



in silico Quest Guided by Physico-Chemical Descriptors of Bedaquiline for New Scaffolds with Potential Inhibitory Capacity against Homology Model of Mycobacterium F₁F₀ ATP Synthase

RAMA KRISHNA MUNNALURI and VIJJULATHA MANGA*

Molecular Modeling and Medicinal Chemistry Group, Department of Chemistry, University College of Science, Osmania University, Hyderabad-500 007, India

*Corresponding author: E-mail: vijjulathamanga@gmail.com

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As the first US FDA approved drug for treating pulmonary multi drug resistant tuberculosis (MDR-TB) in the last 40 years, bedaquiline (TMC207, Sirturo™) stands out as cynosure in the circles of synthetic chemists exploring new therapeutics against tuberculosis. The remarkable efficacy of bedaquiline in treating tuberculosis lies in its ability to target the energy metabolism that affects both replicating as well as dormant forms of *M. tuberculosis* (MTB). Despite its promising antitubercular profile, bedaquiline raises serious concern with its string of side effects and emergence of resistant strains, warrants a quest for better substitutes. In the present work, we employed *in silico* methods like homology modeling and virtual screening to zero in on molecules that exhibit high affinity at the binding site of bedaquiline.

Keywords: Bedaquiline, Pubchem database, MTB F₁F₀ATP synthase, Homology modeling, Virtual screening, Molecular docking.

INTRODUCTION

Tuberculosis (TB) till date remains one of the most dreaded infectious diseases, registering an alarming death count nearing 1.8 million every year worldwide. Latest estimates of World Health Organization (WHO) adds more to this disturbing tale, approximately 10.4 million people across the World are infected with tuberculosis in 2016 [1]. The growing number of infected patients can also be related to increased incidence of HIV as tuberculosis remains one of the most common co-infection and also main cause of mortality in HIV patients [2]. What further complicates the treatment of tuberculosis is emergence of multidrug-resistant tuberculosis (MDR-TB), extensively drug resistant tuberculosis (XDR-TB) and most virulent totally drug resistant tuberculosis (TDR-TB) [3,4]. The current regimen of antituberculosis drugs target different biological pathways important for the survival of the *Mycobacterium tuberculosis* (MTB) and quest for new pathways that can be targeted is on full swing [5]. With most of the drugs in use are rendered ineffective or less effective due to emergence of drug resistant strains, developing new strategies and finding new targets is a pressing need to mitigate the risk of drug resistance [6,7]. One such pathway that has garnered lot of attention lately is energy metabolism in MTB and this obviously is credited to the US FDA approval for bedaquiline, reported to inhibit F₁F₀ ATP synthase [8,9]. Replicating or non-replicating tuberculi need

to maintain threshold levels of ATP for their survival. This probably is the moot point for the incredible success of bedaquiline, known to kill both active as well latent forms of MTB by inter-fereing in ATP synthesis and effecting a rapid depletion of intra-cellular ATP levels [10,11]. Bedaquiline is highly selective towards MTB F₁F₀ ATP synthase and has minimal interaction with human ATP synthase [12].

In the present work, our interest is restricted to F₀, specifically, the inference of subunit-a and rotor, comprising subunit-c, which has been experimentally validated to be the binding site of bedaquiline (Fig. 1). Bedaquiline, despite its superior antituberculosis profile comes with a host of side effects that include arthralgia, nausea, hemoptysis, chestpain and headache [13,14]. Also to quell the risk of emergence of bedaquiline resistant strains, it is imperative to explore molecules that can act as bedaquiline substitutes with fewer side effects.

EXPERIMENTAL

Homology modeling of MTB ac₉ protein complex: A high resolution X-ray diffraction structure of rotor complex in *M. phlei* with c₉ configuration (PDB ID: 4V1G; resolution: 1.55 Å) and the same protein with co-crystallized bedaquiline (PDB ID: 4V1F; resolution: 1.7 Å) (<http://www.rcsb.org/pdb/>) has been solved by Preiss *et al.* [15]. The structure is invaluable in understanding the binding mode of bedaquiline and *M. phlei*

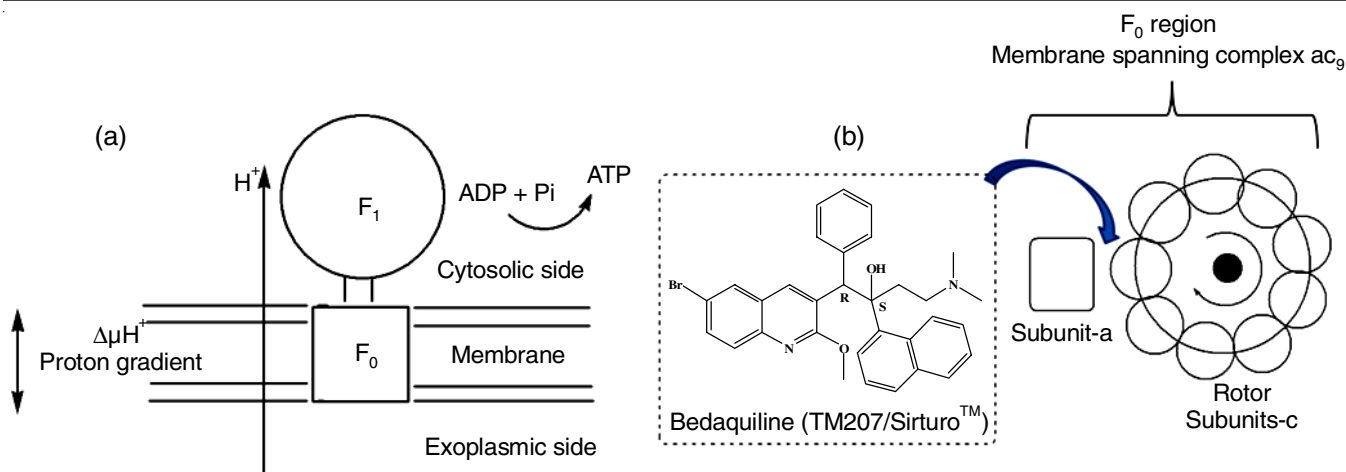


Fig. 1. (a) Skeletal view of F_1F_0 ATP synthase (b) Binding site of bedaquiline at the interface of subunit-a and subunit-c (rotor)

shares an impressive 90 % sequence identity with pathogenic bacterium's rotor sequence. The minimum inhibitory concentration (MIC) values of bedaquiline against MTB (0.06 $\mu\text{g/mL}$) and *M. phlei* (0.05 $\mu\text{g/mL}$) are very close and imply high resemblance in binding mode of the drug with the protein. One short coming in solved X-ray structures of *M. phlei* rotor ring (4V1G or 4V1F) is absence of subunit-a, which forms the interface with the rotor c_9 and it is at this very interface bedaquiline is reported to bind. The molecular docking studies will give meaningful results if the native environment of the protein as *in vivo* conditions can be replicated. To achieve this we planned to build a homology model of pathogenic MTB rotor along with the subunit-a, so as to generate the interface which is known to host the binding site of bedaquiline. Previous workers performed molecular docking studies employing homology model of ac_{12} protein complex using *E. coli* F_1F_0 ATP synthase subunit-a and rotor complex (PDB ID: 1C17) as template that share relatively low sequence similarity (Table-1) [16-19]. One advantage with 1C17 template, despite low sequence identity with query sequence is presence of both subunit-a and entire rotor with c_{12} configuration.

TABLE-1
TEMPLATE SEQUENCE IDENTITY FOR
SUB UNITS a AND c OF MTB

Template PDB code	Organism	Sequence identity to	
		Subunit-a	Subunit-c
1C17 (F_1F_0 ac_{12} complex)	<i>E. coli</i>	38 % (Chain: M)	52 % (Chain: A)
4V1G (rotor- c_9)	<i>M. phlei</i>	–	90 %

Sequence identity calculations were performed using BLAST online server [Ref. 20].

In order to build a homology model that is proximal to the native protein, we planned to build a multi template based composite model that contains C_9 rotor from template 4V1G with high sequence identity and subunit-a from template 1C17 that shared low sequence identity with the target protein. To achieve this objective we build homology model of protein ensemble ac_9 as a hybrid of two homology models both built with query sequences of MTB H37Rv on SWISS MODEL homology modeling server [21-23]. Model-1 was generated

using template 4V1G that contained only subunit-c and model-2 was obtained using template 1C17 that contained subunit-a along with c_{12} rotor. A composite model that contained c_9 rotor and subunit-a, was generated by aligning the interface of ac_{12} of model-2 to c_9 of model 1 by using protein structure alignment option available in maestro panel of Schrödinger suite (Fig. 2). The alignment score was 0.2 on a scale of 0 to 1 where smaller value corresponds to greater alignment and value of 0.2 indicates marginal deviation which is expected owing to difference in templates used. The update coordinates option in maestro panel was used and background c_{12} rotor of homology model 2 was deleted to generate a protein complex now comprising of subunit-a from model-2 (chain M) and rotor c_9 (Chains A to I) from model-1 (Fig. 2). The modeled protein ensemble was saved as a separate entry in project table of maestro module.

Model validation: Homology model validation was carried out using RAMPAGE server (Fig. 3) [24], ProSA web (Fig. 4) [25] and QMEANBrane online server (Fig. 5) (specific for validating membrane spanning proteins) [26]. The model showed reasonably good compliance with the accepted standards and indicates a reliable homology model of ac_9 complex.

Protein preparation: The homology model built is not suitable for immediate use in molecular docking studies and need further processing. This job is accomplished by using protein preparation wizard module in Schrödinger suite [27]. Protein preparation wizard fixes issues with regard to the structure and also allows minimization of protein.

Grid generation: The grid generation is supposedly most important step in molecular docking studies and accuracy of the docking results are invariably dependent on this crucial step. A fair degree of knowledge from biochemical assays and bedaquiline bound PDB structure of *M. phlei* (PDB ID: 4V1F) indicate the binding site to be proximal to Glu-61 (Fig. 6). An initial rotational scan was performed on Arg-186 and other interface residues to select the conformations of these interface residues that do not interfere with binding of bedaquiline [28]. Generation of a grid around Glu-61 was carried out using default settings of the Glide module of Schrödinger suite.

Ligand preparation of bedaquiline and its stereoisomers: A 2D structure of bedaquiline was first built using maestro panel of Schrödinger suite. The structure was then submitted

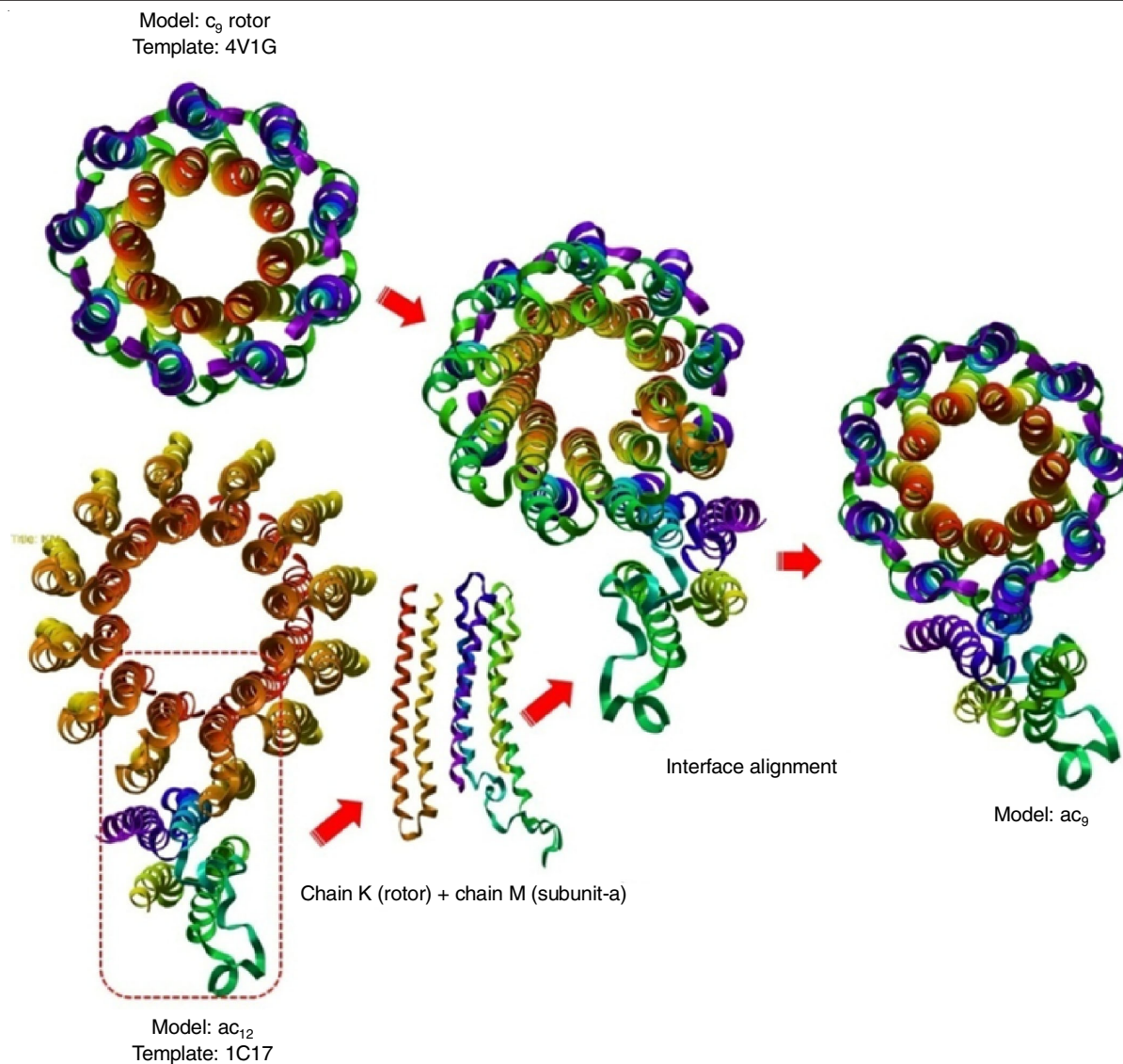


Fig. 2. Graphical representation of scheme employed in generating model ac_9 protein complex

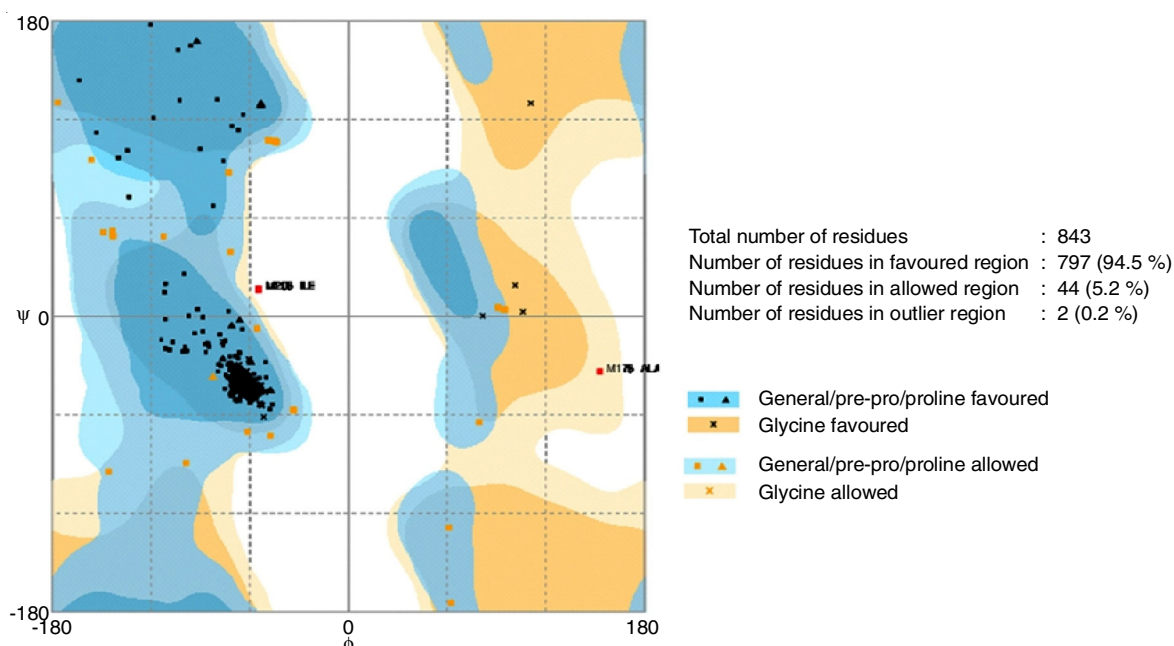


Fig. 3. Ramachandran plot for the modeled protein

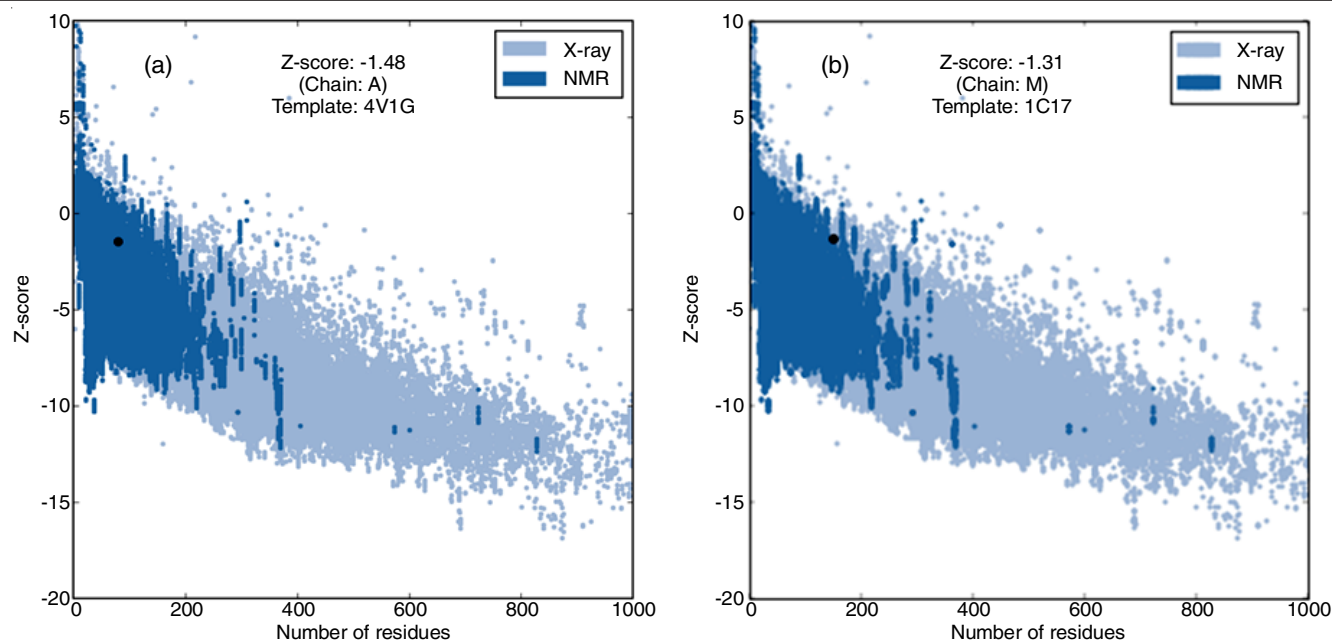


Fig. 4. ProSA plots for the modeled protein for chains A (rotor) and M (subunit-a)

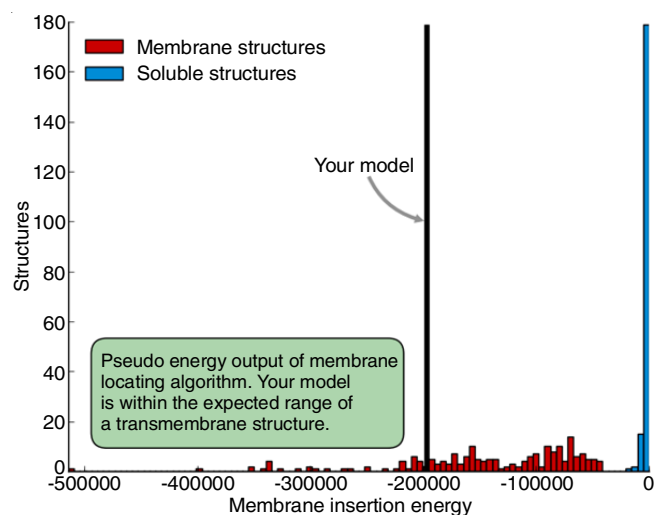


Fig. 5. QMEANBrane plot of membrane insertion energy for the model ac₉

to ligprep module to obtain different 3D structures of stereoisomers, conformers and ionization states for the input structure. Out of these stereoisomers, lowest energy conformers of 4 stereoisomers *i.e.* (R,S) [Bedaquiline], (S,R), (S,S) and (S,S) were selected for further molecular docking studies.

Molecular docking of bedaquiline and its stereoisomers:

Our objective in the present work is to screen molecules with high binding affinity to the MTB F₁F₀ ATP synthase ac₉ protein complex and this would warrant a bench mark to screen actives. For this, first we docked all the four low energy stereoisomers including bedaquiline into the grid by using extra precision (XP) mode available in Glide module. This would allow us to not just arrive at the docking cut-off to screen actives but as well compare the binding affinities of different stereoisomers of bedaquiline at the binding site.

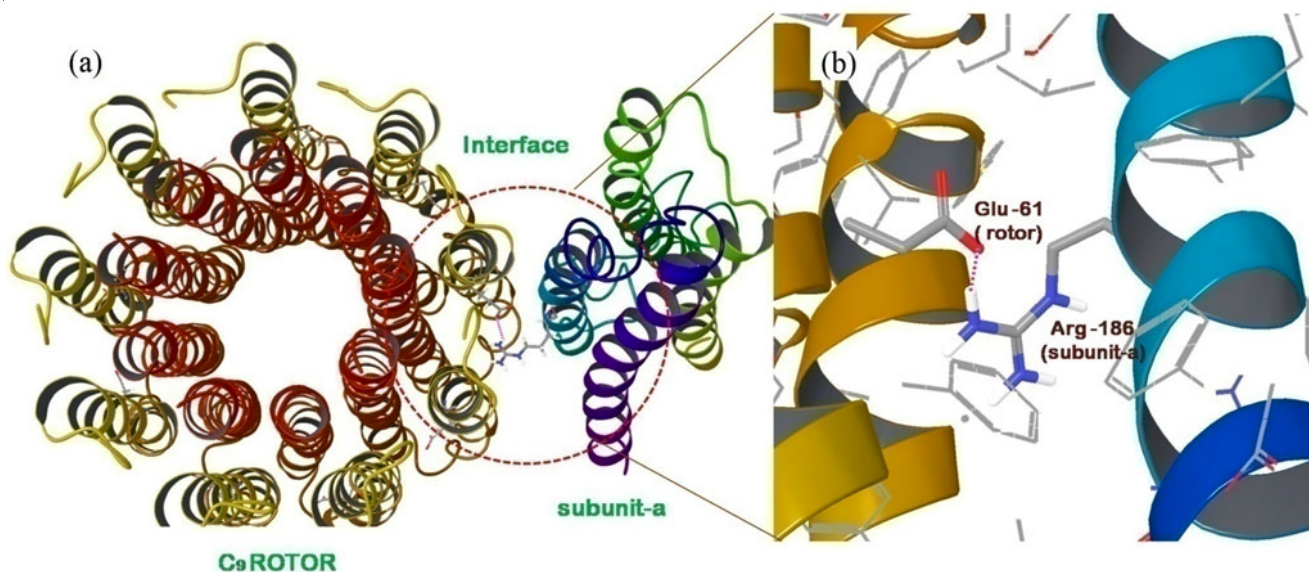


Fig. 6. (a) Interface of subunit-c (rotor) and subunit-a (b) The crucial interaction between Glu-61 (subunit-c of rotor) and Arg-186 (subunit-a)

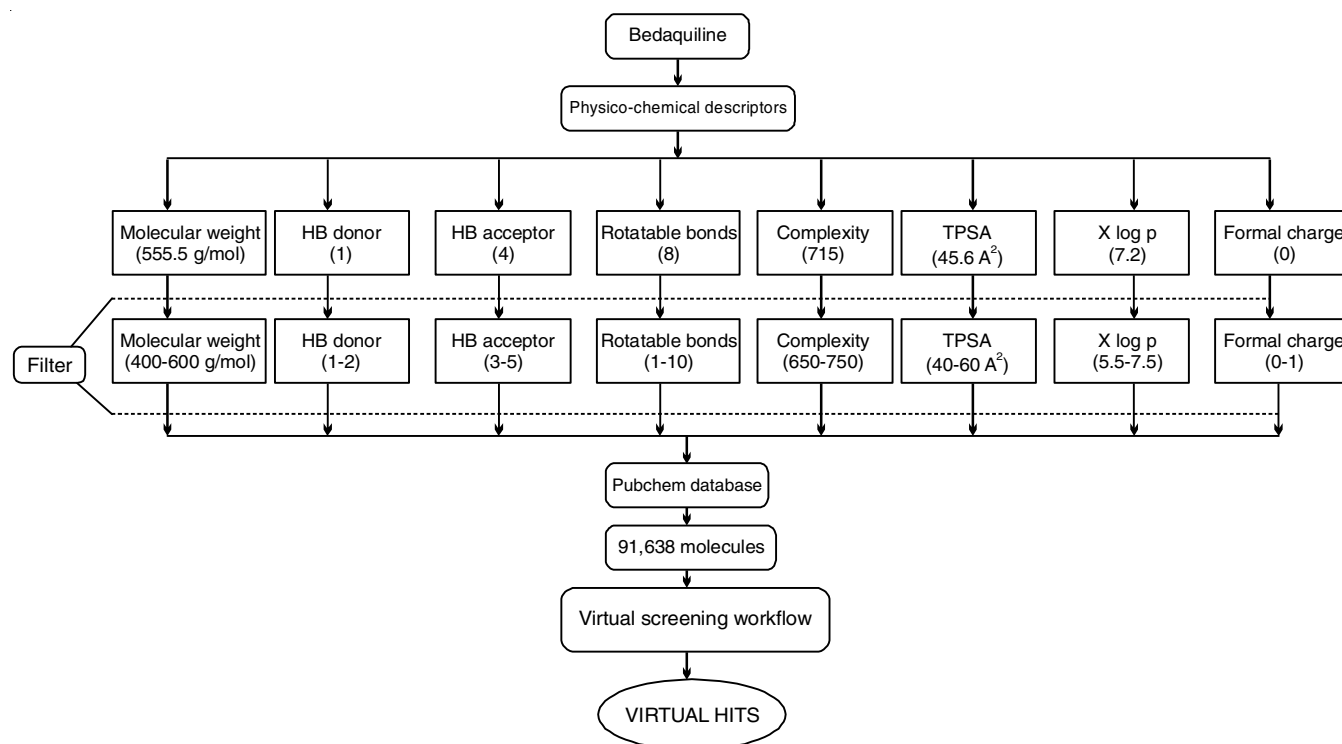


Fig. 7. Schematic showing the criterion employed for extracting a focused library from pubchem database

Preparation of focused library: Pubchem database is a voluminous collection of more than 79 millions compounds with unique structures tagged with their bioactivity information and is maintained by National Center for Biotechnology Information (NCBI) (website info: <https://pubchem.ncbi.nlm.nih.gov/>) [29]. Freely accessible, web user interface allows selective download of the molecules as per ones specification of physico-chemical or biological data. It is a well established fact that biological activity of any compound can be related it to its structural framework and we applied this tenet to prepare a focused library of compounds which share common physical or structural features with bedaquiline [30,31]. In the present context bedaquiline is seen not just as a single molecule but as a representative of wide range of diarylquinoline (DARQ) class of compounds that commonly share a significant antituberculosis profile. The selection criterion for the focused library preparation has been outlined in Fig. 7.

A library of 91,638 molecules was downloaded in sdf format from the PubChem website. Before further use, all these molecules in the library were prepared by using ligprep module and then advanced into virtual screening protocol.

Virtual screening: In the present work we have carried out structure based virtual screening where in the molecules from the focused library after ligand preparation were passed through dock score based filters. The flow chart for virtual screening is presented in Fig. 8.

In Virtual screening exercise we employed different docking protocols to identify virtual hits. First filter is glide high throughput virtual screening (HTVS) docking which rapidly screens actives from a large database and relatively less accurate with processing time of 2 sec/compound (CPU time). Second filter being Glide standard precision (SP) docking that is more refined compared to HTVS docking mode and processing time is 10

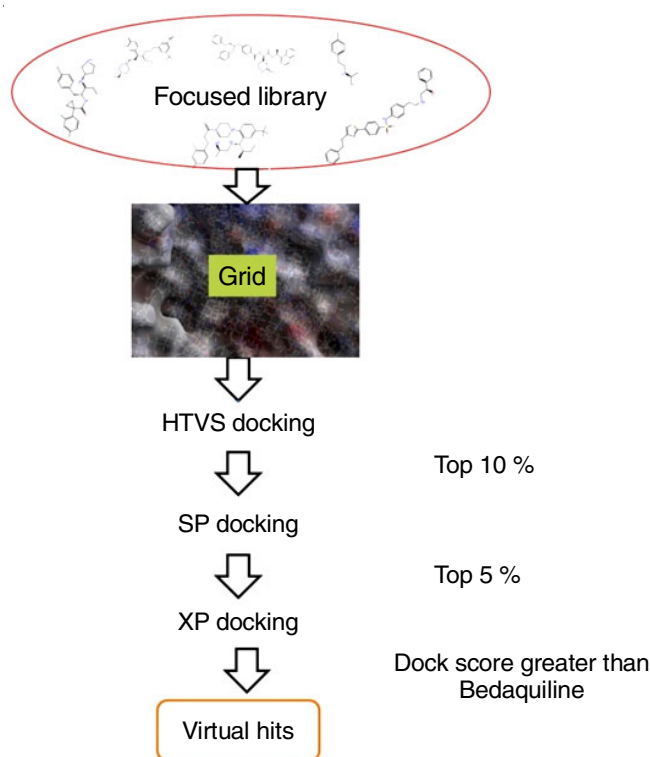


Fig. 8. Virtual screening work flow

sec/compound. Third filter is Glide XP docking performs more extensive sampling than SP and HTVS docking mode. Its advanced scoring function necessitates greater ligand-receptor complementarities to escape penalization. The processing speed in XP docking is 2 min/compound and has greater accuracy in spotting the actives in comparison to either SP/ HTVS mode of docking.

Different docking methods like HTVS, SP and XP described earlier are rigid docking protocols where receptor is held rigid and ligand is flexible. In reality receptor is a dynamic entity and undergoes conformation changes during ligand binding. Induced fit docking (IFD) allows flexibility of residues at the ligand binding site and also uses a softened potential allowing close interaction of ligand with the binding site. Induced fit docking greatly reduces false positives obtained through a virtual screening exercise and binding affinity estimate of the ligand-receptor complex can be obtained from induced fit dock score.

ADME prediction for virtual hits: ADME (absorption, distribution, metabolism and excretion) properties of a compound are very critical in determining its druggability. QikProp module of Schrödinger suite was used to predict the ADME properties of the virtual hits. QikProp prediction program predicts various pharmaceutically relevant physico-biological descriptors for a molecule and compares these parameters against 95 % of the known drug candidates. This information gives a qualitative picture of molecules in question with respect to their drug-likeness.

RESULTS AND DISCUSSION

Molecular docking of bedaquiline and its stereoisomers:

Bedaquiline and its stereoisomers after ligand preparation were docked into the grid generated around glu-61. The results of

XP docking are shown in Fig. 9 and details summarized in Table-2.

The docking results (Table-2) show a good agreement with the experimental activity of the bedaquiline and its stereoisomers. This positive correlation fortifies the reliability of XP docking in finding molecules that can bind effectively to MTB F₁F₀ ATPase.

Results of virtual screening: A total volume of 91,638 molecules were loaded into virtual screening workflow of Schrödinger suite. Ligprep module generated 2,16,318 conformers in total and 21,425 entries made out of HTVS docking filter. These output molecules dwindled down to 4,834 after passing through SP docking filter. These doubly filtered molecules on Glide XP docking yielded four molecules which showed higher XP dock score compared to bedaquiline (-9.38 Kcal/mol). The structures of the virtual hits are shown in the Fig. 10. Physico-chemical descriptors of virtual hits are shown in Table-3.

One glaring structural aspect in atleast three out of four output molecules is a piperazine moiety with a proximal hydroxy group, can be seen in the Fig. 10. (highlighted).

Summary of different docking parameters obtained from Glide XP, dockposes (Fig. 11) and with a detailed hydrogen bond interaction profile has been presented in Tables 4 and 5. Induced fit docking scores can be seen in the same table. (Bedaquiline showed IF dock score of -5.4 and is not indicated in Table-4).

TABLE-2
RESULTS OF XP DOCKING OF BEDAQUILINE AND ITS STEREOISOMERS

Stereoisomer configuration	(MIC ₉₀) (µg/mL)	XPdock score (Kcal/mol)	Glide Ecoul (Kcal/mol)	Glide Evdw (Kcal/mol)	Glide energy (Kcal/mol)
RS(BQ)	0.07	-9.38	-11.80	-52.30	-64.10
RR(SS)	2.78	-8.83	-8.20	-48.15	-56.31
SS(RR)	2.78	-9.12	-11.38	-50.70	-61.20
SR	44.13	-7.40	-6.65	-40.32	-46.97

BQ: bedaquiline; EvdW = lipophilic van der Waals energy; Ecoul = coulomb energy; Biological activity [Ref. 28]

TABLE-3
THE PHYSICO-CHEMICAL DESCRIPTORS OF THESE VIRTUAL HITS

S. No.	PCID	GMW	HBD	HBA	RB	Complexity	TPSA (Å ²)	xlog p	FC
1	123254118	453.97	1	4	9	650	58.3	6.1	0
2	54289035	531.70	1	5	10	736	52.7	6.0	0
3	73351228	522.69	1	5	10	745	53.0	6.5	0
4	20079045	558.52	1	5	6	695	48.8	5.6	0

Legend: PCID: PubChem data base ID; GMW: Gram molecular weight; HBD: Hydrogen bond donors; HBA: Hydrogen bond acceptors; RB: Rotatable bonds; TPSA: Topological polar surface area; FC: Formal charge.

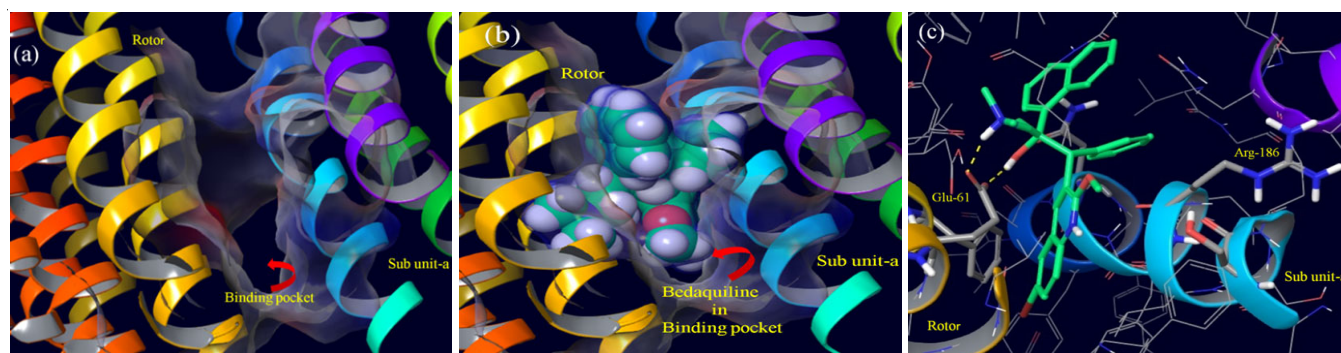


Fig. 9. (a) Binding cavity at the interface of C₉ rotor and subunit-a (b) Bedaquiline (CPK model) docked in the binding cavity (c) Dock pose of bedaquiline at the interface of rotor and subunit-a

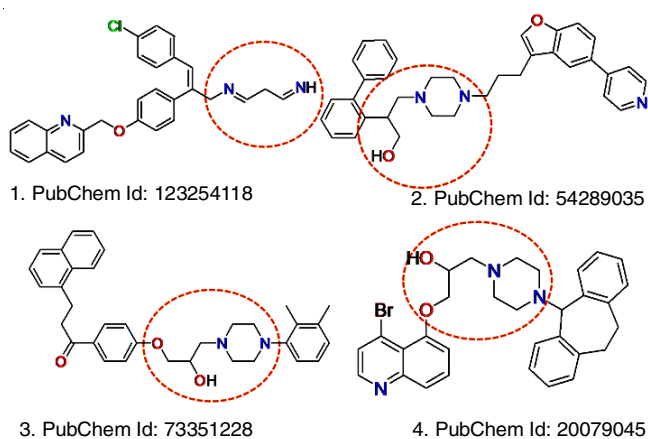


Fig. 10. Graphical depiction of 2D Structures of virtual hits

Results of ADME prediction using QikProp: *in silico*-ADME prediction study using QikProp module of Schrödinger suite, cleared all the four virtual hits with respect to their drug likeliness. It can be seen from Table-6, that at least two virtual hits have issues (indicated with asterisk in Table-6) with aqueous solubility (QPlogS) and this is probably due to high xlogp (measure of hydrophobicity), an obvious feature for molecules with extended molecular frame work. Nevertheless, these molecules show decent human oral absorption and preparing streamlined analogs of these virtual hits could afford candidates with better ADME properties (Table-6).

Recently literature [32] has emerged showing less lipophilic, 6 cyano substitutes of bromine in bedaquiline and related analogs to be equally effective against MTB. This observation

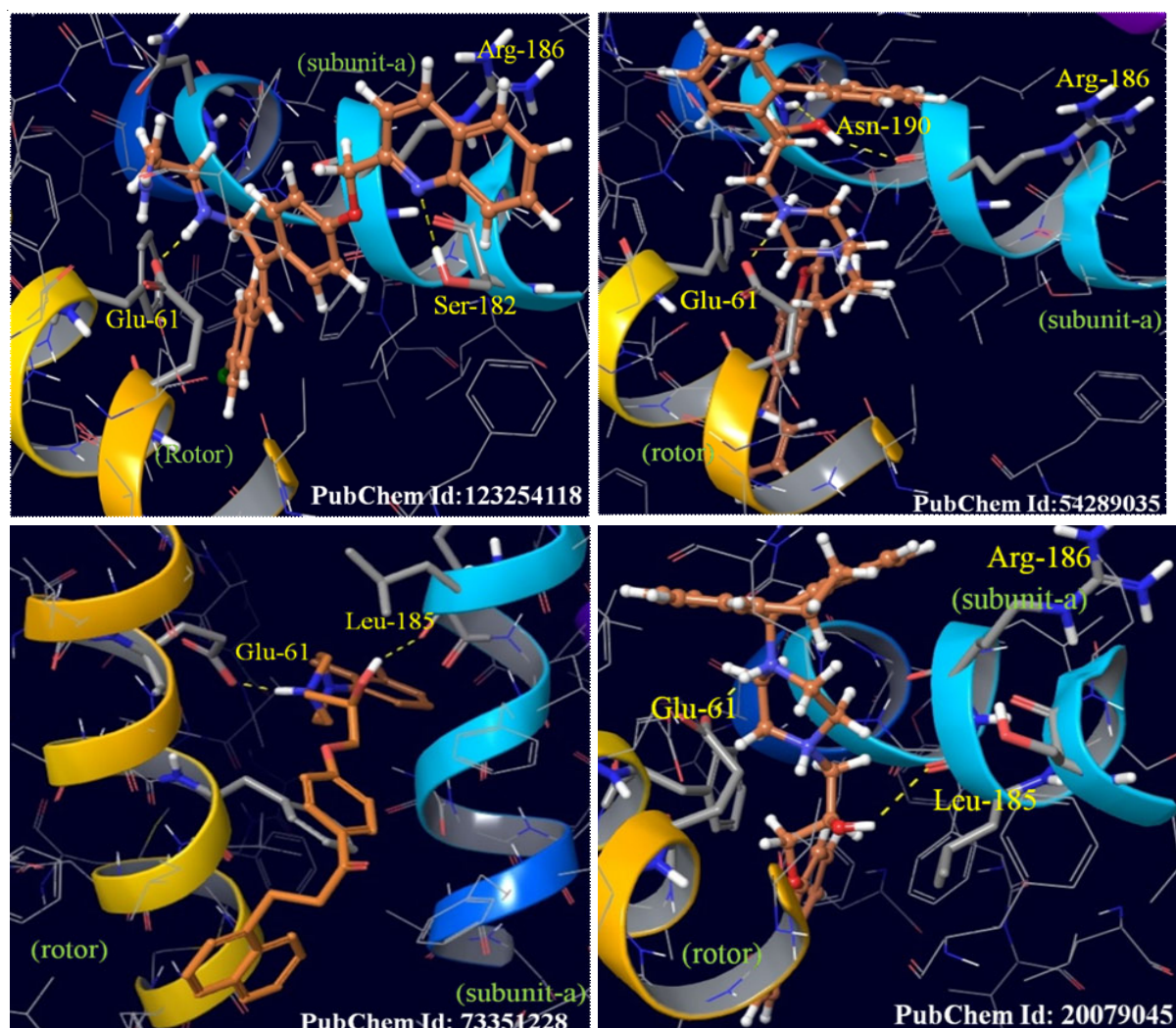


Fig. 11. Dock pose of virtual hits at the interface of subunit-a and rotor-c

TABLE-4
RESULT OF XP DOCKING OF THE VIRTUAL HITS

PC Id	XPdock score (Kcal/mol)	Glide Ecoul (Kcal/mol)	Glide EvdW (Kcal/mol)	Glide energy (Kcal/mol)	IF Dock score (Kcal/mol)
123254118	-12.40	-21.00	-47.37	-68.31	-8.26
54289035	-9.51	-18.58	-37.90	-56.46	-6.71
73351228	-10.65	-8.95	-44.01	-53.04	-5.65
20079045	-9.55	-11.28	-40.32	-52.30	-5.60

EvdW = lipophilic van der Waals energy; Ecoul = coulomb energy

TABLE-5
HYDROGEN BOND INTERACTION PROFILE OF VIRTUAL HITS (XP DOCKING)

PubChem ID	No. of hydrogen bonds	Interacting residues	H-bond acceptor	H-bond donor	Distance (Å)
123254118	2	Glu-61, Ser-182	Glu-61(O of COO ⁻) Lig(N)	Lig(=N-H) Ser(O-H)	1.5 2.4
54289035	3	Glu-61, Asn-190 Arg186	Glu-61(O of COO ⁻) Arg-186(O of C=O) Lig(O)	Lig(N-H) Lig(O-H) Asn-190(N-H)	1.6 2.1 2.2
73351228	2	Glu-61, Leu-185	Glu-61(O of COO ⁻) Leu-185(O of C=O)	Lig(N-H) Lig(O-H)	1.9 2.0
20079045	2	Glu-61	Glu-61(O of COO ⁻) Leu-185(O of C=O)	Lig(N-H) Lig(O-H)	1.7 2.4

Lig stands for ligand and C=O stands for carbonyl group of peptide linkage.

TABLE-6
ADME PREDICTION FOR VIRTUAL HITS

Compound ^a	QPlogHERG ^b	QPPCaco ^c	QPlogS ^d	% HOA	Number of violations	
					Lipinski's rule of five ^f	Jorgensen's rule of three ^g
Bedaquiline	-6.8	1045	-6.0	96.5	2	1
123254118	-7.9	1126	-8.1*	100	1	1
54289035	-9.6	153	-5.5	75	2	1
73351228	-8.8	321	-7.7*	83	2	2
20079045	-8.8	170	-5.4	72	2	1

^aVirtual hits along with bedaquiline.

^bQPlogHERG K⁺ channel blockage: log IC₅₀ (concern below -5).

^cPredicted Caco cell permeability in nm/s (acceptable range: < 25 is poor and > 500 is great).

^dQPlogS, predicted aqueous solubility in mol/L (acceptable range: -6.5 to 0.5).

^e%HOA (percentage of human oral absorption) (acceptable range: < 25 is poor and > 80 % is high).

^fLipinski rule of 5: Maximum number of violation in 95 % of drugs is 4.

^gJorgensen rule of 3: Maximum number of violation in 95 % of drugs is 3.

allows designing less lipophilic compounds and expands scope for designing new inhibitors against MTB F₁F₀ ATP synthase with better pharmacokinetic properties.

Conclusion

With a systematic integration of homology modeling and virtual screening tools with multiple filters, an attempt has been made to find molecules that can effectively inhibit MTB F₁F₀ ATPase. In this quest, we obtained four virtual hits that fared better than bedaquiline in terms of their binding affinity. Some distinct features of these virtual hits that allow key interaction with glu-61 are shown in Fig. 12.

In summary we suggest that designing of novel MTB F₁F₀ ATP synthase inhibitors with diverse scaffolds that share a close resemblance to the physical descriptors of bedaquiline

and incorporating structural features indicated (Fig. 12) could afford potent inhibitors against MTB F₁F₀ ATP synthase.

As always any *in silico* finding need to be experimentally quantified and our findings does provide synthetic chemists, an insight into the structural aspects to be envisaged while designing novel MTB F₁F₀ ATPase inhibitors.

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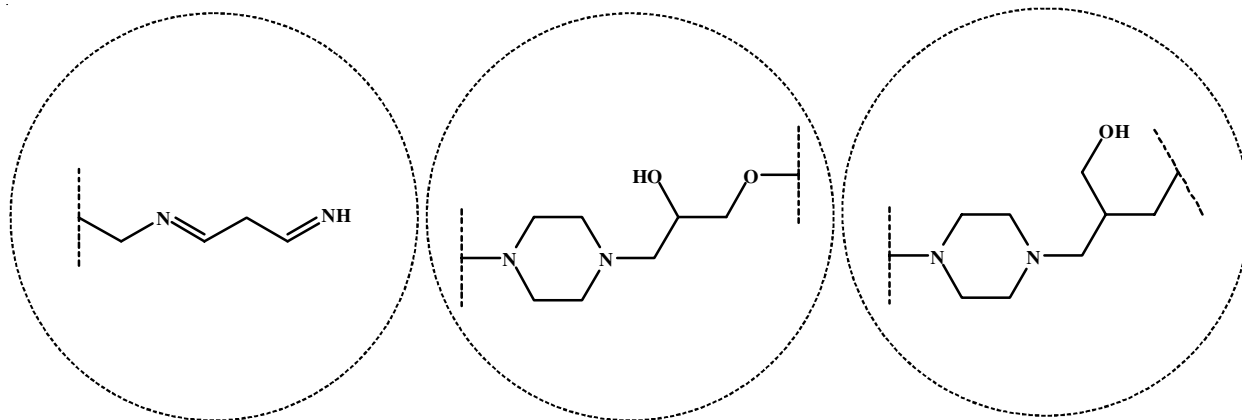


Fig. 12. Important structural features seen in four virtual hits

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