one carboxylic acid. Carboxylic acids are an important structure because they are involved in specific charge-charge interactions, and therefore play a significant role in binding to target proteins. Image Source: Reference [23].

These four compounds were deemed "lead" compounds, and studies have been conducted on the lead compounds themselves, or derivatives of the lead compounds. SB-FI-26, is the only lead compound containing an alpha truxillic acid, and there have been many derivatives created

based off of SB-FI-26. SB-FI-103, for example, is a compound that was created, using SB-FI-26's alpha truxillic acid as a core.

1.5 FABP5-inhibiting compound requirements

In order to create a FABP5-inhibiting drug with the potential to be tested in clinical trials, the compound has to have a cLogP below or around 5 and a lower free binding energy than previously created FABP5 inhibiting compounds.

cLogP is a measure of a compound's hydrophobicity derived from its partition coefficient in an octanol-water system [24]. According to Lipinski's Rule of Five (a typical guideline used to create novel drugs), compounds should aim to have a cLogP of less than or around five, to ensure successful absorption via oral delivery [25].

A compound's "docking" score is obtained from the computer program, Autodock. To measure the the free binding energy of a particular compound, the entropic contribution is subtracted from the enthalpic term [26]. When a ligand binds to a protein, the enthalpic term decreases because of favorable intermolecular interactions. However, the entropic contributions increase due to loss of degrees of freedom (based on tightness of fit). Therefore, the more negative a binding score is, the more favorably it binds to FABP5.

Finally, the created compound has to make canonical interactions with FABP5. Past studies have shown that FABP5-inhibiting compounds that contain carboxylic acids form a salt bridge with Arginine 129 and hydrogen bonds with Tyrosine 131 [27]. Therefore, in order to have a viable FABP5 inhibiting drug, the compound should also be predicted to bind at those specific locations.

1.6 Project Rationale

The impact of FABP5 inhibiting compounds on the progression of breast cancer is still unknown. In this study, breast cancer cells were treated in varying concentrations with FABP5 inhibitors SB-FI-26 and SB-FI-103. Understanding the extent of the relationship between FABP and breast cancer is crucial towards the development of breast cancer treatments. Furthermore, this study also aimed to computationally create novel FABP5 inhibiting compounds based off of SB-FI-31, an understudied FABP5 inhibitor. Creating these analogs of SB-FI-31 will lead to an advancement in potential FABP5 inhibiting compound structures.

2. Methodology

2.1 Cell Lines, Culture, and Treatment

MCF-7, a human breast cancer cell line, was obtained from the American Type Culture Collection (ATCC HTB-22). Cells were kept in Roswell Park Memorial Institute (RPMI) 1640 medium with 10% Fetal Bovine Serum (FBS) and 1% Penicillin G Sodium Salt and incubated in 5% Carbon Dioxide (CO_2) at 37 °C. Medium was changed every 3 days.

2.2 Cell Viability Assay (MTT Assay)

MCF-7 cell cultures were inspected and confluent plates that exhibited the best cell growth were chosen for MTT assay. Cells were detached using Trypsin-EDTA and plated onto a cell counting plate. Cells were counted to a final concentration of 7,000 cells per 100 μL of RPMI solution. The cell solution was pipetted into the wells and the 96 well plate was then incubated for 24 hours.

After a 24-hour incubation period, serial dilutions of SB-FI-26 and SB-FI-103 were prepared with 0.50% DMSO in RPMI medium containing 1% FBS. Drug solutions were then pipetted into the 96 well plate in increasing concentrations, from 0 μM (control) to 100 μM .

After incubation, MTT powder was dissolved in RPMI solution to a final concentration of 0.5 mg/mL. The existing medium in the 96 well plate was then aspirated out and 100 μL of MTT solution was added to each well. After a 4 hour incubation, the MTT solution was aspirated out and the remaining crystals were solubilized using DMSO. The plate was then read using a VICTOR Multilabel Plate Reader at an absorbance of 570 nm.

2.3 Computational Design of SB-FI-31 Analogs

Chemdraw 18.0 - a PerkinElmer software product - was used to computationally design novel FABP inhibiting compounds, in which the lead compound SB-FI-31 was used as a scaffold in the design process. 2-D analogs were primarily made by adding constituents onto different portions of SB-FI-31 (Fig. X). ChemDraw was also utilized to obtain the cLogP score of the created compound.

2.4 Energy Minimization of Designed Compounds

To visualize the created analogs, a three-dimensional molecule was generated with Avagadro 1.90.0, a molecular editor and visualizer program. Using Avogadro, the SB-FI-31 derivatives were refined through the minimization of intermolecular energies (using the MMFF94S force field), optimization of bond angles, and the adjustment of hydrogens to mimic the human physiological pH of 7.4.

2.5 Molecular Docking of Created Compounds

The program AutoDock-1.5.6, was used to molecularly "dock" the created compounds with the target protein (FABP5), in order to investigate favorable ligand/protein interactions. Autodock was able to generate a variety of binding poses for the compounds, which were then docked within FABP5. Autodock then "scored" each compound to show the estimated free binding energy.

2.6 Visualization of Docked Compounds with Fatty Acid Binding Protein 5

The highest scoring compounds were visualized with FABP5 in the program UCFS Chimera. Through Chimera, the bonds between the compounds and FABP5 were visualized. The analogs were then checked to see if they made canonical interactions with Arginine 129 and Tyrosine 131 in FABP5.

2.7 Prediction of Pharmacokinetic Properties

The highest scoring compounds that made canonical interactions were then screened *in silico* through a pharmacokinetic properties predictor - pkCSM. Through pkCSM, the ADMES toxicity, hepatotoxicity, and the Oral Rat Acute Toxicity (LD50) of the selected compounds were predicted. Additionally, compounds were tested to see if they were HERGI or HERGII inhibitors. Testing these specific properties of the created compounds ensures that these derivatives are safe to use in future clinical trials.

3. Results and Discussion

3.1 Effects of SB-FI-26 on MCF-7 Cell Viability

MCF-7 cells treated with SB-FI-26 (Fig. 5A) for 48 hours showed a decrease in cell viability. At SB-FI-26's lowest concentration of 0.39 μM, 90% of MCF-7 cells were still viable; while SB-FI-

26's highest concentration was able to decrease MCF-7 cell viability to 47%. However, data taken from MCF-7 with SB-FI-26 at 48 hours was inconclusive and the predicted cell survival curve was unable to converge to a MCF-7 viability less than 50%. Therefore, an IC_{50} was unable to be determined from the data (Fig. 5B).

MCF-7 cells were treated with SB-FI-26 for 72 hours, and at its highest concentration of 100 μM, SB-FI-26 reduced MCF-7 cell viability to 30%, which is 17% lower than 100 μM at 48 hours. A cell survival curve was generated and the IC50 was determined to be 11.55 μM (Fig. 5C).

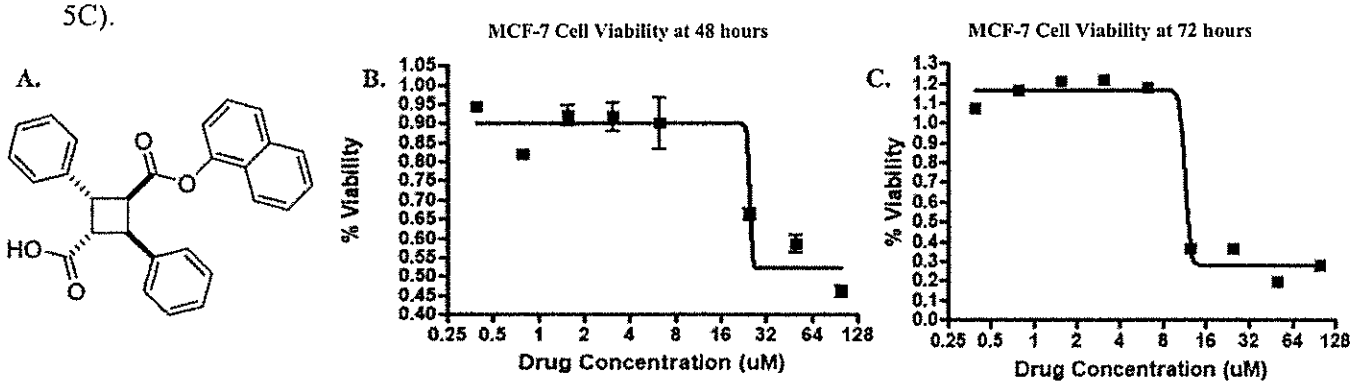


Fig. 5 SB-FI-26 decreases MCF-7 Cell Viability at 48 and 72 hours (A) SB-FI-26 is an α-truxillic acid 1-naphthyl monoester. **(B)** SB-FI-26 was unable to kill 50% of the MCF-7 cells when treated for 48 hours. Therefore, an IC_{50} was unable to be obtained. MTT assays were conducted in triplicate. Data was organized using GraphPad Prism. (C) SB-FI-26 was able to kill more than 50% of MCF-7 cells when incubated for 72 hours, with an IC_{50} of 11.55 μM. Data was organized using GraphPad Prism.

The results of MCF-7 incubation with SB-FI-26 for 48 and 72 hours indicates that SB-FI-26 is capable of prevent cell growth in breast cancer cells due to the inhibition of FABP5. However, SB-FI-26 is more effective when incubated at 72 hours compared to an incubation of 48 hours. This implies that SB-FI-26 may not be a fast-acting drug and would need more time within the body to properly restrict cancer growth.

3.2 Effects of SB-FI-103 on MCF-7 Cell Viability

SB-FI-103 (Fig. 6A) is a FABP5 inhibiting derivative of SB-FI-26 that has exhibited a higher binding specificity to FABP5 than SB-FI-26 (unpublished laboratory data). Due to the

higher binding specificity to FABP5, it was hypothesized that SB-FI-103 would be more successful than SB-FI-26 in inhibiting MCF-7 viability.

Incubation of SB-FI-103 with MCF-7 cells for 48 hours yielded an IC_{50} of 20.87 μM, while an incubation of 72 hours yielded an IC_{50} of 7.886 μM (Fig. 6B). At the lowest concentration of 0.0781 μM of SB-FI-103, the 48 hour trial had 95% MCF-7 cell viability, while

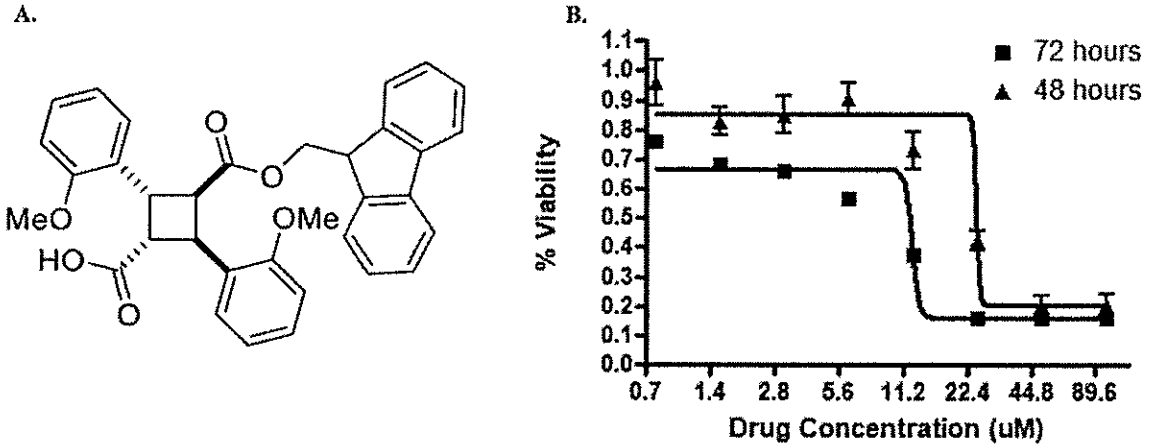


Fig. 6 SB-FI-103 decreases MCF-7 Cell Viability at 48 and 72 hours (A) SB-FI-103 is a derivative of the α-truxillic acid 1-naphthyl monoester SB-FI-26. Studies have shown that SB-FI-103 exhibits a higher binding specificity to FABP5 than SB-FI-26 (B) SB-FI-103 had an IC50 of 20.87 μM when incubated with MCF-7 cells for 48 hours. MTT assays were conducted in triplicate. Data was organized using GraphPad Prism. (C) SB-FI-103 was able to kill more than 50% of MCF-7 cells when incubated for 72 hours, with an IC_{50} of 7.886 μM. MTT assays were conducted in triplicate. Data was organized using GraphPad Prism.

the 72 hour trial had 75% cell viability. However, at the highest concentration of SB-FI-103, the 48 hour and 72 hour trial had cell viabilities of 22% and 20% (respectively). While there is a difference of 2% in MCF-7 viability at 48 and 72 hours at the highest concentration of SB-FI-103, the lowest concentration of SB-FI-103 at 48 and 72 hours had a difference of 17%. This implies that low concentrations of SB-FI-103 are more effective in impeding MCF-7 viability when incubated for longer periods of time.

SB-FI-103 had a lower IC_{50} compared to SB-FI-26 at 72 hours and is therefore a more potent MCF-7 inhibitor. However, data from both SB-FI-26 and SB-FI-103 suggests that FABP5

inhibiting compounds' ability to decrease MCF-7 viability increases as time increases from 48 to 72 hours.

3.3 Creation of Preliminary SB-FI-31 Analogs

Preliminary compounds were created and compared with SB-FI-31 (Fig. 7). It was discovered through SB-CY-30, that replacing the indole with an indane in SB-FI-31 resulted in a

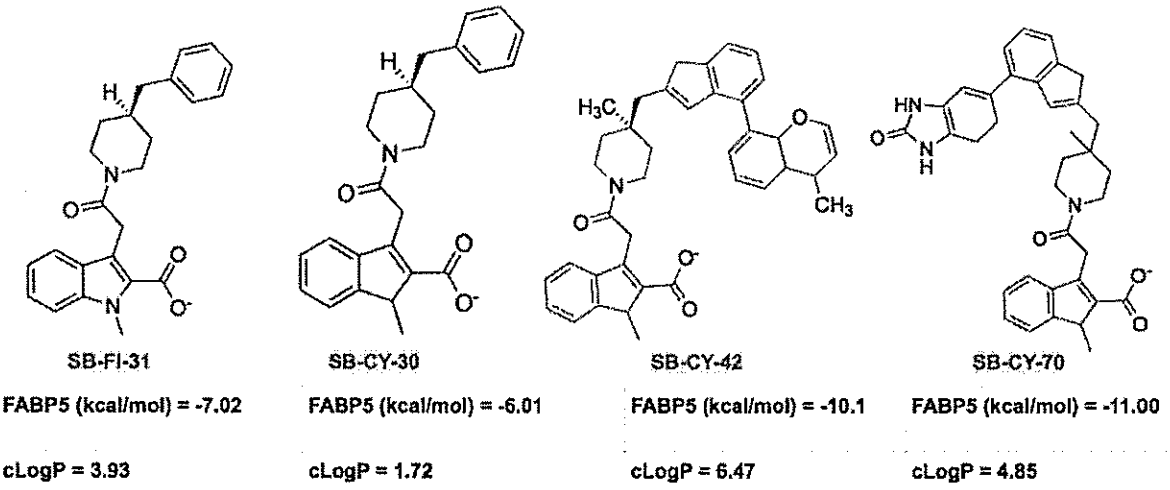


Fig. 7 First Generation Analogs of SB-FI-31 First generation analogs of SB-FI-31 include SB-CY-30, SB-CY-42, and SB-CY-70. SB-CY-30's structure replaced the indole in SB-FI-31 with an indane. This resulted in a higher solubility due to a 2.2 decrease in cLogP. SB-CY-42's replaced the methyl-benzene constituent of SB-FI-31 with a 5-methyl-3,4-dihydro-2H-chromene. SB-CY-42 had a lower solubility compared to SB-FI-31 and SB-CY-30, however its free binding energy was lower than both. SB-CY-70 had an optimal solubility and free binding energy, however upon visualization with FABP5, SB-CY-70 wasn't capable of making canonical interactions. Image generated by ChemDraw.

decrease of 2.21 in cLogP and a 1.01 kcal/mol increase in free binding energy. With SB-CY-42, a 5-methyl-3,4-dihydro-2H-chromene was incorporated into the methyl-benzene constituent of SB-FI-31. This led to a favorable

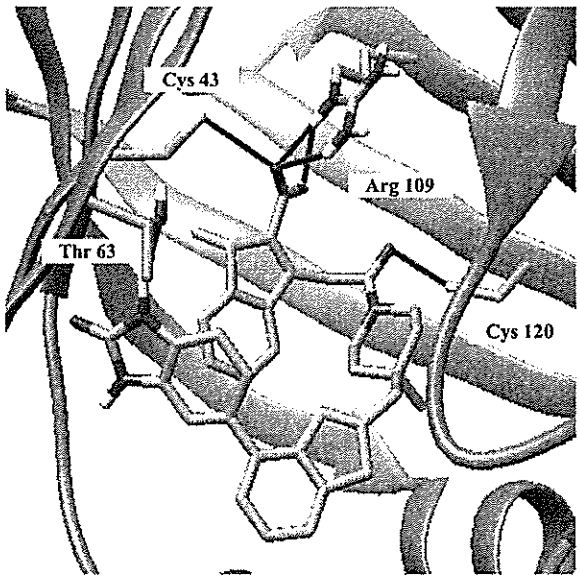


Fig. 8 SB-CY-70's interactions with FABP5 The carboxylic acid of SB-CY-70 makes interactions with Cys 43 and Arg 109. Cys 120 binded with the carbonyl constituent. Thr 63 binded with 1,3,4,5-tetrahydro-2H-benzo[d]imidazole-2-one. SB-CY-70 was unable to make canonical interactions with Arg 129 and Tyr 131, which is located in the binding pocket of FABP5. SB-CY-70 was visualized with FABP5 using UCSF Chimera.

3.01 kcal/mol decrease of binding energy compared to SB-FI-31 but a cLogP of 6.47, which exceeds Lipinski's rule. SB-CY-70 took the structure of SB-CY-42, but replaced the 5-methyl-3,4-dihydro-2H-chromene with a 1,3,4,5-tetrahydro-2H-benzo[d]imidazole-2-one. SB-CY-70 had a cLogP of 4.85 and a free binding energy that was -3.98 kcal/mol lower than SB-FI-31's. SB-CY-70 became a potential interest because it satisfied the solubility requirements for a drug like compound and it was predicted to have a tighter binding fit than SB-FI-31. Upon visualization of the SB-CY-70 and its interactions (Fig. 8), it was found that the carboxylic acid constituent of SB-CY-70 made interactions with Cys 43 and Arg 109. There are additional interactions between the carbonyl and Cys 120, and the 1,3,4,5-tetrahydro-2H-benzo[d]imidazole-2-one with Thr 63. However, none of the interactions were canonical and thus, SB-CY-70 was deemed unfit for further analysis.

3.4 Selection of SB-CY-138 for Further Studies

This study created and analyzed the docking score of 138 compounds in relation to SB-FI-31 (Fig. 9). Out of the 138, 111 compounds had a lower free binding energy with FABP5 compared to SB-FI-31, with -8.44 kcal/mol being the average binding energy for the compounds.

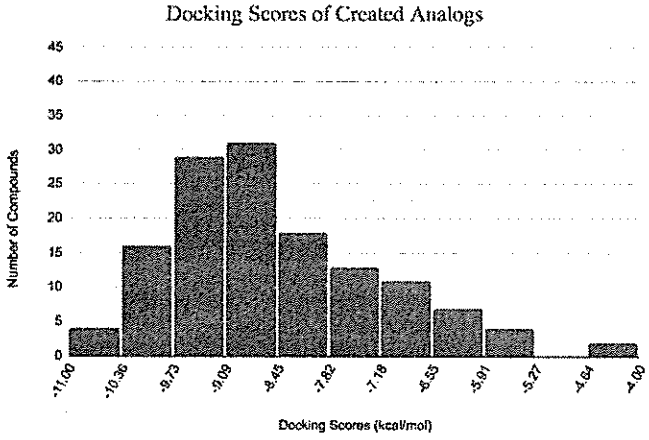


Fig. 9 Docking Scores of Created Analogs Over 130 FABP5 inhibiting compounds were generated over the course of this study. 111 compounds had a lower free binding energy than SB-FI-31's binding energy of -7.02 kcal/mol. The average free binding energy was -8.44 kcal/mol. Out of all the created compounds, only 20 had a free energy binding score of -9.73 kcal/mol or higher. Investigations into these 20 compounds revealed that SB-CY-138 was a potential candidate for further analysis, as it had a cLogP below 5 and a free binding energy of -10.11 kcal/mol.

However, only 20 compounds had desirable free binding energies, ranging from -9.7 to -11.00 kcal/mol.

Out of those 20 compounds, SB-CY-106, SB-CY-134, SB-CY-138 were chosen as candidates for further analysis (Fig. 10). SB-CY-106, SB-CY-134, and SB-CY-138 exhibited free binding energies of -9.70 kcal/mol, -10.30 kcal/mol, and -10.11 kcal/mol respectively. Both SB-CY-106 and SB-CY-134 had a cLogP of 5.1609 while SB-CY-138 had a lower cLogP of 4.7752.

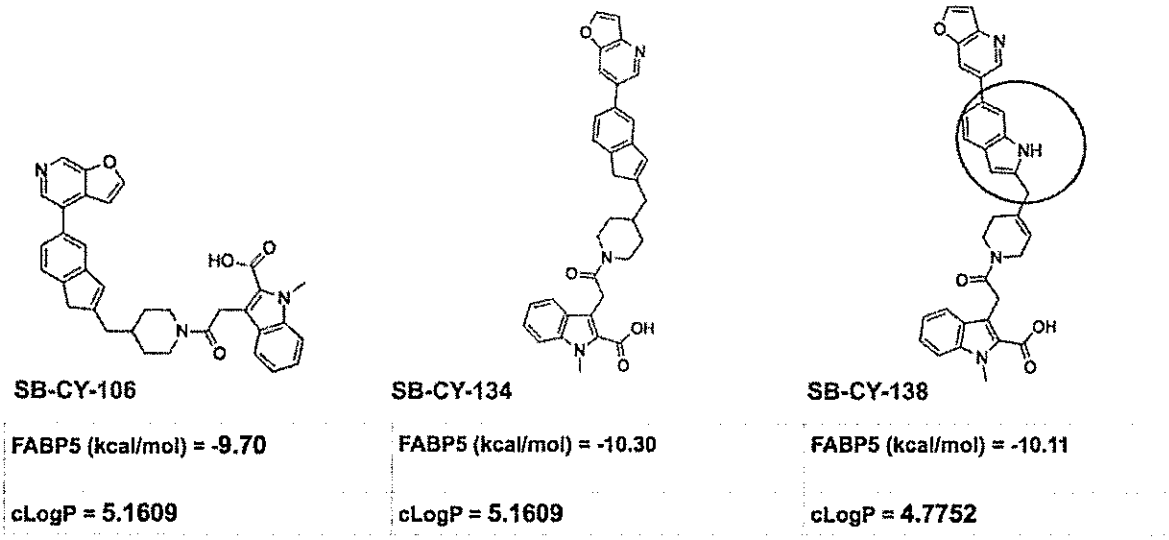


Fig. 10 2-D Structure of SB-CY-106, SB-CY-134, and SB-CY-138 SB-CY-106, SB-CY-134, and SB-CY-138 all have the indole constituent incorporated back into their structures, compared to earlier designs, such as SB-CY-42 and SB-CY-70, which contain an indane. SB-CY-134 exhibited the lowest free binding energy, while SB-CY-138 had the highest solubility out of the three compounds. SB-CY-134 is a structural isomer of SB-CY-106, and it exhibited a 0.60 kcal/mol decrease in free binding energy. SB-CY-138 is similar in structure to SB-CY-134, however, it contains an indole instead of an indane (circled in red). The added indole increases free binding energy by 0.19 kcal/mol and increases solubility of the compound, as demonstrated through a lower cLogP score compared to SB-CY-134. Image generated by Chemdraw.

While preliminary compounds contained indanes instead of indoles, for later compounds like SB-CY-106, the indole was incorporated back to optimize free binding energy. In lieu of the methyl benzene constituent in SB-FI-31, SB-CY-106 contains a furo-pyridine with an additional indane structure. The structural isomer of SB-CY-106, SB-CY-134 demonstrated a 0.60 kcal/mol decrease in free binding energy while retaining a cLogP of 5.1609 . SB-CY-138 had the same design as SB-CY-134, except the indane constituent of SB-CY-134 was replaced by an indole

(circled in red). The additional indole was found to increase solubility without seriously affecting binding potential. SB-CY-138 was chosen as the compound for further investigations because upon creating synthesis plans, SB-CY-138's structure allowed for the easiest synthesis route.

3.5 Analysis of SB-CY-138's chemical and pharmacokinetic properties

Although SB-CY-138 had an ideal cLogP and free energy binding score, the compound was visualized with FABP5 in UCSF Chimera (Fig. 11A) to ensure that there were canonical

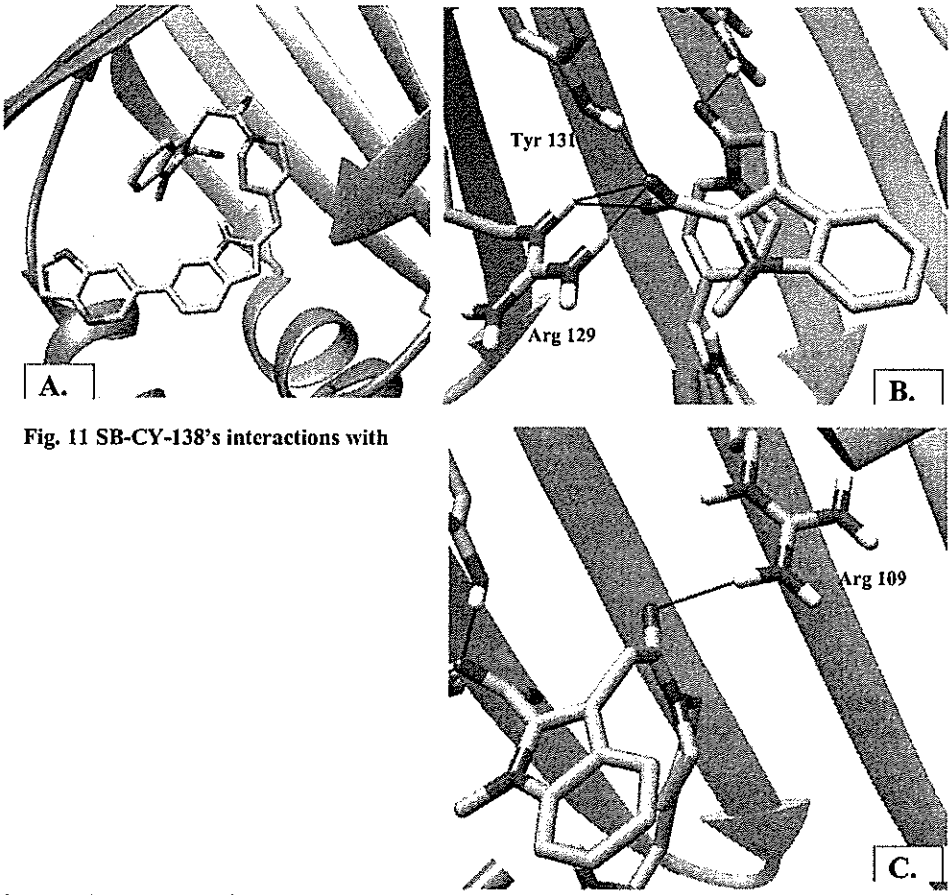
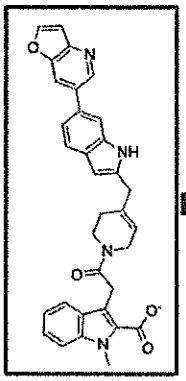


Fig. 11 SB-CY-138's interactions with

interactions. The carboxylic acid of SB-CY-138 was found to interact with Arginine 129 and Tyrosine 131 (Fig. 11B) in a similar manner compared to SB-FI-31's carboxylic acid constituent. There was an additional interaction between the carbonyl constituent of SB-CY-138 and Arginine 109 (Fig. 11C). By fulfilling all the requirements needed for a suitable FABP5

inhibiting compound, SB-CY-138 becomes an extremely probable candidate for chemical synthesis and biological testing.

To analyze the pharmacokinetic properties of SB-CY-138, the compound was run through pkCSM tests (Fig. 12). SB-CY-138 tested negative for AMES toxicity, which implies



Predicted Pharmacokinetic Properties of SB-CY-138

AMES Toxicity	No
hERG I Inhibitor	No
hERG II Inhibitor	No
Oral Rat Acute Toxicity LD50 (mol/kg)	2.71

Fig. 12. Predicted Pharmacokinetic

that SB-CY-138 is unlikely to act as a carcinogen. Additionally, SB-CY-138 tested negative as a hERGI and II inhibitor. Human either-a-go-go genes (hERG) encode potassium channels and inhibition of these channels will result in fatal ventricular arrythmia.

4. Conclusion and Future Work

Although past studies have shown that FABP5 plays a significant role in the metastasis of breast cancer [28]. The decrease in viability of breast cancer cells due to inhibition of FABP5 has never been demonstrated. By exposing MCF-7 cells to FABP5 inhibitors, this study highlighted the substantial role that FABP5 plays in the progression of breast cancer.

Application of FABP5 inhibitors SB-FI-26 and SB-FI-103 to MCF-7 cells hours resulted in a decrease in MCF-7 cell viability, which further strengthens FABP5's potential as a target protein for clinical treatment of breast cancer. However, both SB-FI-26 and SB-FI-103 exhibited a greater effectiveness at 72 hours compared to 48 hours, indicating that FABP5 inhibitors are

slow acting drugs. SB-FI-103 was observed to be more successful in decreasing MCF-7 viability than SB-FI-26, which could be attributed to SB-FI-103's increased binding specificity to FABP5. Future studies would aim to optimize the effect of FABP5 inhibitors on MCF-7 cells, through different concentrations and time periods. Past studies have implicated FABP5 in other types of cancer, such as prostate, cervical, and gastric cancer [29]. Additional MTT assays can be conducted to evaluate the significance of FABP5 in the progression of these cancers. The results of the MTT assays can then determine which cancer, FABP5 plays the most significant role in- based on the overall decrease in cell viability.

Over 130 FABP5 inhibitors based off of SB-FI-31, were created over the duration of this study. Preliminary analogs explored the importance of the indole and methyl constituent of SB-FI-31. Many prominent drugs on the market today, like Sumatriptan and Ondansetron, contain indoles [30]. This is due to the indole's favorable heterocyclic structure, which resembles essential metabolites [31]. This study showed that the conversion of an indole to an indane in FABP5 inhibiting compounds like SB-CY-30, SB-CY-42, and SB-CY-70 had negative effects on different aspects of the compound's effectiveness. However, substituting the methyl substituent in SB-FI-31 with other structures demonstrated a marked decrease in free binding energy of the compound with FABP5. Through analysis of canonical interactions, free binding energy, and solubility, SB-CY-138 was ultimately chosen as the compound with the most drug-like potential. SB-CY-138 utilized two indole structures and a furo-pyridine substituent. *In-silico* simulations showed that SB-CY-138 was able to make canonical interactions with FABP5, while fulfilling the solubility conditions for an ideal drug. Upon closer inspection of SB-CY-138's fit in FABP5's binding pocket, it is revealed that there is still free space within the binding pocket that SB-CY-138 hasn't utilized. Future work can investigate the free binding energy of

compounds that capitalize on this space by incorporating substituents onto the furo-pyridine (Fig. 13A) and the carbonyl of SB-CY-138 (Fig. 13B).

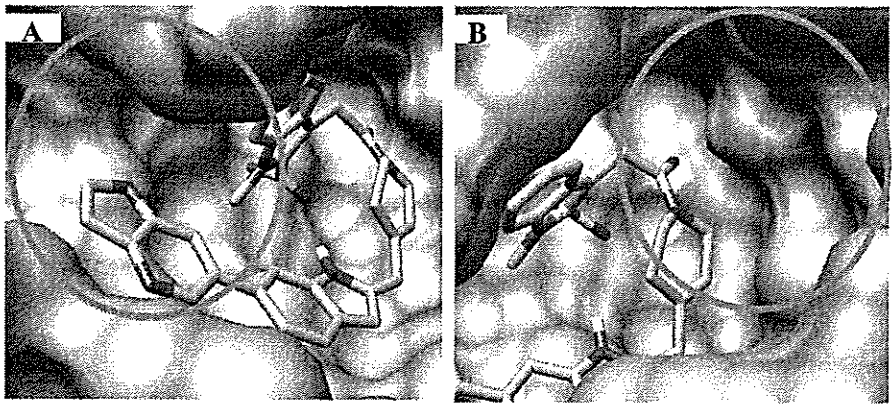
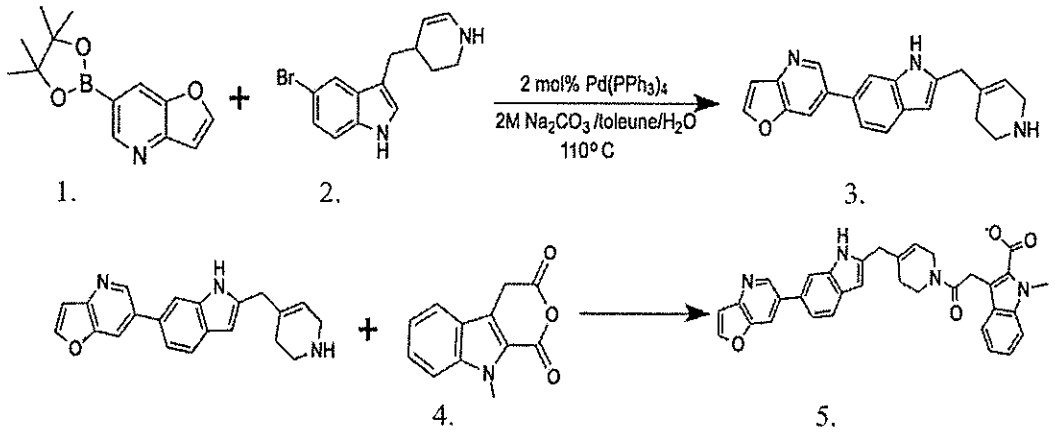


Fig. 13 Additional Space within FABP5 binding pocket Upon visualization of SB-CY-138's binding pose within FABP5, it is revealed that there is still space within FABP5's binding pocket that hasn't been utilized by SB-CY-138. Additional structures can be incorporated into the furo-pyridine **(A)** and carbonyl **(B)** of SB-CY-138. Supplementary structures may result in a lower free binding energy due to the tighter fit, however it will also decrease solubility. Therefore, a balance between solubility and free binding energy should be taken into account when creating future compounds. Image generated by UCFS Chimera.

To further investigate SB-CY-138's potential, future work would include the synthesis of SB-CY-138. Due to the similarities between SB-FI-31 and SB-CY-138, a multi-step synthesis plan, using SB-FI-31 as a scaffold, was created (Fig. 14).

Fig. 14 Synthesis scheme of SB-CY-138



1. 6-bromofuro[3-2b] pyridine underwent Miyaura borylation in which the bromine constituent of the furo-

After synthesis, SB-CY-138 would be tested with MCF-7 to observe the effect of SB-CY-138 on breast cancer cell viability. Understanding the impacts of FABP5 and creating novel FABP5 inhibiting compounds is pivotal to the advancement of anti-cancer treatments.

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