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Do drugs for Mycobacterium Tuberculosis cofactor F420 prevent the evolution of resistance

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ABSTRACT

Tuberculosis (TB) is an infectious disease that affects the majority of the world. The current treatment TB involves a combination of 5 to 6 antibiotics due to the development of resistance to many of these drugs in Mycobacterium tuberculosis (Mtb). These drugs usually consist of first-line drugs like isoniazid and rifampicin and second-line drugs like fluoroquinolones. Hence, drugs that currently exist to combat Tuberculosis only work when combined with other antibiotics, since extensively-drug resistant tuberculosis (XDR TB) has rendered them less effective against Mycobacterium Tuberculosis(Mtb).

In this paper, I have investigated how different drugs that exist could inhibit cofactor F420 in the metabolism of Mtb. These include Tamiflu, AZD6482, Arfomoteral, and Minocycline. The binding affinity of these drugs, which are similar in structure to cofactor F420, was found against different F420-containing proteins in Mtb, and hence their possible use in the future was investigated. Hence, hypothetically if such drugs are able to bind to cofactor F420-containing enzymes, they can inhibit them. This could give rise to the theory of allowing mtb to undergo antibiotic stress, since F420 has been found to be crucial in pathways that combat this stress. Therefore, mtb would undergo stress, decreasing its chances of survival and subsequently decreasing the possibility of cells surviving and mutating. However, this is an indirect relation and needs further experimental investigation.

INTRODUCTION

The prevalence of Tuberculosis is a pressing issue, and reasons for this largely include, the evolution of Mycobacterium Tuberculosis (Mtb), specifically in providing resistance to antibiotics, is why it is still thriving in many countries. Mtb is dependent on cofactor F420 for enzymatic activities, and specifically its role in Mtb's resistance to drugs. There are many proteins that could bind to F420- dependent enzymes, and through analysing different publicly available proteins, it was determined which of those could act as suitable drugs or be designed into drugs that would bind to F420-dependent enzymes.

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LITERATURE REVIEW

The unique metabolism of Mtb and the role of Cofactor F420 in its metabolism: Mycobacterium Tuberculosis consists of a unique cofactor - cofactor F420 - which is essential in its redox metabolism and pathogenesis. Cofactor F420 to microbial persistence, even after antimicrobial treatment. While this feature of cofactor F420 is known, the molecular understanding behind it is not complete. This cofactor enhances Mtb's survival under antimicrobial conditions because it broadens the range of natural products Mtb can reductively detoxify. Hence, it may indirectly affect the development of resistance. [1] Furthermore, it has been found that cofactor F420-dependent anti-oxidative mechanisms protect Mtb from Oxidative stress. Hence, the presence of cofactor F420 in Mtb is one factor contributing to its prevalence and survival are so high under hostile conditions.

The physical changes Mtb undergoes in hosts: When the conditions in a host are unfavourable to Mtb, the Mtb goes into a dormant stage in which it adapts itself to survive. This is another reason as to why Mtb is so common as it may activate at any time. In this dormant stage Mtb changes to slow its metabolic rate, increase cell volume, and makes structural changes to itself to handle stress. Moreover dormant Mtb can also use host cholesterol for energy. While there is a vaccine for TB called the BCG vaccine, it does not provide protection when dormant Mtb is activated hence a more effective way of doing so is still needed. [2]

The role evolution of Mtb plays: The treatment of Mtb requires a combination of 3 to 4 antibiotics over several months. This treatment has led to the development of different types of resistance in Mtb to these drugs hence it is becoming harder to treat. It was found in a research study that the transition bias in the number of mutational events associated with resistance to drugs in Mtb. It has also been found that the treatment of Mtb induces strong selection pressure for the emergence of antibiotic-resistant variations of Mtb. Through this study it has been found that this characteristic of Mtb is mainly attributed to the indels. [3] Hence, the treatment of Mtb currently, and in the long run, is not entirely effective hence its evolution and prevalence are ubiquitous. With this research paper, I hope to investigate a possible basis to decrease the evolution of Mtb.

How the presence of Cofactor F420 affects the treatment of Mtb: It was noted in a study that the mutant strains of Mtb unable to produce cofactor F420, were hypersusceptible to certain antibiotics like isoniazid. Similarly, the inactivation of a gene within Mtb, leads to the unavailability to cofactor F420 due to which, Mtb is hypersensitive to oxidative stress. It has also been suggested in a study that the inhibition of pathways involving cofactor F420 could lead to reduced fitness of Mtb and enhanced impact of antimicrobial drugs. The sporadic presence and abundance of cofactor F420 in Mtb suggests that it is crucial for redox reactions and so a possible target. However the investigation on such a matter is limited and so the aims of this research paper are to explore how drugs for Mycobacterium Tuberculosis cofactor F420 prevent the evolution of resistance. Here I will be analysing different molecules, similar in structure to cofactor F420,could be possibly used for inhibition.

The link between cofactor F420 and the evolution to resistance in Mtb: The physiology of redox cofactor F420 in Mycobacteria plays a critical role in its metabolism . In a previous study, upon testing cofactor F420 [7,8dodidemethyl-8-hyroxy-5-deazariboflavin-5'-phosphoryllactyl(glutamyl)n glutamate] found in bacteria, certain properties were revealed which contribute to its role. The properties of this cofactor F420 such as its ability mediate hydride transfer between organic carbon compounds or challenge reduction reactions, are why it is utilised by bacteria in an array of metabolic reactions. In bacteria, this cofactor is found secondary in importance during such reactions; however, it is found that certain metabolic reactions do depend on it. Studies that have been done specifically on Mtb cofactor F420 show how it is essential for its survival when facing antibiotic stress and oxidative stress. [4]

In Mtb, a metabolic pathway of combating oxidative stress includes the F420H2- dependent quinone reductase catalysing an F420H2-specific obligate two-electron reduction of endogenous quinones and this prevents the formation of cytotoxic semiquinones. [5]

The effect of cytotoxic semiquinones radicals will cause an oxidative burst which releases toxic chemicals, Reactive Oxygen Species, inflicting DNA damage and protein oxidation on the bacteria. [6]

This detoxification system helps the bacteria survive when placed under antibiotic-induced stress conditions. The survival of bacteria when placed under stress from antibiotics is the primary reason for selection pressures that may lead to the expansion of mutant bacteria. Hence, inhibiting F420-dependent pathways may reduce the ability for the bacteria to survive under antibiotic stress, reducing the pool of cells which can cause resistant variants to arise.

However, the relation between F420 inhibition and reduced mutation in bacteria has not been investigated experimentally. Hence these links are suggestive and purely based on the ability of F420 to cause stress tolerance in Mtb. This limitation is an important one and proves the need for further experimentation.

METHODS

Collecting data:

The process of data collection for this study first follows using RCSB PDB to search for suitable proteins consisting of cofactor F420. The collection of the proteins used in this study is done through X-ray crystallography which is considered a high standard, however it is just representing a model. [7]

Following this, the SMILE codes for each protein were obtained.

Next, by using the structural similarity search on Chemmine Tools, each of their SMILES codes was inserted separately, and a similarity cutoff was used to generate results. With the results, suitable drugs that fit an established criteria were chosen.

The 3D structure of each was downloaded and uploaded separately as ligands on CB-Dock 2. On CB-Dock2, the suitable cofactor f420-containing proteins found in Mtb were uploaded as the protein and blind docking was performed.

Then from these results, the protein-ligand with the most negative Vina score was selected and its protein-ligand PDB file was downloaded.

On Pymol, the cofactor f20-containing proteins were uploaded first, and their waters were from the structure. Then the protein-ligand file was opened and aligned with the cofactor f420-containing protein to see if they intersect. If they intersected, I the next step was carried out. However, if they did not then the process would be repeated again with the second most negative Vina score

Once a Vina score which allows for the protein-ligand and cofactor f420-containing protein to align was identified, the protein-ligand section on prodigy was utilised.

Using the data available on the aligned complex on Pymol, the data needed on prodigy was acquired. Then, the results generated showing binding affinity were noted and analysed.

Justification and explanation:

This study poses a structure-based visual output to evaluate potential interactions between the selected F420 binding sites and the chosen drug molecules.

On Chemmine Tools, the structural similarity search ensures the identification of compounds that share structural features with the cofactor F420 being studied. This similarity search hence increase the chances of finding a molecule that could potentially bind to a binding site a cofactor F420 molecule would bind to, suggesting possible inhibition. However, there are limitations and this would need to be considered experimentally.

The docking was performed on CB-Dock2 and the binding affinity scores were obtained from here. To ensure more accuracy the docking was repeated and the scores were used. This ensures the predicting binding score is correct.

This study was done under the assumption of semi-flexible binding with CB-Dock 2 giving results for binding that have been shown to be up to 85% accurate. However, there is still a chance for inaccuracy and false results for this process. [8]

The Vina scores that were achieved were then ranked from most negative (highest predicted binding affinity) to least negative (lowest predicted binding affinity). Those with the most negative Vina scores were chosen and tested visually using Pymol to see interactions between drug and cofactor F420 showing how similar they are. This was followed by using prodigy to get numerical data for the interactions.

Choosing subjects:

The data available from RCSB PBD was used to identify 5 different types of cofactor f420-containing proteins. The 5 chosen were: 4XOQ, 4QVB, 7KL8, 5JAB, and 3B4Y.

Then, to use the similarity search by inputting the SMILES code for each of these, a similarity of 0.6 to 0.7 and an output of 100 structures was used.

The reason why a higher similarity percentage was not selected was because at higher percentages, the only structures that would come up were other cofactor F420 molecules and nucleotides commonly found within the human body, while what was needed were potential drugs that could be used.

The criteria for structures not used to test binding affinity were applied with structural similarity being a filter for those which did not have a similar structure to the cofactor F420 being used. Other criteria included: selection against structures with inappropriate molecular size comparison with F420, drugs that did not have files available hence could not be uploaded onto pymol, those drugs which were already approved were selected where possible.

After going through different drugs that were coming up the 4 which fit the criteria were chosen. The 4 chosen were Tamiflu, AZD6482, Arformoterol, and Minocycline. Each underwent the process mentioned above and was tested relative to each of the cofactor F420-containing proteins.

Conducting analysis:

After the binding affinity for each of the protein-ligand complexes was determined using prodigy, the best 2 out of 4 drugs with the highest binding affinity with each of the 5 cofactor f420-containing proteins was chosen. After these steps were carried out, for the overall analysis, took the 2 drugs that were most commonly the among the drugs with high binding affinities for each cofactor F420-containing proteins, and suggest comments for these.

Limitations:

This study is done based on just quantitative data and was not done exponentially. The tools used do not give results which are entirely accurate and the connections made are based on the function of cofactor F420 in the metabolism of mtb and under antibiotic stress. There were also no considerations for pharmacokinetics, permeability across cell surface membranes and target accessibility. Hence, there would need to be experimentation to test if this is accurate.

Use of controls:

A known cofactor F420 molecule is used as a control to measure how accurate the binding was. This showed how different the other results were to this. The control used is Folic acid. [26]

RESULTS

The cofactor F420 containing proteins present in Mycobacterium Tuberculosis chosen were: 4XOQ [14], 4QVB [15], 7KL8 [16], 5JAB [17], and 3B4Y [18]. The criteria these structures needed to fulfill included being from Mtb and containing cofactor F420. As such these were the only suitable structures available.

The four drugs chosen when a similarity search for cofactor F420 was carried out were: Tamiflu, AZD6482, Arfomoteral, and Minocycline. [19]

Tamiflu:
Uses:Tamiflu is a drug used to treat the flu.
Side effects: It could cause nausea, vomiting, headaches and pain [9]
Previous uses: Tamiflu has not been used previously to treat Mycobacterium Tuberculosis

AZD6482:
Uses: used to treat thrombosis
Side effects: it is currently under investigation [10]
Previous uses: Has not been used previously for Mycobacterium Tuberculosis

Arformoteral:
Uses: It is inhaled and used for coughing and wheezing.
Side effects: flu like symptoms [11]
Previous uses: Has not been used previously for Mycobacterium Tuberculosis

Minocycline:
Uses: It is used to treat infections.
Side effects: nausea, vomiting, itching, hair loss [12]
Previous uses: It is currently under investigation for treatment of drug resistant Tuberculosis. [13]

Then, each of the proteins was docked with each of the drugs through molecular docking [20] using CB-Dock 2, to ensure whether these drugs were able to competitively inhibit binding sites for cofactor F420. The type of docking being done was rigid body docking.

As a result, 5 values for the Vina score were obtained. The first and most negative one was chosen and then checked on Pymol. If there was no interaction with cofactor F420 site, the next was chosen and tested until one was found to interact. The Vina scores for the molecular docking for each are:

	4XOQ	4QVB	7KL8	5JAB	3B4Y
Tamiflu	-7.3	-6.0	-6.2	-5.9	-7.0
AZD6482	-9.9	-8.0	-8.2	-8.2	-10.9
Arformoterol	-8.3	-6.6	-6.4	-6.6	-8.1
Minocycline	-10.6	-7.9	-8.5	-7.5	-8.5

TABLE I This table shows the Vina scores

The protein-ligand complexes were then obtained from CB-Dock 2 and downloaded and then uploaded onto prodigy. The tool was run for each of the complexes and their binding affinities were checked to identify strengths.

Key:

C:Carbon

O:Oxygen

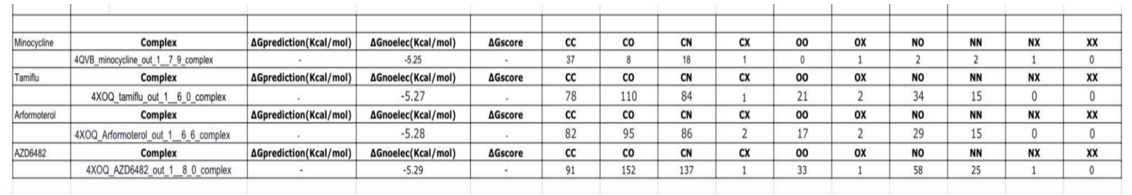
N:Nitrogen

X:all other atoms.

Each pair of these atoms is demonstrating the types of interactions taking place between the cofactor F420 and the drug being tested.

4XOQ:

I ran each of the 4 chosen ligands, along with 4XOQ through the required steps and obtained the following results:



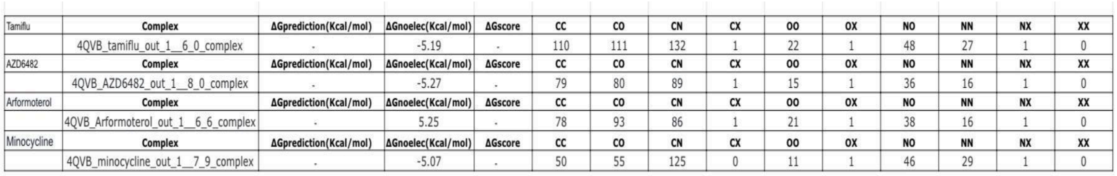
Minocycline	**Complex**	**ΔGprediction(Kcal/mol)**	**ΔGnoelec(Kcal/mol)**	**ΔGscore**	**CC**	**CO**	**CN**	**CX**	**OO**	**OX**	**NO**	**NN**	**NX**	**XX**
	4QVB_minocycline_out_1__7_9_complex	-	-5.25	-	37	8	18	1	0	1	2	2	1	0
Tamiflu	**Complex**	**ΔGprediction(Kcal/mol)**	**ΔGnoelec(Kcal/mol)**	**ΔGscore**	**CC**	**CO**	**CN**	**CX**	**OO**	**OX**	**NO**	**NN**	**NX**	**XX**
	4XOQ_tamiflu_out_1__6_0_complex	.	-5.27	.	78	110	84	1	21	2	34	15	0	0
Arformoterol	**Complex**	**ΔGprediction(Kcal/mol)**	**ΔGnoelec(Kcal/mol)**	**ΔGscore**	**CC**	**CO**	**CN**	**CX**	**OO**	**OX**	**NO**	**NN**	**NX**	**XX**
	4XOQ_Arformoterol_out_1__6_6_complex	.	-5.28	.	82	95	86	2	17	2	29	15	0	0
AZD6482	**Complex**	**ΔGprediction(Kcal/mol)**	**ΔGnoelec(Kcal/mol)**	**ΔGscore**	**CC**	**CO**	**CN**	**CX**	**OO**	**OX**	**NO**	**NN**	**NX**	**XX**
	4XOQ_AZD6482_out_1__8_0_complex	-	-5.29	-	91	152	137	1	33	1	58	25	1	0

TABLE II This table shows the data generated when the binding affinity for each protein-ligand complex was tested, with the protein being 4XOQ.

The above table shows the values that were generated when the protein-ligand was inserted into prodigy. From the table it can be determined what ΔGnoelec(Kcal/mol) -which tells the binding energies of each of the 4 proteins- was for the protein-ligand being tested. The more negative the value that is produced, the better the binding affinity for the molecule. Each of the other values tell the number of interactions between certain atoms as mentioned in the key above. Hence, the results express the possible areas where the two structures overlap which can better tell how compatible they would be to bind to each other.

It is therefore seen that the ΔGnoelec(Kcal/mol) for Arfomoterol and AZD6482 are best. Each of the values follows closely behind one another within the range of -5.24<ΔGnoelec<-5.30 meaning that each of the ligands has similar binding affinity to this molecule.

4QVB:

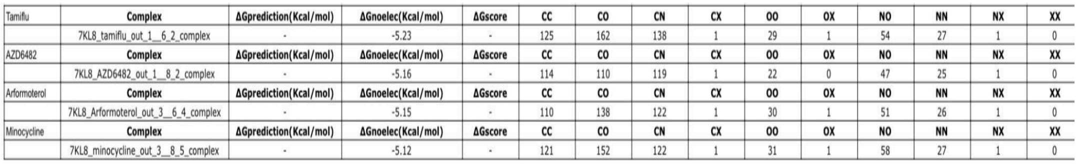


Tamiflu	**Complex**	**ΔGprediction(Kcal/mol)**	**ΔGnoelec(Kcal/mol)**	**ΔGscore**	**CC**	**CO**	**CN**	**CX**	**OO**	**OX**	**NO**	**NN**	**NX**	**XX**
	4QVB_tamiflu_out_1__6_0_complex	-	-5.19	-	110	111	132	1	22	1	48	27	1	0
AZD6482	**Complex**	**ΔGprediction(Kcal/mol)**	**ΔGnoelec(Kcal/mol)**	**ΔGscore**	**CC**	**CO**	**CN**	**CX**	**OO**	**OX**	**NO**	**NN**	**NX**	**XX**
	4QVB_AZD6482_out_1__8_0_complex	.	-5.27	.	79	80	89	1	15	1	36	16	1	0
Arformoterol	**Complex**	**ΔGprediction(Kcal/mol)**	**ΔGnoelec(Kcal/mol)**	**ΔGscore**	**CC**	**CO**	**CN**	**CX**	**OO**	**OX**	**NO**	**NN**	**NX**	**XX**
	4QVB_Arformoterol_out_1__6_6_complex	.	5.25	.	78	93	86	1	21	1	38	16	1	0
Minocycline	**Complex**	**ΔGprediction(Kcal/mol)**	**ΔGnoelec(Kcal/mol)**	**ΔGscore**	**CC**	**CO**	**CN**	**CX**	**OO**	**OX**	**NO**	**NN**	**NX**	**XX**
	4QVB_minocycline_out_1__7_9_complex	.	-5.07	.	50	55	125	0	11	1	46	29	1	0

TABLE III This table shows the data generated when the binding affinity for each protein-ligand complex was tested, with the protein being 4QVB.

It is seen through table III that the ΔGnoelec(Kcal/mol) for Arfomoterol and AZD6482 are slightly better due to a higher binding energy. All the values for ΔGnoelec(Kcal/mol) are seen to be within the range of -5.05<ΔGnoelec<-5.30. Hence from these results it can be seen that the ΔGnoelec(Kcal/mol) for Arfomoterol and AZD6482 are the most negative, meaning that these proteins have the highest binding affinity.

7KL8:

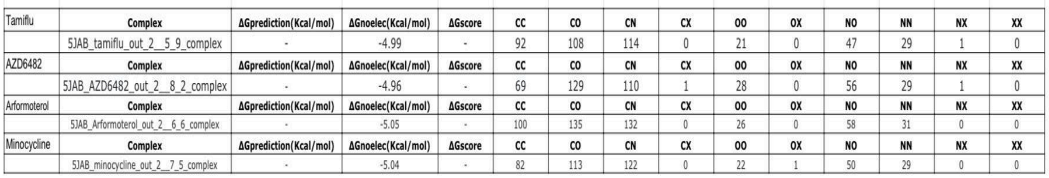


Tamiflu	Complex	ΔGprediction(Kcal/mol)	ΔGnoelec(Kcal/mol)	ΔGscore	CC	CO	CN	CX	OO	OX	NO	NN	NX	XX
	7KL8_tamiflu_out_1__6_2_complex	-	-5.23	-	125	162	138	1	29	1	54	27	1	0
AZD6482	Complex	ΔGprediction(Kcal/mol)	ΔGnoelec(Kcal/mol)	ΔGscore	CC	CO	CN	CX	OO	OX	NO	NN	NX	XX
	7KL8_AZD6482_out_1__8_2_complex	-	-5.16	-	114	110	119	1	22	0	47	25	1	0
Arformoterol	Complex	ΔGprediction(Kcal/mol)	ΔGnoelec(Kcal/mol)	ΔGscore	CC	CO	CN	CX	OO	OX	NO	NN	NX	XX
	7KL8_Arformoterol_out_3__6_4_complex	-	-5.15	-	110	138	122	1	30	1	51	26	1	0
Minocycline	Complex	ΔGprediction(Kcal/mol)	ΔGnoelec(Kcal/mol)	ΔGscore	CC	CO	CN	CX	OO	OX	NO	NN	NX	XX
	7KL8_minocycline_out_3__8_5_complex	-	-5.12	-	121	152	122	1	31	1	58	27	1	0

TABLE IV This table shows the data generated when the binding affinity for each protein-ligand complex was tested, with the protein being 7KL8.

It is seen through table IV that the ΔGnoelec(Kcal/mol) for Tamiflu and Minocycline are most. The ΔGnoelec(Kcal/mol) for each of these values is high, specially for Tamiflu, AZD6482 and Minocycline, who all show comparatively high ΔGnoelec(Kcal/mol) values within the range of -5.12<ΔGnoelec<-5.23. Out of all 5 cofactor F420 containing proteins, the highest ΔGnoelec(Kcal/mol) hence highest binding affinity is seen for 7KLI.

5JAB:

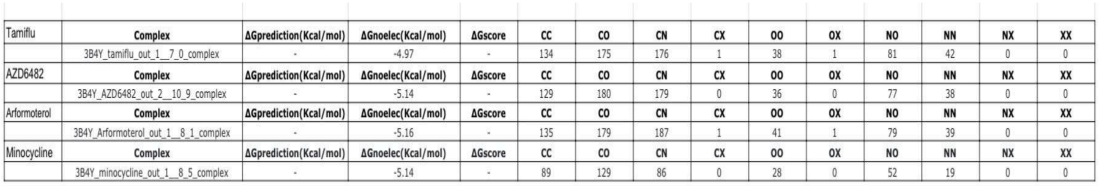


Tamiflu	Complex	ΔGprediction(Kcal/mol)	ΔGnoelec(Kcal/mol)	ΔGscore	CC	CO	CN	CX	OO	OX	NO	NN	NX	XX
	5JAB_tamiflu_out_2__5_9_complex	-	-4.99	-	92	108	114	0	21	0	47	29	1	0
AZD6482	Complex	ΔGprediction(Kcal/mol)	ΔGnoelec(Kcal/mol)	ΔGscore	CC	CO	CN	CX	OO	OX	NO	NN	NX	XX
	5JAB_AZD6482_out_2__8_2_complex	-	-4.96	-	69	129	110	1	28	0	56	29	1	0
Arformoterol	Complex	ΔGprediction(Kcal/mol)	ΔGnoelec(Kcal/mol)	ΔGscore	CC	CO	CN	CX	OO	OX	NO	NN	NX	XX
	5JAB_Arformoterol_out_2__6_6_complex	-	-5.05	-	100	135	132	0	26	0	58	31	0	0
Minocycline	Complex	ΔGprediction(Kcal/mol)	ΔGnoelec(Kcal/mol)	ΔGscore	CC	CO	CN	CX	OO	OX	NO	NN	NX	XX
	5JAB_minocycline_out_2__7_5_complex	-	-5.04	-	82	113	122	0	22	1	50	29	0	0

TABLE V This table shows the data generated when the binding affinity for each protein-ligand complex was tested, with the protein being 5JAB.

It is seen through table V that the ΔGnoelec(Kcal/mol) for Arfomoterol and Minocycline are most. The values for ΔGnoelec(Kcal/mol) in this case are comparatively on the low side, however they still lie close together in the range of -4.95<ΔGnoelec(Kcal/mol)<-5.06. Hence, while the values for ΔGnoelec(Kcal/mol) are not very high for 5JAB, it can still be suggested that since the values lie close together, they may be similarly effective in binding with a more compatible cofactor F420 containing molecule, as seen above.

3B4Y:



Tamiflu	**Complex**	**ΔGprediction(Kcal/mol)**	**ΔGnoelec(Kcal/mol)**	**ΔGscore**	**CC**	**CO**	**CN**	**CX**	**OO**	**OX**	**NO**	**NN**	**NX**	**XX**
	3B4Y_tamiflu_out_1__7_0_complex	-	-4.97	-	134	175	176	1	38	1	81	42	0	0
AZD6482	**Complex**	**ΔGprediction(Kcal/mol)**	**ΔGnoelec(Kcal/mol)**	**ΔGscore**	**CC**	**CO**	**CN**	**CX**	**OO**	**OX**	**NO**	**NN**	**NX**	**XX**
	3B4Y_AZD6482_out_2__10_9_complex	-	-5.14	-	129	180	179	0	36	0	77	38	0	0
Arformoterol	**Complex**	**ΔGprediction(Kcal/mol)**	**ΔGnoelec(Kcal/mol)**	**ΔGscore**	**CC**	**CO**	**CN**	**CX**	**OO**	**OX**	**NO**	**NN**	**NX**	**XX**
	3B4Y_Arformoterol_out_1__8_1_complex	-	-5.16	-	135	179	187	1	41	1	79	39	0	0
Minocycline	**Complex**	**ΔGprediction(Kcal/mol)**	**ΔGnoelec(Kcal/mol)**	**ΔGscore**	**CC**	**CO**	**CN**	**CX**	**OO**	**OX**	**NO**	**NN**	**NX**	**XX**
	3B4Y_minocycline_out_1__8_5_complex	-	-5.14	-	89	129	86	0	28	0	52	19	0	0

TABLE VI This table shows the data generated when the binding affinity for each protein-ligand complex was tested, with the protein being 3B4Y.

It is seen through table VI that the ΔGnoelec(Kcal/mol) for Arfomoterol and Minocycline are most. The ΔGnoelec(Kcal/mol) values for AZD6482, Arfomoterol and Minocycline are in the midranges from -5.13<ΔGnoelec(Kcal/mol)<-5.16. This suggests that the binding affinity for these proteins with 3B4Y might not be the highest, however they are still effective in binding to it. [21]

These results show how for all of the cofactor F420-containing proteins, Arfomoterol and Minocycline are the most effective as their binding affinities and interactions are the highest. These drugs often show the most interactions between different carbons and their NO interactions are amping the highest for all of the proteins.

However, each has values that are close and have a small difference. This suggests the cofactor F420 molecules interact similarly with each of the drugs tested. This could be insignificant however. This data is not completely accurate and reliable and further testing and experimental evidence would be needed.

FIGURES

Below are images of the protein-ligand complex, generated through Pymol [22], for each of the 4 cofactor F420 containing molecules with the 2 proteins (Arfomoterol and Minocycline).

4XOQ:

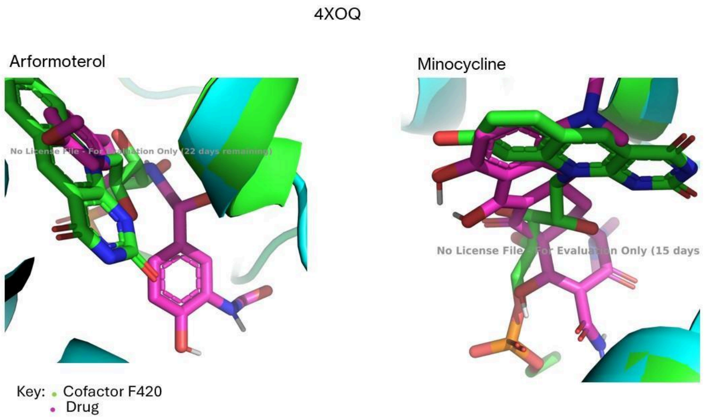


Figure I This figure shows the binding of each of these drugs to cofactor F420 in 4XOQ

4QVB:

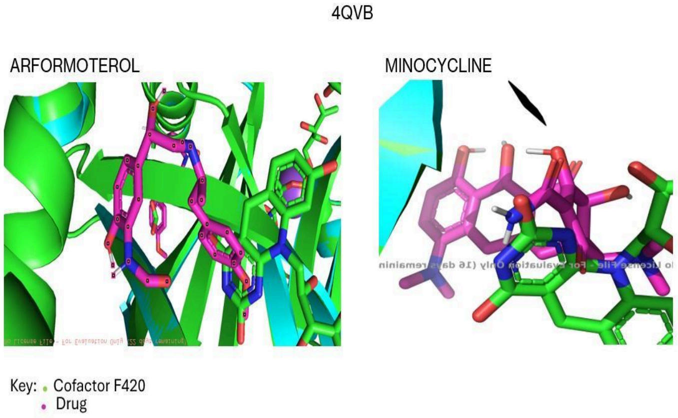


Figure II This figure shows the binding of each drug to cofactor F420 in 4QVB

<u>7KL8</u>:

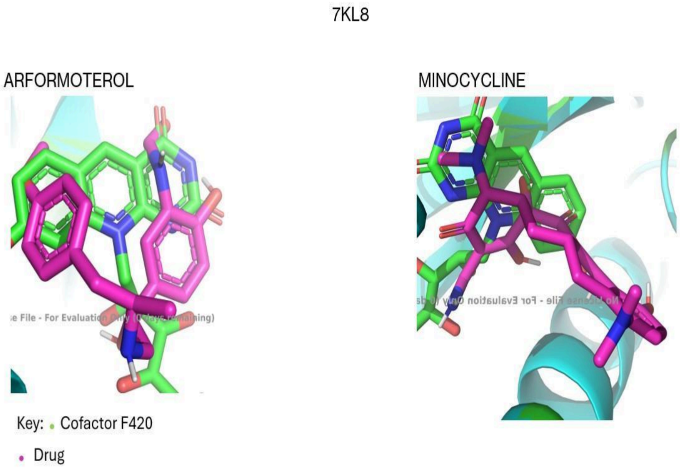


Figure III This figure shows the binding of each drug to cofactor F420 in 7KL8

<u>5JAB</u>:

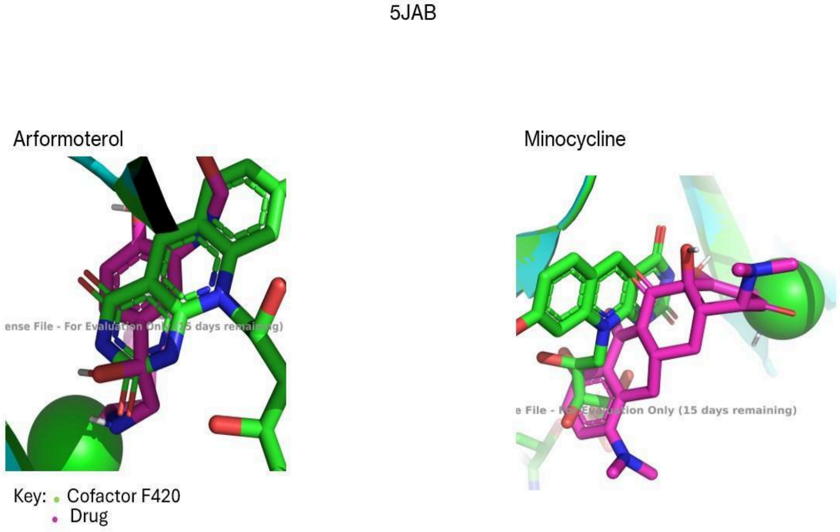


Figure IV This figure shows the binding of each drug to cofactor F420 in 5JAB

<u>3B4Y:</u>

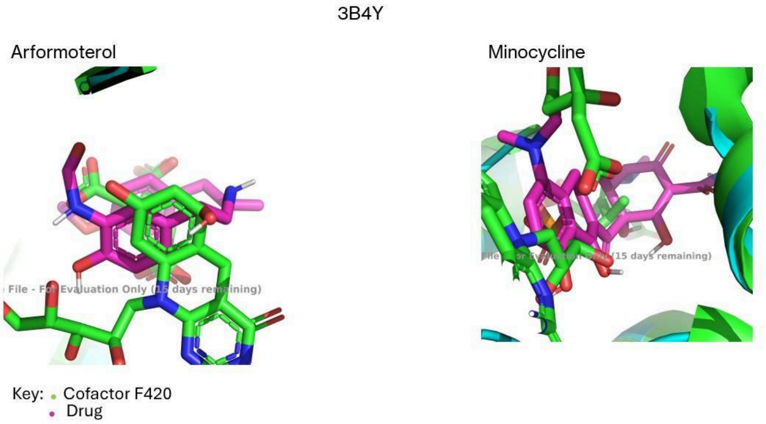


Figure V This figure shows the binding of each drug to cofactor F420in 3B4Y

<u>4XOQ and control:</u>

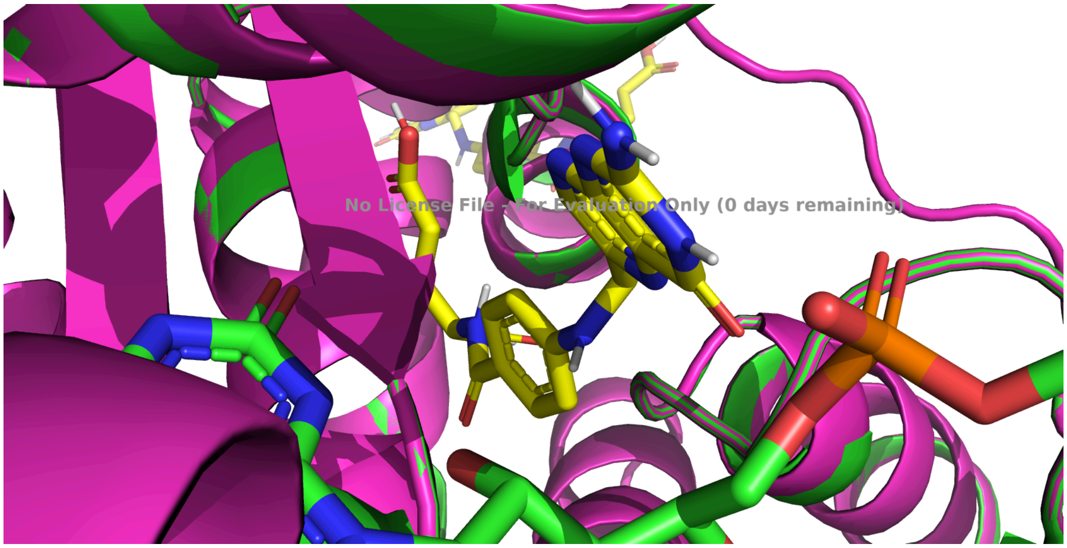


Figure VI This figure shows the binding of the control to cofactor F420 in 4XOQ

DISCUSSION

When determining whether the drug being investigated would bind to the cofactor F420 in the protein being used, there were 5 Vina scores that came up through molecular docking and the most negative values were tested first. For most of the cases, the most negative Vina scores would successfully form a protein-ligand complex that would bind to cofactor F420. However, when tested on Pymol, this was not always the case for the first complex, instead it would bind for the second Vina score. This suggests that the predicted binding site would not be where the cofactor F420 was present.

The results obtained show how Arfomoterol and Minocycline show the higher binding affinities. This means that these two drugs bind to cofactor F420 most effectively and hence they would be most likely to bind to the binding sites of cofactor F420 dependent enzymes effectively. However, this data was collected through preliminary bioinformatics and is not completely accurate. Hence, further experiments would need to be carried out to see if this is accurate. However, this could mean that if these drugs, when introduced into somebody infected with Mtb, they could compete with cofactor F420 in binding to these enzymes. As a result, this suggests a possible pathway that could influence the way the enzymes work. Hence, hypothetically the interactions may lead to alternative enzyme activity. However, such results would need to be seen through experimentation and trials.

The docking of the molecules is shown to work due to the control used. While it might not be completely accurate, the control is seen to not interact with cofactor F420 in the image shown before.

For some of the results however, the values for binding affinity were comparatively low but still close in value to each other. This could suggest that these drugs do not bind as well to cofactor F420 present in 5JAB, but still might bind to cofactor F420 present in other molecules as shown in the rest of the results.

However, there are certain limitations to these results. This data and these results were gathered online and so there was a significant amount of bioinformatics involved which is not entirely reliable and can be complicated to use. There are also no real applications of this. Hence this data could serve as a basis for future experimentation and data analysis in order to use drugs to inhibit the use of cofactor F420 and therefore decrease the metabolism of Mtb, causing it to work less effectively in its host.

Minocyline, which was one of the drugs that presented the best results in this study, is already being tested to treat tuberculosis. This means that it could be a potential mechanism of action. In previous studies it has been found that minocycline effectively kills enough Mtb when somebody is suffering from latent tuberculosis. It is also suggested that using minocycline to replace second-line drugs for tuberculosis could help shorten the span of its treatment and since it is available world wide for lower prices it would be more accessible for patients too. [23]

CONCLUSION

In a recent study published, the effect of deep-sea metabolites as anti-tubular agents was investigated. The results of this study showed how three particular metabolites Upenamide, Aspyronol and Fiscpropionate F. show promise in binding affinity to cofactor F420-dependent enzymes [24]. Such results show how there is potential in inhibiting these enzymes and hence slowing the function on Mtb [25]. However, there would need to be thorough experimentation to prove this.

This paper has investigated, through the use of publicly available data and bioinformatics, whether existing drugs could be used to inhibit enzymes used by cofactor F420. After obtaining data and conducting analysis, it can be seen that the drugs investigated showed to have binding affinities with cofactor F420 in the proteins tested. Hence it could be possible that the use of these drugs could compete with cofactor F420 in Mtb and thus would decrease its metabolism. This could in turn decrease the evolution of Mtb which is seen to increase with the use of a combination of antibiotics.However, there are many other considerations to be taken into account when such experimentation is being done that have not been accounted for in this paper.

In the future it would be suggested that more practical experimental data is collected to gather whether these drugs can actually inhibit the function of cofactor F420.

REFERENCES

[1] Jirapanjawat, T. *et al.* (2016) ‘The redox cofactor F 420 protects mycobacteria from diverse antimicrobial compounds and mediates a reductive detoxification system’, *Applied and Environmental Microbiology*, 82(23), pp. 6810–6818. doi:10.1128/aem.02500-16.

[2] Liu, Y. *et al.* (2024) ‘Gene regulatory mechanism of mycobacterium tuberculosis during dormancy’, *Current Issues in Molecular Biology*, 46(6), pp. 5825–5844. doi:10.3390/cimb46060348.

[3] Godfroid, M. *et al.* (2020) ‘Insertion and deletion evolution reflects antibiotics selection pressure in a mycobacterium tuberculosis outbreak’, *PLOS Pathogens*, 16(9). doi:10.1371/journal.ppat.1008357.

[4] *Jirapanjawat TNey BTaylor MC, Warden AC, Afroze SRussell RJ, Lee BM, Jackson CJ, Oakeshott JG, Pandey G, Greening C2016.The Redox Cofactor F420 Protects Mycobacteria from Diverse Antimicrobial Compounds and Mediates a Reductive Detoxification System. Appl Environ Microbiol82:.*https://doi.org/10.1128/AEM.02500-16

[5] Nih.gov. (2024). Checking your browser - reCAPTCHA. [online] Available at: https://pmc.ncbi.nlm.nih.gov/articles/PMC3567243/ [Accessed 26 Apr. 2026].

[6] Meera Gurumurthy, Rao, M., Mukherjee, T., Srinivasa, Boshoff, H.I., Dick, T., Barry, C.E. and Manjunatha, U.H. (2013). A novel F $_{420}$ *‐dependent anti‐oxidant mechanism protects Mycobacterium tuberculosis against oxidative stress and bactericidal agents. Molecular Microbiology, 87(4), pp.744–755. doi:https://doi.org/10.1111/mmi.12127.*

[7] Smyth, M.S. and Martin, J.H.J. (2000). X Ray Crystallography. Molecular Pathology, 53(1), pp.8–14. doi:https://doi.org/10.1136/mp.53.1.8.

[8] Liu, Y., Yang, X., Gan, J., Chen, S., Xiao, Z.-X. and Cao, Y. (2022). CB-Dock2: improved protein–ligand blind docking by integrating cavity detection, docking and homologous template fitting. Nucleic Acids Research, 50(W1). doi:https://doi.org/10.1093/nar/gkac394.

[4] Xu, X., Duan, R. and Zou, X. (2023) 'Template‐guided method for protein–ligand complex structure prediction: Application to casp15 protein–ligand studies', *Proteins: Structure, Function, and Bioinformatics*, 91(12), pp. 1829–1836. doi:10.1002/prot.26535.

[9] *Oseltamivir (Tamiflu): Uses, side effects, interactions, pictures, warnings & dosing* (no date) *WebMD*. Available at: https://www.webmd.com/drugs/2/drug-17765-5294/tamiflu-oral/oseltamivir-oral/details (Accessed: 12 September 2025)

. [10] *Ncats inxight drugs - AZD-6482* (no date) *Inxight Drugs*. Available at: https://drugs.ncats.io/drug/78G6MP5PZ5 (Accessed: 12 September 2025).

[11] *Arformoterol oral inhalation: Medlineplus Drug Information* (no date) *MedlinePlus*. Available at: https://medlineplus.gov/druginfo/meds/a607061.html (Accessed: 12 September 2025).

[12] *Minocycline: Medlineplus drug information* (no date) *MedlinePlus*. Available at: https://medlineplus.gov/druginfo/meds/a682101.html (Accessed: 12 September 2025).

[13] Deshpande, D. *et al.* (2018) 'Minocycline immunomodulates via sonic hedgehog signaling and apoptosis and has direct potency against drug-resistant tuberculosis', *The Journal of Infectious Diseases*, 219(6), pp. 975–985. doi:10.1093/infdis/jiy587.

[14] wwPDB.org (no date) *PDB entry - 4xoq (pdb_00004xoq)*, *wwPDB: pdb_00004xoq*. Available at: https://doi.org/10.2210/pdb4xoq/pdb (Accessed: 12 September 2025).

[15] wwPDB.org (no date a) *PDB entry - 4QVB (pdb_00004qvb)*, *wwPDB: pdb_00004qvb*. Available at: https://doi.org/10.2210/pdb4qvb/pdb (Accessed: 12 September 2025).

[16] Bank, R. P. D. (n.d.). *RCSB PDB - 7KL8: Structure of F420 binding protein Rv1558 from Mycobacterium tuberculosis with F420 bound*. https://www.rcsb.org/structure/7KL8

[17] wwPDB.org (no date c) *PDB entry - 5jab (pdb_00005jab)*, *wwPDB: pdb_00005jab*. Available at: https://doi.org/10.2210/pdb5jab/pdb (Accessed: 12 September 2025).

[18] wwPDB.org (no date a) *PDB entry - 3B4Y (pdb_00003b4y)*, *wwPDB:*

pdb_00003b4y. Available at: https://doi.org/10.2210/pdb3b4y/pdb (Accessed: 12 September 2025).

[19] *Chemmine Tools* (no date) *ChemMine Tools*. Available at: https://chemminetools.ucr.edu/ (Accessed: 12 September 2025).

[20] (No date) *Apache2 ubuntu default page: It works*. Available at: http://cadd.labshare.cn/cb-dock2/ (Accessed: 12 September 2025).

[21] *Prodigy* (no date) *Prodigy Webserver*. Available at: https://rascar.science.uu.nl/prodigy/ (Accessed: 12 September 2025).

[22] *Introducing pymol 3.1* (no date) *PyMOL*. Available at: https://www.pymol.org/ (Accessed: 12 September 2025).

[23] *Minocycline Immunomodulates via Sonic Hedgehog Signaling and Apoptosis and Has Direct Potency against Drug-Resistant Tuberculosis | The Journal of Infectious Diseases | Oxford Academic*, *academic.oup.com/jid/article/219/6/975/5263980. Accessed 28 Oct. 2025*.

[24] Deep-Sea Marine Metabolites as Promising Anti-Tubercular Agents: CADD-Guided Targeting of the F420-Dependent Oxidoreductase

Ria Desai, Amane A. Alaroud, Gagan Preet, Rishi Vachaspathy Astakala, Rainer Ebel, Marcel Jaspars

[25] Nih.gov. (2024). Checking your browser - reCAPTCHA. [online] Available at: https://pmc.ncbi.nlm.nih.gov/articles/PMC2953692/ [Accessed 1 Apr. 2026].

[26] ---. "Folic Acid." Pubchem.ncbi.nlm.nih.gov, pubchem.ncbi.nlm.nih.gov/compound/135398658#section=2D-Structure.