Designing molecules to inhibit Mycobacterium Tuberculosis

By

Eujin Lim

Middlesex County College

Edison, NJ

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Research Advisor: Dr. Phalguni Ghosh, Dr. Steven Rowley and Dr. Brian Lavey

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Abstract:

Tuberculosis is the ninth deadliest disease in the world, killing 1.4 million people yearly. The mycolic acid coating the outside of the bacteria is what makes the tuberculosis difficult to treat. With the addition of mutations in the genes of the bacteria, traditional first line drugs, such as Rifampicin and Isoniazid, are rendered useless. The goal of my research was to build a new inhibitor for the *Mtb* enzyme, Fatty acid degradation D32, better known as FadD32. This enzyme contributes to the condensation of mycolic acid. The purpose of this research is to not only develop a new inhibitor for this enzyme, but to design new molecules that have never been tested to our knowledge before. If this research is successful, then the inhibitor could potentially be further synthesized into a drug to treat patients with MTB.

Introduction:

This research would not have been possible without an understanding of the ligands relating to the topic at hand. In this case, bioinformatics can be of high value. Bioinformatics is the study of ligands through modeling software to better one's conceptual understanding of them This complex software combines mechanics, biology, and computer science to give user more intuitive sense of understanding the Drug Discovery Process.

Several Bioinformatic softwares are used for this research. The first tool used was the Protein Data Bank, otherwise known as the PDB. This is used to search the 3D crystal structures of the target protein being studied. UCSF Chimera, another bioinformatic, is used to view the 3D molecule structure. Chem3D was used to design and minimize the energy of the designed ligand. Once all the files are made using the previous softwares, I used the online website SwissDock, operated by the Swiss Institute of Bioinformatics, to view the binding characteristics and interactions. Each of these softwares will be discussed in detail further.

The Drug Discovery Process today is an expensive and time-consuming process. Most pharmaceutical companies follow the same guidelines when introducing a new drug to the market. Researchers would first need to identify the target protein to know exactly where the drugs need to interact with. Once that has been accomplished, the Lead Discovery phase is the next step where researchers synthesize molecules that can best fit inside the active site of the target protein. At this stage, most researchers will continue further to the Lead Optimization/Medicinal Chemistry phase. This is where researchers modify the designed molecule to improve its binding abilities to the protein. Once this has been successfully accomplished, researchers can move forward to the In Vitro studies where the designed molecule is used to test the binding assay inside and outside of the cell. If the results are satisfactory, they will move on to the In Vivo studies, where animals, such as rats and mouse, are introduced to the molecule. If the In Vivo studies successful, researchers can move forward to the most important stage, the Pre-Clinical trial. This is when tests are performed to identify the safety of the molecule and its maximum dosage. The last step would be the Lead Optimization phase, where final adjustments to the molecule are done to ensure a better interaction with the protein.

Most pharmaceutical companies would traditionally use the 2D structure-activity relationship (SAR) approach. This is a method which screens large numbers of molecules and relates the chemical structure of the ligand and the biological activities associated with the ligand. The problem with this approach is the amount of time and resources required. However, with bioinformatics, the structure of the protein, in this case Fatty acid Degradation D32, can be used to better understand the active site of the pocket.

With the use of Bioinformatics, designing the inhibitor can be done through 3D Structure Based Design method. This allows researchers to clearly identify the interactions of the small molecules and the target protein, FadD32. In addition, it also allows them to make changes to the scaffold of a ligand more easily because Bioinformatics provides a better image of the interactions. Many would refer to this as the "Lock and Key" method. To open a lock, one would need a key that can fit the inside of the lock to unlock it. 3D Structure based drug design is similar, in the sense that the protein's active site is the lock and ligand the key. (Figure 1)
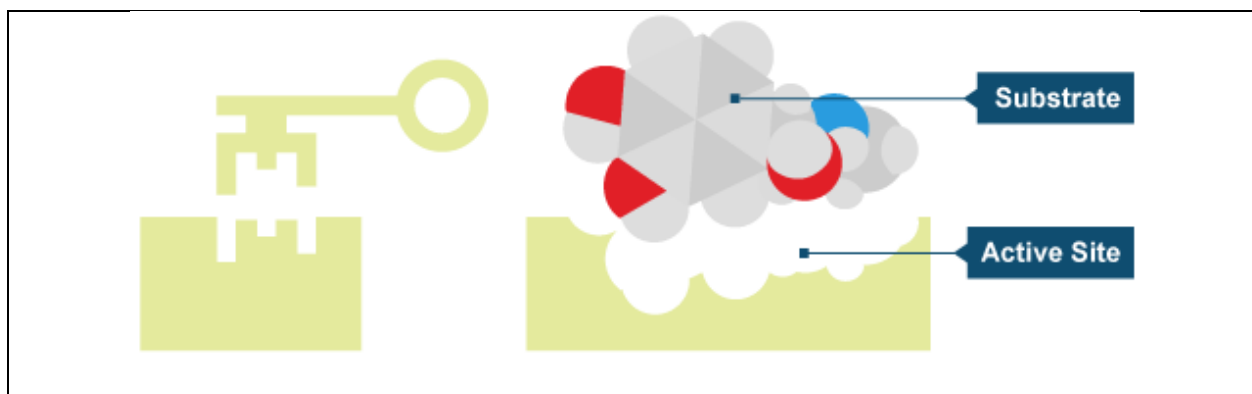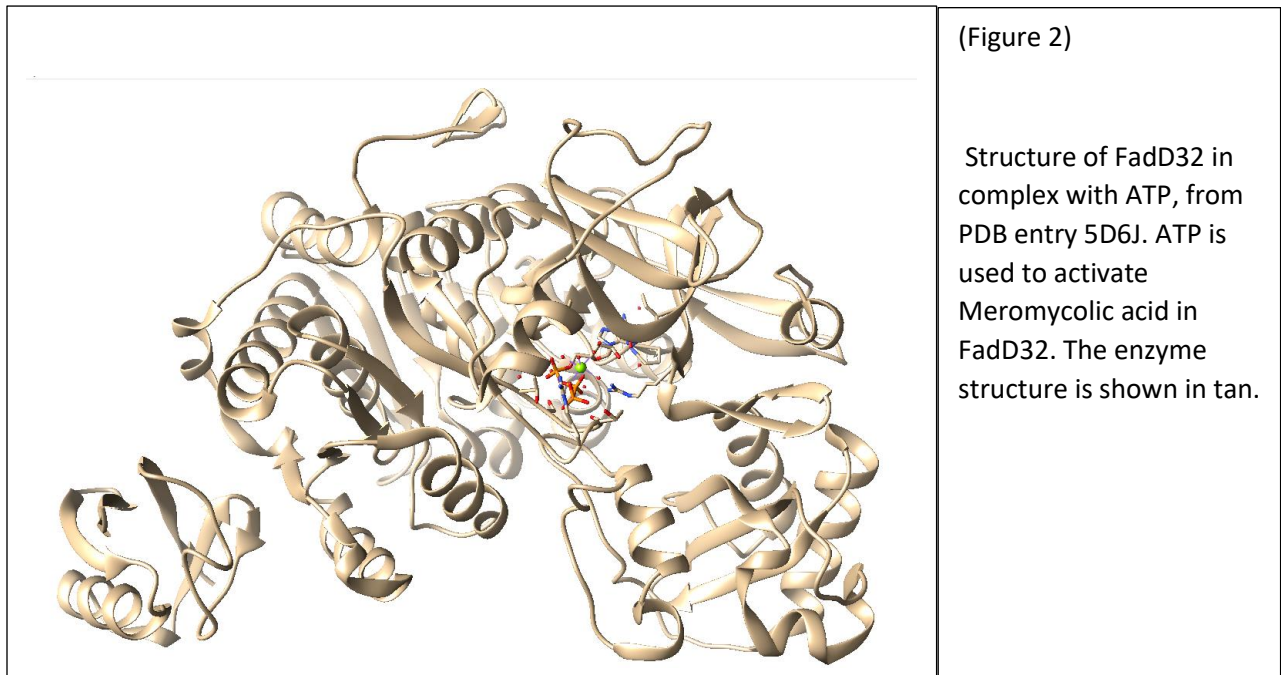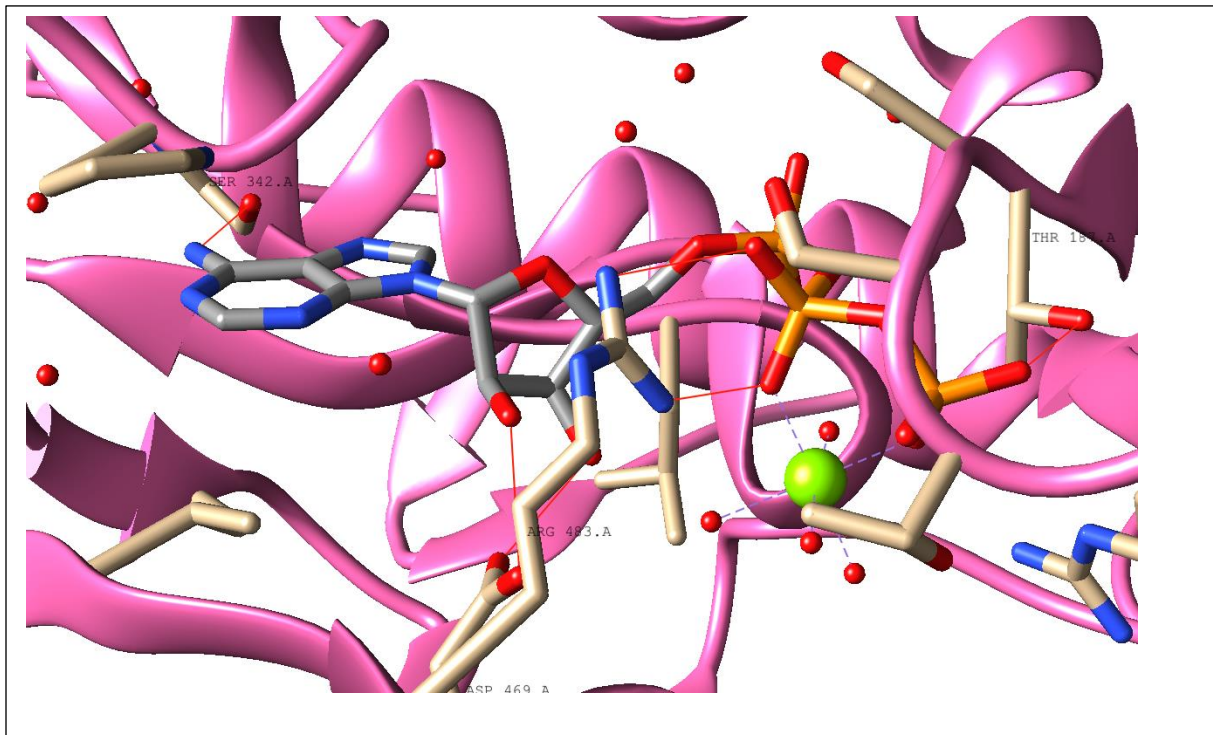

(Figure 1)

3D Drug Design as compared to "lock and key." Protein active site is much like a lock, and the substrate for the protein is like a key fitting that lock.

Fatty acid degradation protein D32, better known as FadD32, is one of the 35 FadD proteins in M. tuberculosis. Like fatty acyl-AMP ligase, to this protein is found in all of the mycobacterium genus. During the biosynthesis of the mycolic acid, FadD32 activates and transfers Meromycolic acid to the final enzyme in biosynthesis of mycolic acid, PKS13 for condensation. The full synthesis of the diverse sets of mycolic acid found in *Mtb* is the result of condensation. If left untreated, anyone who's been infected can be fatal. Once the bacteria makes contact with the alveoli, the immune system sends the wrong macrophage to kill *Mtb*. The macrophages cannot break down the mycolic acid in *Mtb*, thus letting the bacteria to continue to grow without any impediment. Once infection occurs, *Mtb* causes tissue degradation, better known as Necrosis, in the lung and infects other cells throughout the body. To prevent infection, first line drugs such as Rifampicin, a drug that suppresses the initiation of RNA synthesis, is used

to treat patients. However, mutated MTB, ranging from Multi Drug Resistant to Completely Drug Resistant, renders many first and even second line drugs useless.

(Figure 2)

Structure of FadD32 in complex with ATP, from PDB entry 5D6J. ATP is used to activate Meromycolic acid in FadD32. The enzyme structure is shown in tan.

FadD32 will only activate the Meromycolic acid if Adenosine Triphosphate, ATP, is present at the N-terminal domain. During the enzymatic reaction, ATP will enter the pocket of FadD32, and bind in a cleft position. Hydrophobic and hydrogen bonds from several residues such as Proline 315, Serine 342, Aspartic acid 469, and several other residues stabilize the complex for the activation of the substrate. Once the Meromycolic acid enters the enzyme, Lysine 601 reorients itself to help catalyze the formation of the activated substrate from AMP and Meromycolic acid. FadD32 will then transfer the activated substrate to PKS13 for condensation. Currently there are no available drugs on the market that specifically target this enzyme, however researchers theorized binding to the entrance of the phosphopantetheine-binding tunnel can inhibit FadD32.

(Figure 3)

Active site of FadD32; FadD32 in complex with ATP. The molecule is then stripped of two PP resulting in AMP + PP. ATP shown in grey, hydrogen bonded to Ser342, Asp469, and several others to stabilize the structure found in PDB entry 5D6J. 5D6J shown in magenta ribbons, and the resides in beige. Mg+ along with some H20 molecule to help stabilize the triphosphate.

With the assistance of the Protein Data Bank and Chimera, I studied the 3D structure of ATP and an inhibitor called 649 inside the active site of FadD32. After analyzing both interactions shown on Figure 4, I could draw several conclusions on the necessary for designing a new inhibitor for FadD32. On Figure 4, with ATP superimposed with 649 we can see the approximate pocket size of the enzyme. Both ATP and 649 form the essential hydrogen and hydrophobic bonds with the residues around on the protein to stabilize the complex. But, the inhibitors long carbon chain binds in the phosphopantetheine tunnel preventing the entrance of Meromycolic acid. Looking at the pocket, we can see that there's a hydrophilic pocket, which allows the chance of a hydrogen bond. Moving ahead, we know that in order to design a new inhibitor for FadD32, we will need a molecule that can participate in a hydrogen or hydrophobic bond to stabilize the complex. We also know that relatively skinner molecules are favored.

Experimental Section:

Tools used:

1. UCSF Chimera: a visualization software that projects images of various enzyme structures and molecules needed for docking.
2. RCSB PDB (www.rcsb.org): to scrutinize and retrieve the 3D structure of FadD32-various ligands
3. ChemBio3D: Design and minimize the energy of the molecule. Optional* SMILE string
4. SwissTargetPrediction*: An online tool that can predict the targets of a small molecule using a combination of 2D and 3D similarity measures. It compares the query molecule to a library of several thousand compounds active on selected targets from different species
5. SwissDock: A web-based tool that predicts the molecular interactions that may occur between a target protein and a small molecule.

Figure 5 below shows the steps used to design the molecule. First, I dug out the entries of small molecules in complex with FadD32. After opening the entry on Chimera, the interactions of the molecules in FadD32's pocket are now available to see.

After studying the molecule's interaction, I was able to use ChemBio3D to design the molecule. In this specific research, FadD32 cannot be searched on SwissTargetPrediction because SwissTargetPrediction can only check for proteins from mammals. However, it can be still used to check it doesn't bind to other proteins. Then the molecule is then docked inside FadD32 using Swissdock. The software predicts the potential interactions between the designed molecule and the targeted protein.

Once the docking results are completed and returned, we would analyze the results to ensure the bindings mode made sense. If the design is indeed patentable, the next step is to synthesize the molecule, and test the inhibition activity on FadD32. But, before molecules are made into drugs, researchers must test the ADME (Absorption, Distribution, Metabolism, and Excretion) profile. During this process, if there are any unwanted results during any step of the design proves, the molecule might have a chance of undergoing modifications to further improve the process. Because SwissTargetPredication checks ligand on mammal protein and not bacterial, Step 4 cannot be used.
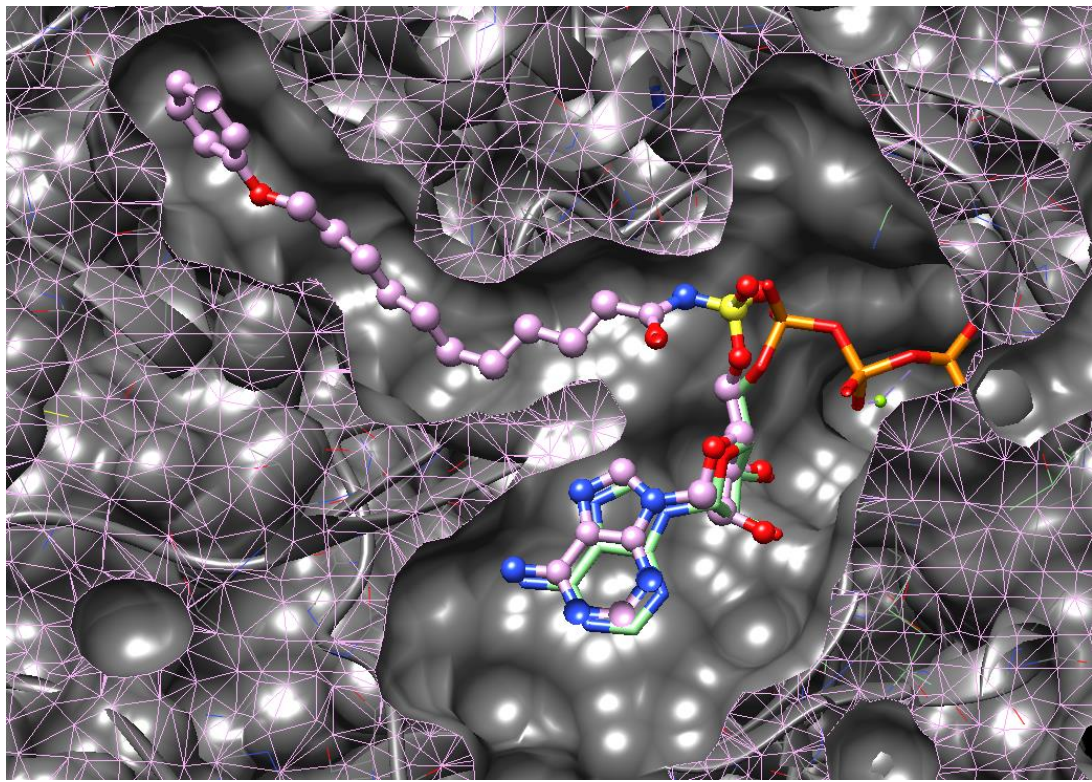
(Figure 4)

PDB ID 5D6J, ATP in green stick model bound to FadD32 shown in surface and ribbons representation and 5HM3 in purple ball-and-stick model bound to FadD32.

(Figure 5)

Process followed in designing new drug-like molecules to inhibit FadD32
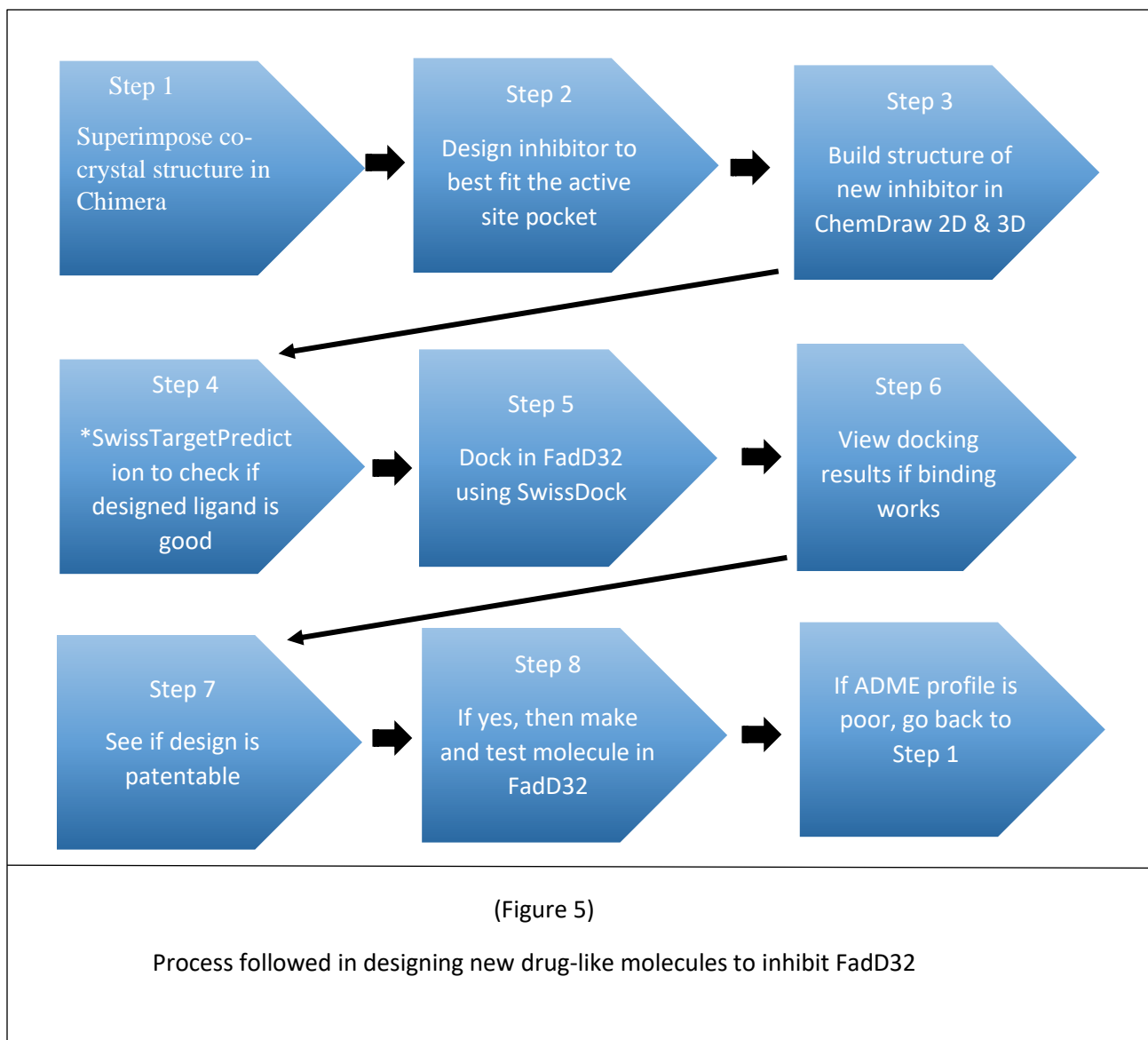
**Results and Discussion:**

Establishing Proof of concepts

Eujin_5D6J_ATP

Eujin_5HM3_649

Utilizing the Protein Data Bank

Enter the PDB's web address (www.RSCB.org). Once the page is loaded, the necessary protein structure can be found using the search bar above, in this case FadD32. The website will

provide a box with previous searches based on the topic at hand. The user can look up ligands, macromolecules, and proteins. On Figure 6, if the user were to click on the blue underlined link, the website will link he/she to a new page showing all available entries involving FadD32. On the bookmark side of the page, click on the tab that spells ligand. This will show a page of various ligands connected to the search topic. This will be crucial since our research focuses on designing a new inhibitor for FadD32. In Figure 7 below, near the top left-hand side of the image, under the PBD logo shows 37 structures of FadD32 archived on the PBD. Starting on the right 13 citations including FadD32, and 34 ligands archived that are known to interact with FadD32. Relating back to drug discovery, on (Figure 8) it shows the pages of what PDB entries the ligand is present in. This is necessary in this research. The pages listing the ligands interacting with FadD32 is what would be referenced back to when we are designing a new molecule to inhibit FadD32.
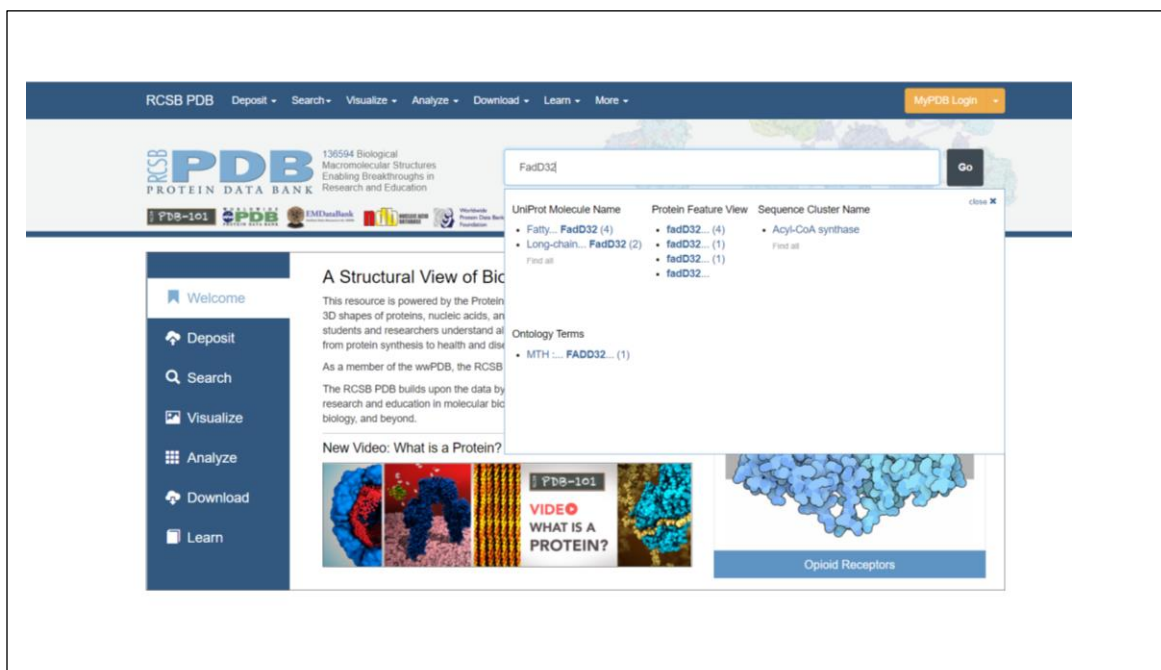
(Figure 6)

The website provides the user with recommended choices based on previous keywords enterd. FadD32 is one the choices providing 5 structures archived on the PDB

(Figure 7)

On the top, left-hand corner of the figure shows, that 37 structures of FadD32 archived on the PBD, 13 citations including FadD32, and 34 ligands archived that are known to interact with FadD32.
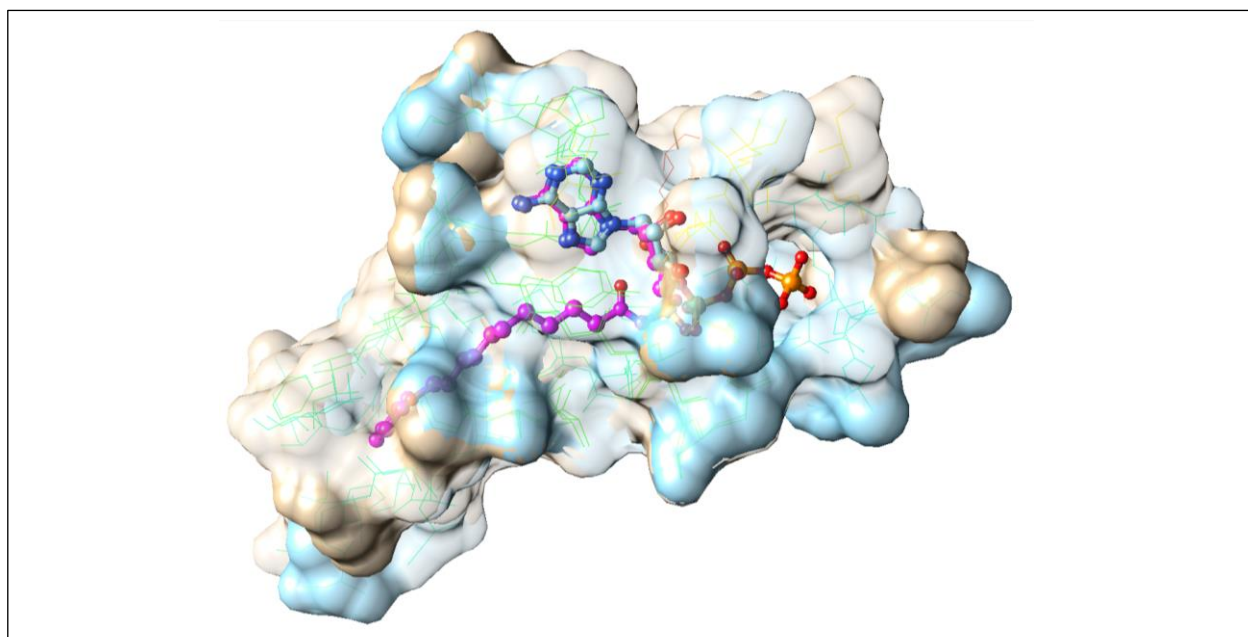

(Figure 8)

Clicking on the "34 Ligands" tab will result in showing this page. This page will list each ligand that has interacted with FadD32 that's been recorded on the PDB archive, and what entries were they found in. This page is vital in our research because this is what will be referring to when designing new molecules.

<u>Using UCSF Chimera for Viewing/Designing Molecules</u>

Chimera needs to be installed beforehand. Chimera extracts 3D structures of enzymes with the molecules inside from previous PDB entries recorded for viewing purposes. The structure can be fetched by the following: select "File" in the toolbar, select "Fetch by ID," a small window will pop up with a long list of options; because the structures is found from the PDB archive with the ID number is given. Select "PDB," this will allow the user to search the desired entry. For the purposes of this research, the first entry I studied was with the PDB ID 5D6J, which is FadD32 in complex with ATP. Chimera will automatically open the 3D structure of FadD32 with ATP inside.

Next, we want to superimpose another ligand with ATP. Doing so will allow us to identify the approximate size of the protein pocket. The same protocol is used to "Fetch" the ID number of the other ligand used. During the design of Eujin_5HM3_649, we fetched the entry with the PDB ID 5HM3, FadD32 with an inhibitor, 649. Once both structure is shown, we can now proceed to superimposed the image, giving us the approximate pocket size.

After analyzing the superimposed image, we wanted to create a ligand based on the interactions inside the pocket. In this case, we want to design a ligand that binds in the area where the ribose and adenine is present to block the entrance of ATP shown below on Figure 9A. We also want to design ligands where it can bind inside the hydrophobic pocket where the 649 ligand is shown below Figure9B.

(Figure 9)

The arrow pointing at the purple chain shows the structure of ligand 649 from PDB entry 5HM3. On the right, shows the structure of ATP from PDB entry 5D6J. Looking at the circle image, both adenine and ribose from 649 and ATP are superimposed. This has lead us to know what regions are hydrophobic vs hydrophilic.

The next step after is to design the molecule using ChemBio 3D.

Using ChemDraw Software to Build Molecules

Middlesex County College provided me ChemBio 3D to design my desired molecule. After conceptualizing the designed molecule, ChemBio 3D is used to draw the 2D structure. With the molecule presented in its 3D structure on the side, we need to minimize the energy of the molecule. This will present the designed molecule in its most stable form. The method doing so is the following: highlight the molecule, then finding the "MM2" tab under the "Calculation" tab by toolbar. The program will adjust the user molecule the most stable conformation of the molecule used. After the program finished, save the program under ".Mol2" file, which is used later for docking.

We also used ChemBio 3D to retrieve the SMILES string for the designed ligand. The SMILES string is a unique identifier for any molecule; all molecules have a SMILES string. To get the molecule's SMILES string, the structure must first be selected using the selection tool. From the Edit menu in the toolbar, select "Copy As" and choose SMILES. The string will automatically copy inside the system. Optionally, the SMILE String should be pasted in a document and saved in a file near the ligand designed. The string is now used to in SwissTargetPrediction to see how the ligand binds to other proteins.

Using SwissTargetPrediction*

SwissTargetPrediction is another bioinformatic tool used to check that the ligand won't bind to other proteins. The tool can be accessed through the Internet at Swisstargetprediction.ch (Figure 10). The SMILES string from ChemBio 3D just gets pasted into the box provided, and within a minute it will provide a report showing what proteins the molecule is predicted to interact with
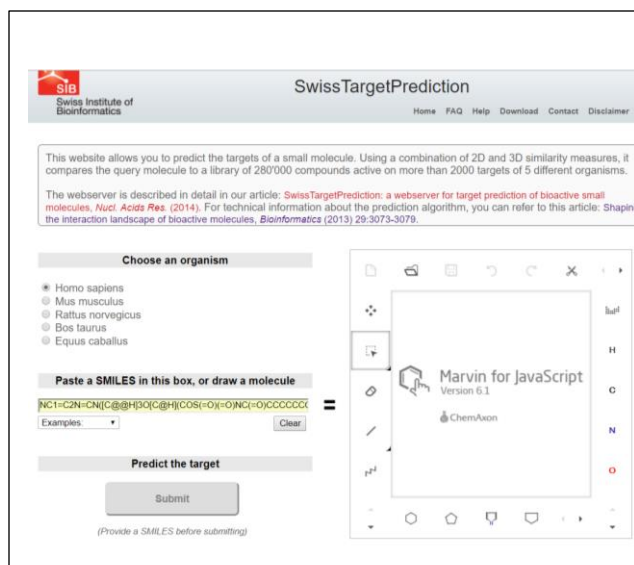
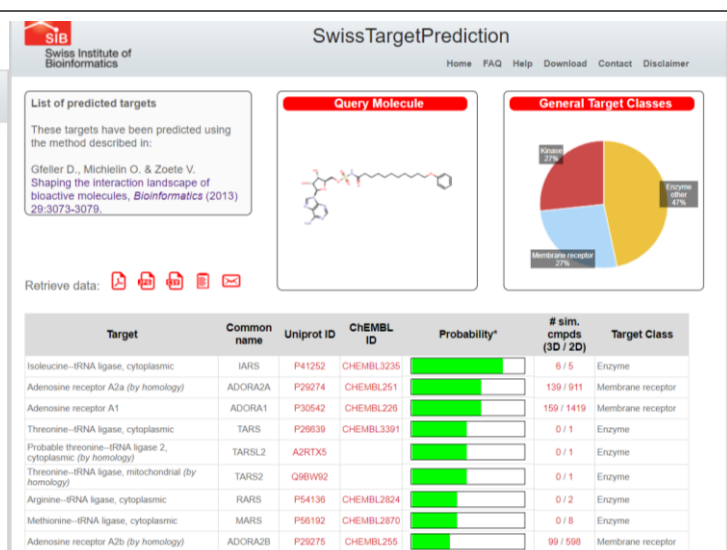| Figure 10: SwissTargetPrediction landing page. Here, the target protein can be selected, and the SMILES string is pasted in the given box. Then select "Submit"	Figure 11: Generated SwissTargetPrediction of Eujin_649 for FadD32. As expected FadD32 will not appear in the predicted interactions.

The list provided by SwissTargetPrediction shows any protein that s predicted to interact with, as well as the probability of interaction (green bars in Figure 11). Based on the results of Eujin_649 (Figure 11), the designed molecule can be moved forward with the molecule and dock it in FadD32. However, regarding our research SwissTargetPrediction cannot predict the interactions.

Once the protein structure and molecule are complete, we can proceed to start docking the designed molecule in FadD32 to view the predicted interactions inside the pocket, through Swissdock. Entering the URL (Swissdock.ch) then selecting the "Submit Docking" tab, the user now needs to submit the information needed for the tool to "run the dock."

Using SwissDock to Dock Molecules

Before docking any molecule, a file of an empty protein structures needs to be made first. To make it, the PDB ID containing the crystal structure of the desired protein, in this case 5D6J, needs to be opened through Chimera. After the structure appears, all nonstandard ligand needs to be deleted. This can be done by the following: Click on the tab "Select," click on "Residue", then select "All Nonstandard." This will highlight all the ligand inside the pocket. Then click on "Actions," then click "Atoms/Bonds," then click "Delete." After, click on "Tools" then click on "Structure Editing," then perform the following: select "AddH." A small window will pop up, press "Ok." Next, select "Add Charge." Again, a small window will pop up, simply select "Ok." Last, select "Dock Prep." A small window will pop up, every option in that window should be

checked. Then, press "Ok," and then "Ok" again on the next window. Chimera will now prompt the user to save the file as a .Mol2 file. Once saved, the user must open the same .Mol2 file and save it again as a .PDB file instead. Figure 12 below, the user needs to put the information necessary for the tool to "Start Docking."

(Figure 12)

SwissDock "Submit Docking" page where information about docking job is input by the user. Once all information is entered, then job will begin by selected "Submit Docking"

Under *Target selection*, the .PDB file of the protein must be uploaded; this file is made through Chimera. The ".PBD" file of the "emptied" protein structure will be used.

Under *ligand selection*, the designed molecule that was saved as ".Mol2" format will be uploaded. SwissDock will run checks for both files to ensure that the file is ready for docking. This will be represented with a green checkmark.

Under *description*, a job name must be created, such as "Eujin_5D6J_ATP" and an email address needs to be entered so that the website can inform the user of the job's termination. Click on the "Show Extra Parameter" to show an area where more information can be added.

Docking type should be set to "Accurate." To *define the region of interest* background work must be done ahead. First, the user needs to identify the center of the protein pocket. This can be done by finding the x,y,z coordinates of the pocket. The coordinates for FadD32 respectively are (3.114, -3.279, -12.081) The coordinate was chosen using Chimera and the PDB.

Chimera provides us the 3D Structure which help identify which atom is the closet in being the center of the pocket. Doing so, "C9" in ligand "649," the coordinates are found. The user then must set must be informed he size of the protein pocket is. Setting the x-y-z sizes to 6 works fine. Under *Flexibility,* the user needs to set some form of flexibility in SwissDock. Setting it to 3 angstroms, cam allow a moderate level of flexibility for both the protein residues and the ligand. If the binding pocket is not clarified, SwissDock will attempt to run "blind docking," where it will dock the small molecule all over the protein, rather than just at the active site, or pocket of the protein. It is important that this information is given before running docking.

After all the protocols has been set, the job is ready to be submitted as a docking job. Most jobs will take over night for it to be terminated. Once the job is terminated, the user should receive an email with that will provide a link with a similar image like Figure 13.

Show	Cluster	Element	FullFitness (kcal/mol)	Estimated ΔG (kcal/mol)
	0	1	-3358.07	-12.03
	0	0	-3358.13	-11.99
	2	3	-3352.67	-10.81
	2	2	-3352.68	-10.81
	2	1	-3352.68	-10.81
	2	0	-3352.68	-10.81
	0	2	-3352.22	-10.80
	0	4	-3349.01	-10.73
	0	3	-3350.44	-10.46
	1	0	-3353.62	-10.38
	0	5	-3336.34	-10.37
	33	7	-3321.56	-10.32
	33	0	-3322.17	-9.88
	33	1	-3322.17	-9.88
	33	2	-3322.17	-9.88
	33	3	-3322.17	-9.88
	33	4	-3322.17	-9.88
	33	5	-3322.06	-9.85
	33	6	-3322.06	-9.85
	3	0	-3346.88	-9.78
	4	0	-3341.48	-9.75
	3	1	-3346.52	-9.72

(Figure 13) Docking results for ATP, the estimated △G value in the highlighted box gives an indication of the tightness of binding

Looking at the figure above, the columns show the different poses, or predicted orientations of the small molecule in the active site of the protein. The program predicted hundreds of poses in the given docking job. Selecting the right pose is quite simpler than it seems. The purpose of this docking job is select the specific job that can match closest with the crystal structure position. The column label "Estimated △G value" shows the stability of the binding mode. In Chemistry, △ G stands for Gibb's free energy. Gibb's law stated that as long the pressure and temperature is constant and if the value generally is in the negative, it means that the reaction will be spontaneous and in increase in stability. Most of the time, the more negative the △ G, the better the binding mode. The results can be analyzed further using

Chimera. On the same page, there's a red link that reads "Launch UCSF Chimera to visualize predicted binding modes," the website will give a file to open the job's docking results in Chimera.

<u>Using UCSF Chimera to analyze Docking Results</u>

The purpose of docking ATP and 649 in FadD32 is to ensure that the protein structure designed can be used to dock other molecules into. In this case, Eujin_5D6J_ATP needs to be superimposed with the 5D6J, and Eujin_5HM3_ATP with 5HM3. After going through the results, we need to identify the which choices will match closest to the PDB entry to ensure whatever pose chosen will make sense. We would apply the same protocol to 649.

After analyzing Eujin_5D6J, the result that shows the best-fit pose in the pocket of the protein was Cluster 0 Element 5, with a $\triangle$ G value of -10.35. It should be noted that my docked result forms almost the same hydrogen-bond and hydrophobic bonds compared to the crystal structure. Since SwissDock cannot "dock" nonessential ligands such as Mg+ and H2O, certain hydrogen bonds will not form. Also, the triphosphate, from my docked results, flexibility can bind to different residue, but it was expected. Following the same protocol from ATP to 649, results show that Cluster 0 Element 2 best match pose in the pocket with a $\triangle$ G of -11.85. The docked result nearly superimposed on the known structure, with my docked results sulfide slightly shifting up due to the flexibility. It should also be noted that generally the larger ligand, chances of additional interactions will occur. The best fit pose for both can be seen below, on Figure 14 and 15. The residue interaction can be shown below on Figure 14b and 15b.
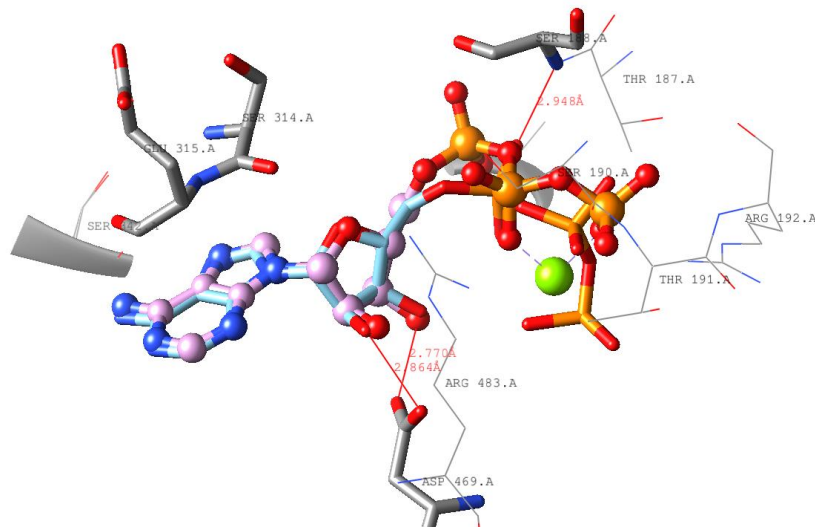
(Figure 14)

Best docked pose of Eujin_5D6J_ATP, shown in blue stick, superimposed with ATP, purple ball and stick, from PDB entry 5D6J.
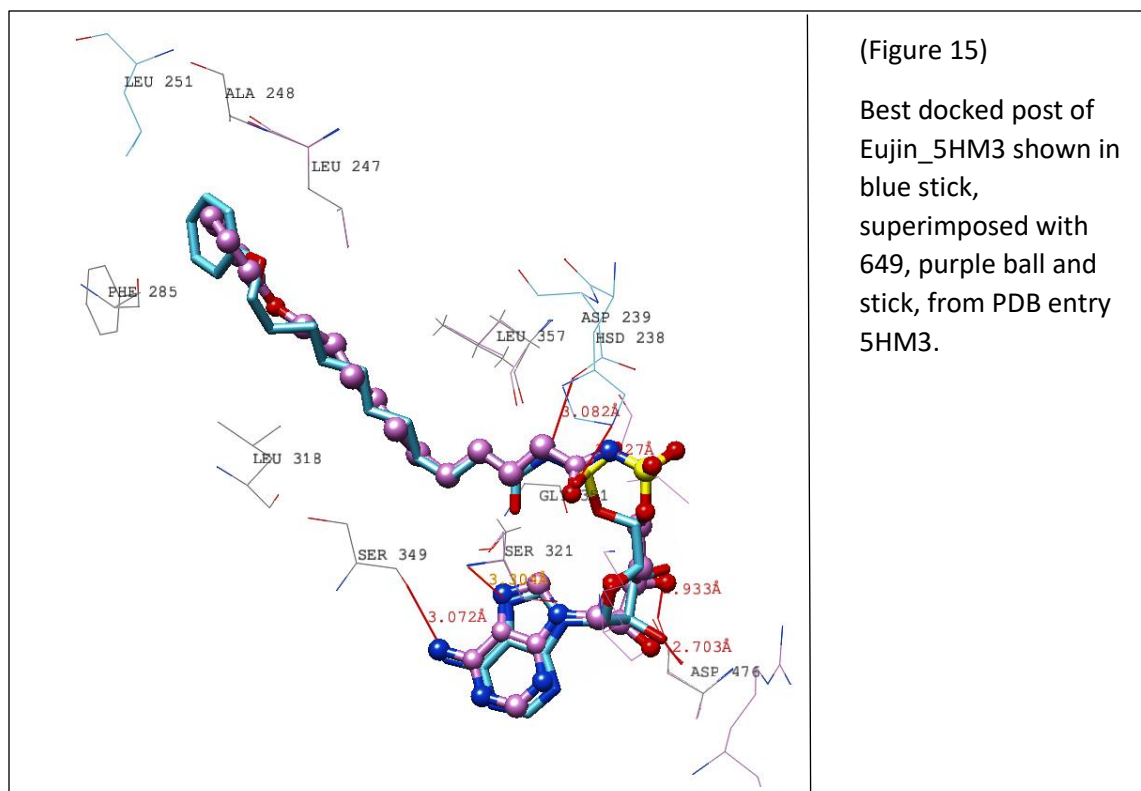
Eujin_5D6J_ATP	5D6J Crystal Structure
Ser342	Ser342
Asp469	Asp469
Ser188	Ser188
Thr191	Thr191
Arg192	Arg192
Ser314	Ser314
Arg483	Ser190
	Thr187
	Arg483

(Figure 14b.)

The table above on Figure 14b shows the individual ligand-residue interaction between my

(Figure 15)

Best docked post of Eujin_5HM3 shown in blue stick, superimposed with 649, purple ball and stick, from PDB entry 5HM3.

Eujin_5HM3_649	5HM3 Crystal Structure
Ser349	Ser349
Asp476	Asp476
Ser321	Ser321
Gly351	Gly351
	HSD238
Asp239	Asp239
Leu357	Leu357
Leu318	Leu318
Leu247	Leu247
Leu251	Leu251

(Figure 15b.)

The table above on Figure 15b. shows the individual ligand-residue interaction between my Docked result and the crystal structure.
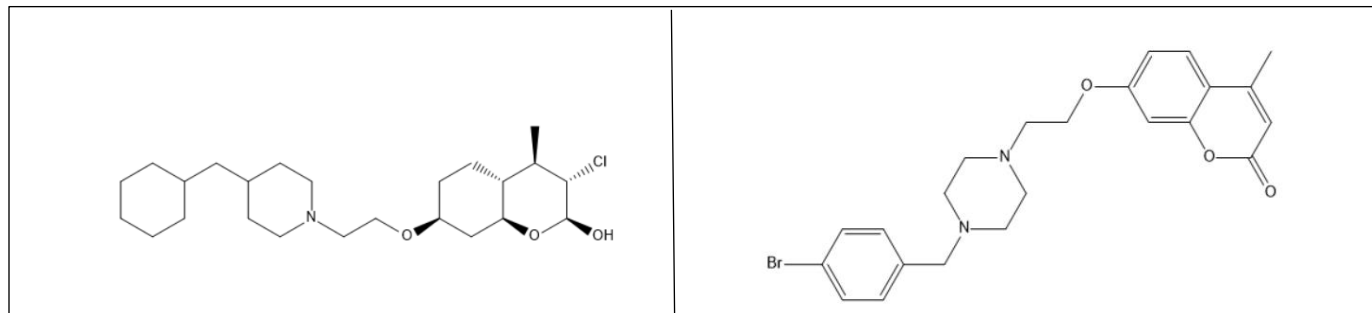
Eujin CM8

Eujin CM12

**Eujin CM14 (Dock Results Pending* Results unavailable at the moment)**

Eujin CM15

The molecules below on Figure 15 and 16 were synthesized to test the inhibition activity on *Mtb,* and noted that these four specifically are the most potent. I docked these four to compare the binding mode to the protein and compare it with other inhibitors.

(Figure 16)

Image shown in the figure are the 2D Crystal Structure of CM8 and CM12 respectively
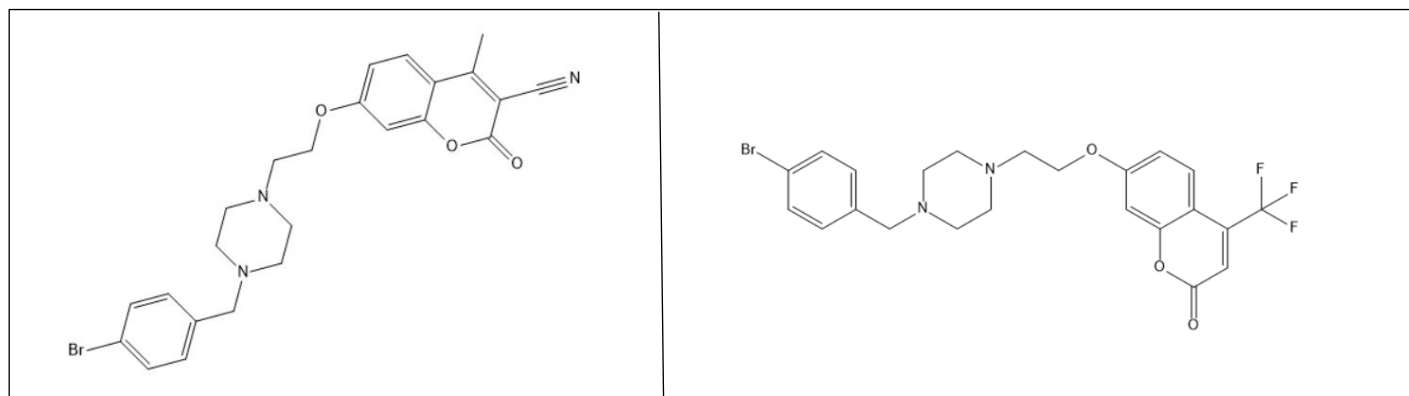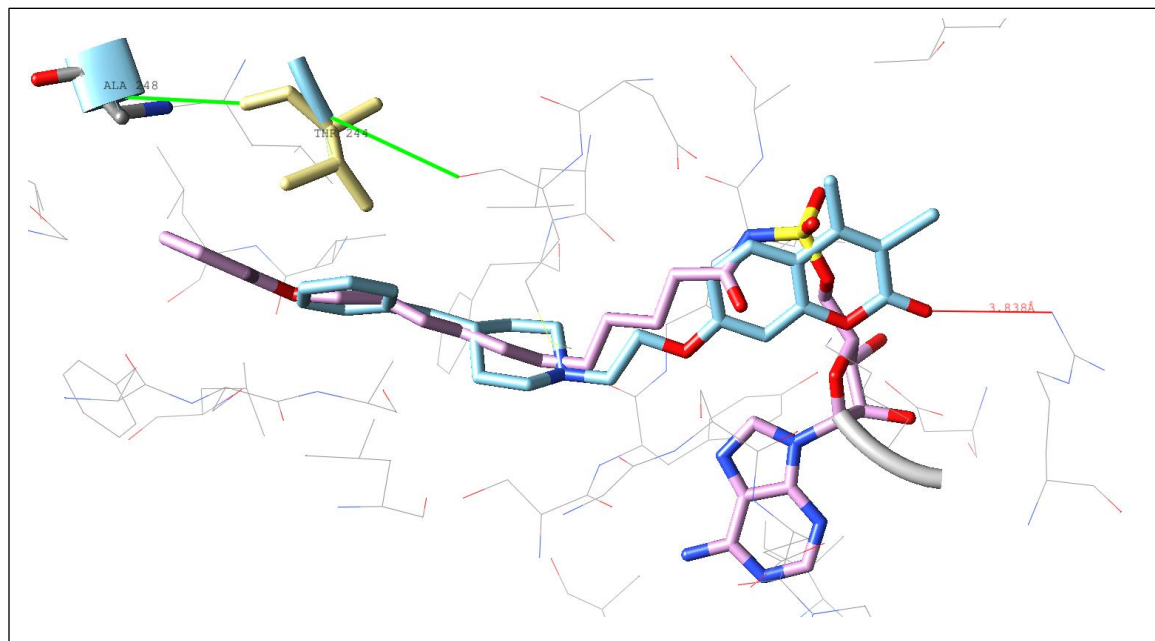

(Figure 17)

Image above are the 2D Crystal Structure of CM14 and CM15 respectively.

The docking results chosen with 649 in complex was FadD32 can be seen below on Figure 18. Analyzing articles with information's of using coumarin derivative to *Mtb*, the coumarin base is used as a scaffold for the position outside to inhibit the bacteria. The best fit

pose chosen for all four provided ok results. It could suggest that coumarin although versatile, should not be used as an inhibitor for Mtb. The $\triangle$ G value on all four are labeled on Figure 18, 19, and 21.
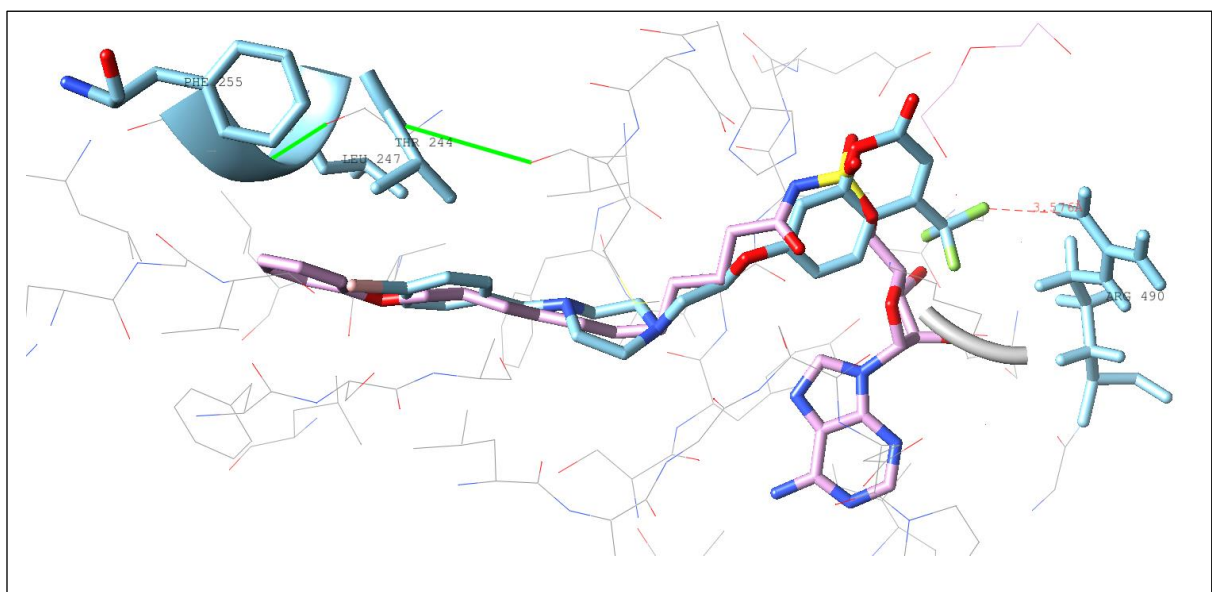

(Figure 18)

Best docking pose of Eujin_CM8 (blue stick) superimposed with 649 (purple stick) from PDB entry 5HM3 with a $\triangle$G of -7.23
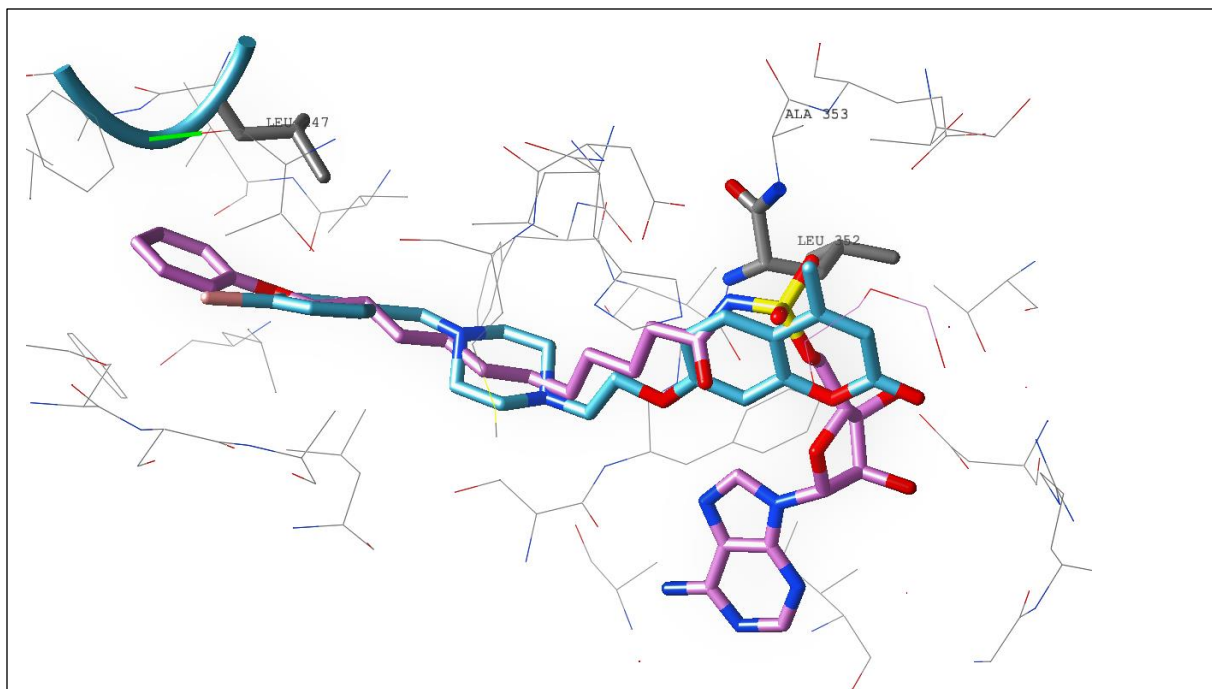
(Figure 19)

Best docking pose of Eujin_CM12 (blue stick) superimposed with 649 (purple stick) from PDB entry 5HM3 with a △ G value of -7.64

(Figure 21)

Best docking pose of Eujin_CM15 (blue stick) superimposed with 649 (purple stick) from PDB entry 5HM3 with a △ G value of -7.99

7MGTP

7BenzGTP

The following molecules were designed in a scientific journal by Dr. Ghosh. Used previously as an anti-viral agent, the designed molecules have similar structural characteristics as ATP. The idea is due to the similarity, and it could potentially be used a viable inhibitor. The 2D structure of the molecules is shown below on Figure 21.
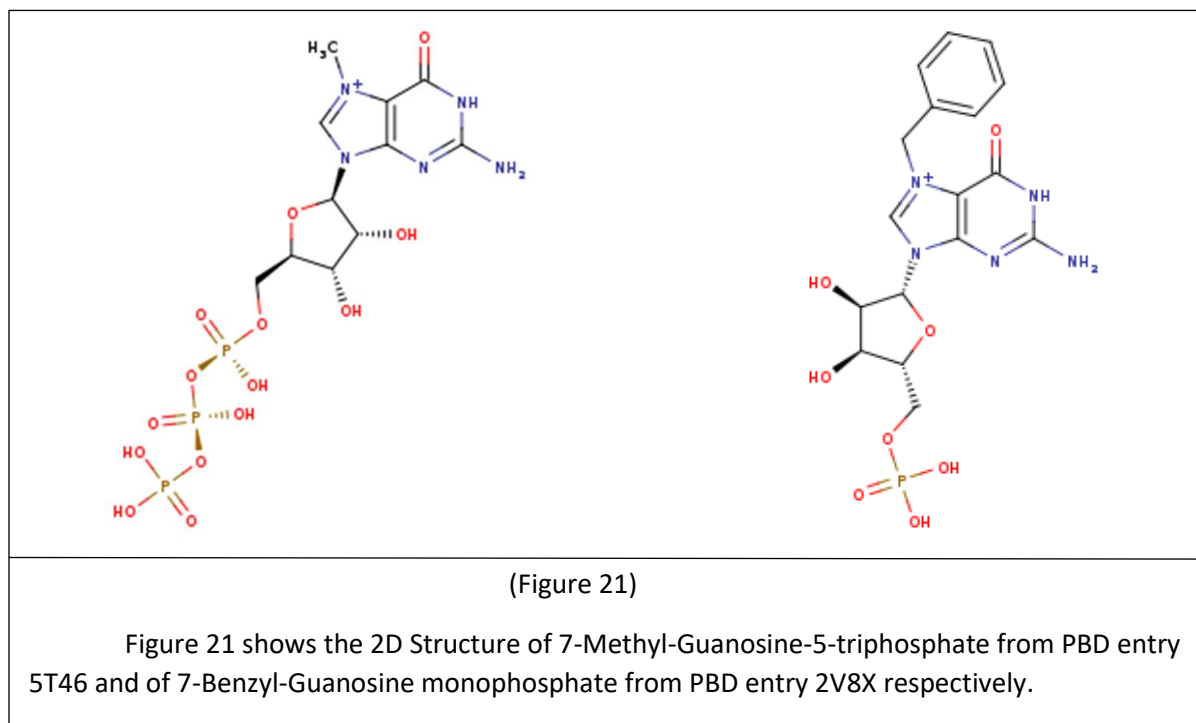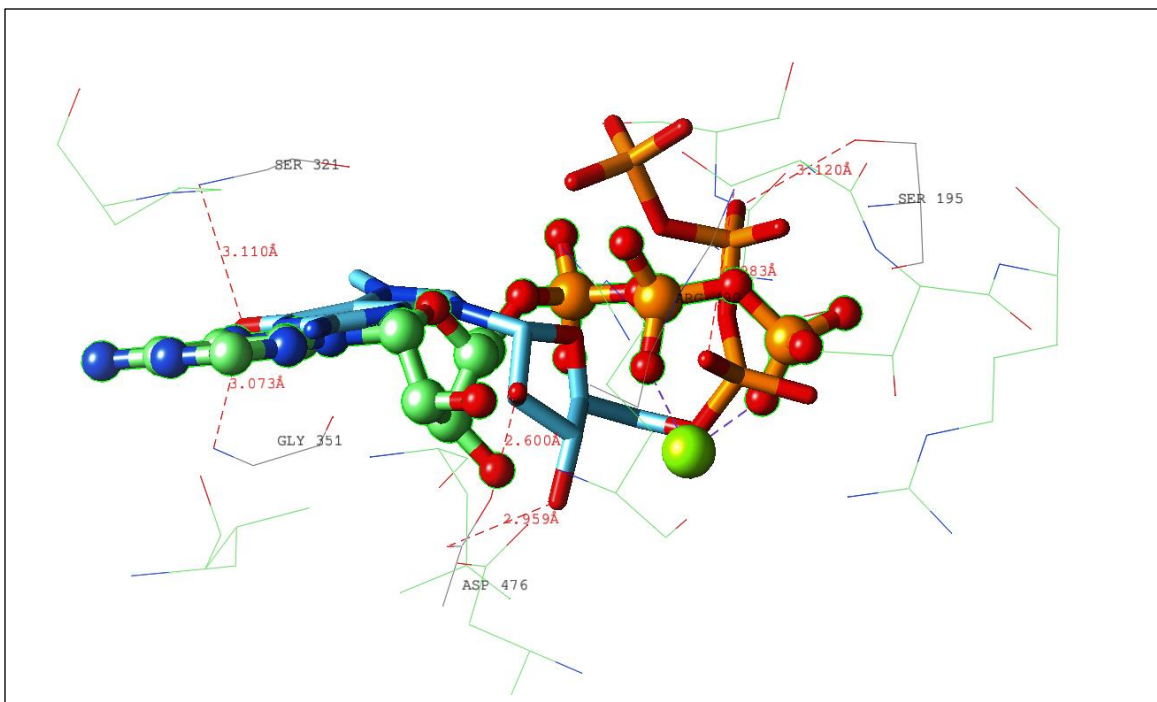

(Figure 21)

Figure 21 shows the 2D Structure of 7-Methyl-Guanosine-5-triphosphate from PBD entry 5T46 and of 7-Benzyl-Guanosine monophosphate from PBD entry 2V8X respectively.

Figure 22 below shows the best fit pose for 7MGTP superimposed with ATP from PBD entry 5D6J.The chosen pose superimposed with each ligand shows the expecting binding pose for FadD32. Analyzing the docking pose, it was found to form a bidentate bond with Serine 321 and Glycine 351, to help stabilize the complex. Without Mg+ and/or H2O not present in the protein pocket; a decrease of possible interactions will be expected. Also, the triphosphate forms a hydrogen in a different position, compared to ATP, with Ser195 for additional stability. With a △ G value of -13.14, this structure shows promising results. There are always room for improvement. The residue interaction of 7MGTP and ATP is shown below on Figure22b.
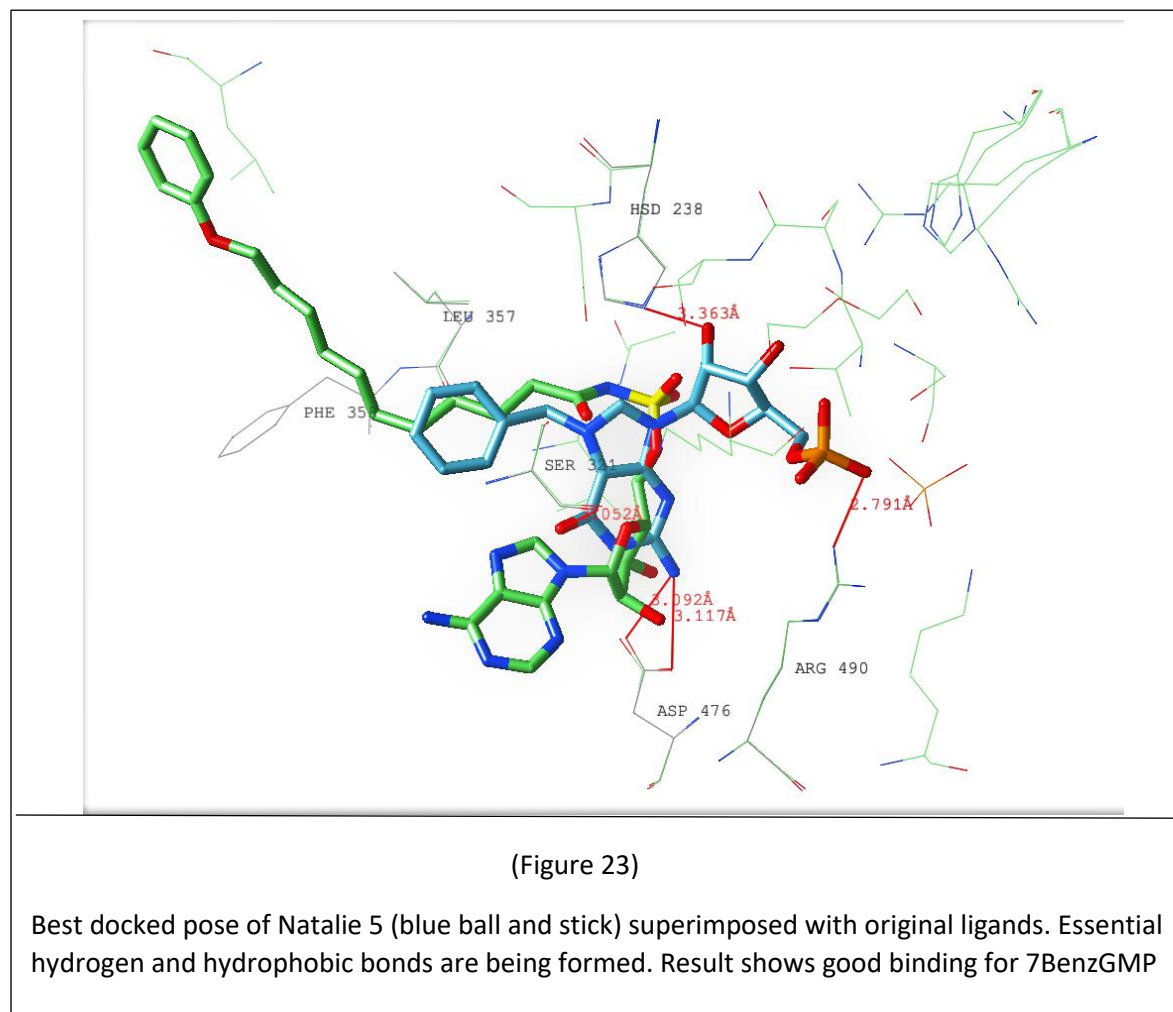
(Figure 22)

7MGTP docking results. The bidentate bond with Ser321 and Gly351, and other hydrogen bonds are being formed. Results show good binding for 7MGTP

7MGTP	5D6J Crystal Structure
Ser321	Ser342
Gly351	Asp469
Asp476	Ser188
Arg483	Thr191
Ser195	Arg192
	Ser314
	Ser190
	Thr187
	Arg483

(Figure 22b.)

The table above on Figure 22b shows the individual ligand-residue interaction between 7MGTP and the crystal structure of ATP.

Figure 23 below is the best fit pose for 7BenzGMP, superimposed with 649 from PDB entry 5HM3. Comparing the superimposed image, the charged guanine forms hydrogen bond with Asp476 rather than the ribose. The ribose binds to HSD238 and phosphate with Arg490 to form hydrogen bonds. This will help keep the ligand in position. Lastly, the benzyl ring from the guanine form the same hydrophobic interaction as 649 on PHE358 with a △ G value -10.77. Despite having a smaller △G value compared to other ligand, the size of 7BenzGMP is also much smaller than others which decreases the number of interactions formed. It also suggests that the △G value of 7BenzGMP is a result of the specific residues that bind, rather than the number of residues. Improvements to this molecule can happen. Comparing the results with the previous coumarin molecule, it can be theorized that the charged guanine should perhaps be used as a scaffold rather than coumarin derivatives. The residue interactions of 7BenzGMP is shown below on Figure 23b.
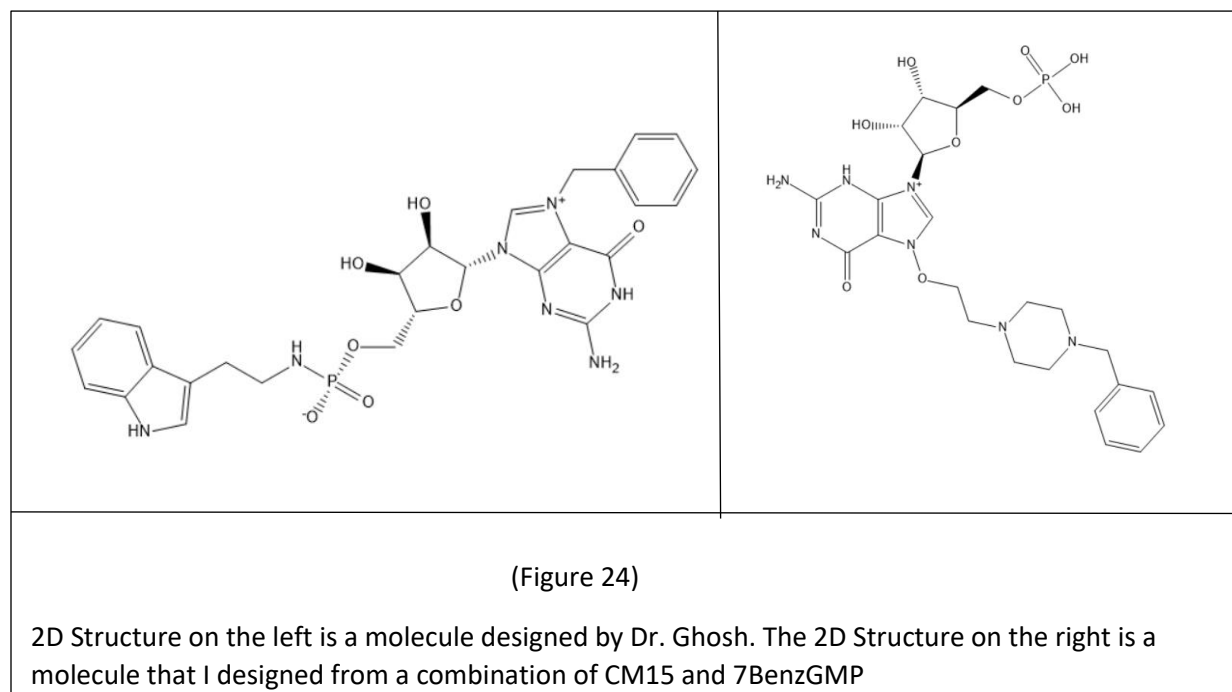

(Figure 23)

Best docked pose of Natalie 5 (blue ball and stick) superimposed with original ligands. Essential hydrogen and hydrophobic bonds are being formed. Result shows good binding for 7BenzGMP

7BenzGMP	5HM3 Crystal Structure
Phe358	Ser349
Asp476	Asp476
Ser321	Ser321
	Gly351
HSD238	HSD238
Arg490	Asp239
Leu357	Leu357
	Leu318
	Leu247
	Leu251

(Figure 28)

The table above on Figure 28 shows the individual ligand-residue interaction between 7BenzGMP and the crystal structure of 649.

Failed Docking Attempts:

The following molecules on Figure 24 below was not able to dock because of Topology errors when loading the file on SwissDock.

(Figure 24)

2D Structure on the left is a molecule designed by Dr. Ghosh. The 2D Structure on the right is a molecule that I designed from a combination of CM15 and 7BenzGMP

Conclusion:

Docking a lot of molecules, there were some interesting results. Through these methods, it can be argued that using 2D approaches for Drug Discovery is not optimal. It does not consider the 3D the active site of the protein, thus requiring more resources and time. With the use of bioinformatic software to study the 3D structure, more accurate results in designing a new inhibitor for FadD32. Also by following this approach, we reduce the amount of time and resources during the discovery process. With the use of bioinformatic tools, users can make more accurate design choices compared to 2D Structure Based Drug Design.

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