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Validation of the Novel QSAR-SBDD Integrated Methodology in Quest To Develop NCES as Antitubercular Agents

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ABSTRACT

A novel hybrid methodology combining Quantitative Structure–Activity Relationship (QSAR) and Structure-Based Drug Design (SBDD) methodologies has been established for the identification of new antitubercular medicines. This comprehensive technique resulted in the development of a predictive QSAR model characterized by five essential parameters: the amino acid residues Glu65, Ala66, Phe69, the water molecule HOH2018, and an indicator variable. Validation of the model using a structurally heterogeneous external dataset resulted in a correlation coefficient of 0.79, indicating robust prediction performance. Structural and binding research highlighted the essential significance of stereochemistry in ligand-target interactions, with Glu65 identified as the most significant residue influencing both binding affinity and biological activity. These findings underscore the efficacy of the QSAR-SBDD integration and offer a logical framework for the development of effective and selective antitubercular agents.

Keywords: *Tuberculosis, ATP synthase, QSAR, SBDD, Docking, quinoline*

1. INTRODUCTION

Tuberculosis (TB) is a dreadful disease which is caused by bacterium *Mycobacterium tuberculosis* (Mtb). In 2023, tuberculosis (TB) resulted in 1.25 million fatalities, which included 161,000 individuals co-infected with HIV. In 2023, approximately 10.8 million individuals worldwide were diagnosed with tuberculosis, comprising 6.0 million men, 3.6 million women, and 1.3 million children [1]. Globally, tuberculosis has regained its status as the leading cause of death from a single infectious agent, after being surpassed by coronavirus disease (COVID-19) for past few years [2]. TB is usually treated with first line drugs namely, isoniazid, ethambutol, rifampicin, and pyrazinamide (Fig.1).

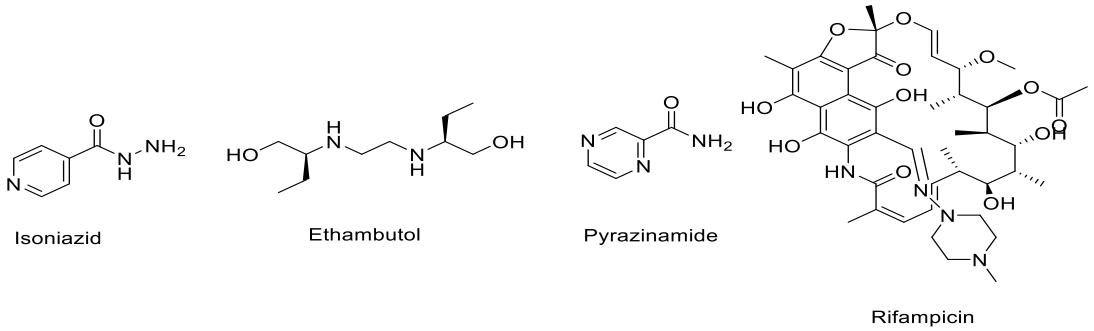


Figure 1. First-line drugs for the treatment of TB.

Multidrug-resistant tuberculosis (MDR-TB) is a variant of tuberculosis caused by a strain of Mycobacterium tuberculosis complex that exhibits resistance to rifampicin (RR-TB) and isoniazid. Pre-extensively drug-resistant tuberculosis (pre-XDR-TB) denotes multidrug-resistant or rifampicin-resistant tuberculosis (MDR/RR-TB) that exhibits resistance to a fluoroquinolone, while extensively drug-resistant tuberculosis (XDR-TB) isolates demonstrate further resistance to additional critical medications, including bedaquiline and/or linezolid [3]. The two factors contributing to the ongoing emergence and proliferation of MDR/RR-TB are inadequate management of tuberculosis treatment and interpersonal transmission. The majority of individuals with tuberculosis are healed through a six-month treatment regimen, which is administered to patients receiving sufficient care. Improper or erroneous administration of tuberculosis medications, utilization of ineffective drug formulations (including monotherapy, substandard medicines, or inadequate storage conditions), and premature cessation of treatment can lead to drug resistance, which may subsequently be transmitted, particularly in densely populated environments. Individuals with pulmonary tuberculosis can transmit the infection through coughing, sneezing, or even speaking and inhalation of only a minimum quantity of the pathogens can lead to infection [4]. To combat the prevalence of resistant TB, the WHO has come up with BPaLM regimen in patients with MDR/RR-TB whether or not there is extra resistance to fluoroquinolones. It is a 6-month all-oral treatment regimen comprising of bedaquiline, pretomanid, linezolid, and moxifloxacin (Figure 2) [5]. One of the second line drugs, bedaquiline, which also constitutes BPaLM regimen has recently been reported for resistance [6-10]. Literature indicate that resistance to bedaquiline may be attribute to atpE mutation, a gene responsible for encoding the target of bedaquiline as well as mutations in Rv0678 which activates the transcription of the MmpL5-MmpS5 efflux transporter [11].

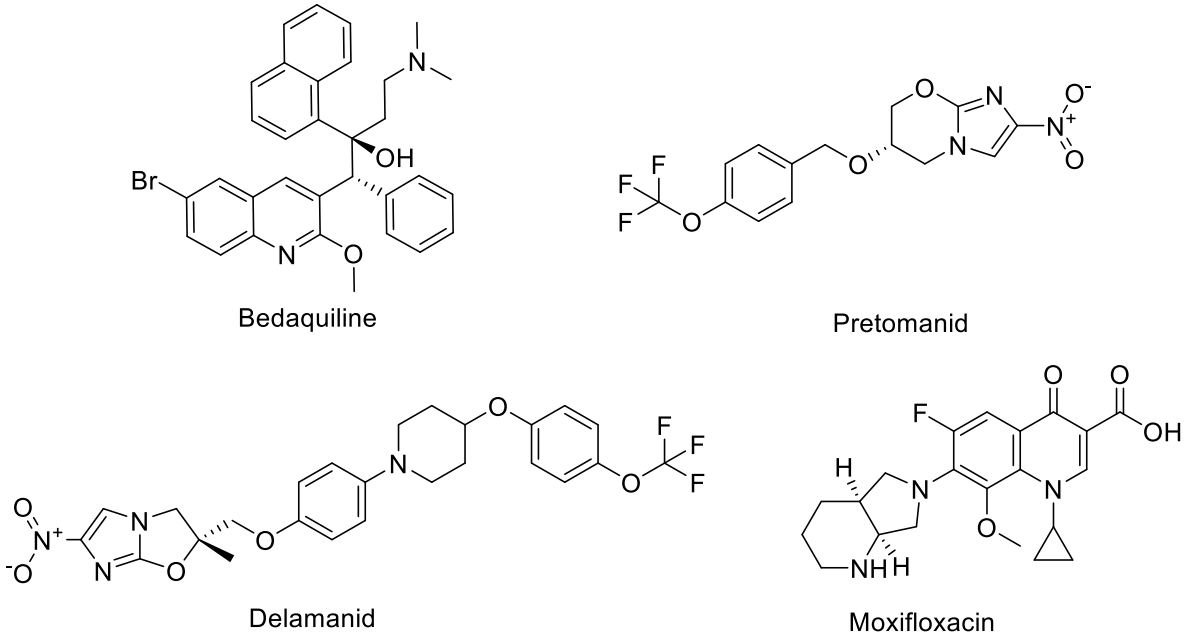


Figure 2. Structures of some of the second-line drugs used for treating TB.

Bedaquiline is known to inhibit mycobacterial ATP synthase, an enzyme crucial for generating ATP. Bedaquiline specifically acts on the mycobacterial ATP synthase and exerts its action by stopping the flow of proton followed by changes in conformation [12]. Resistance to bedaquiline, warrants search for novel chemical entities which may be developed for the treatment of TB. Computer-aided Drug Design (CADD) is a state-of-the-art technique for the drug discovery and development. CADD has been instrumental in the discovery of various drugs which are currently used clinically [13]. One of the branches of CADD, the Structure-based drug design (SBDD) deals with the knowledge of drug target. SBDD although a very effective technique, has certain limitations, such as the docking score/energy not correlating with biological activity, in addition to little knowledge about the important amino acids out of all the interactions observed during the docking studies. These limitations may be due to various factors such as imperfect scoring functions, accounting of protein flexibility, and limited representation of water molecules, etc. [14-17]. It was imperative to device strategies to deal with these limitations through the already available information and sometimes using the data already embedded in the docking results. One such methodology was developed by the authors. This novel technique involved the amalgamation of SBDD with QSAR and utilizes the information obtained through the docking results in the form of number of interactions between the amino acid residue and atoms of the molecule/ligand is considered in this methodology [18]. The methodology takes into account the number of combined interactions between the amino acid residues and the ligand as an independent parameter and the biological activity as dependent parameter. The methodology has been used in the development of models which take into

account the total number of interactions between the amino acid residues (AARs) and the ligand where the number of interactions is considered as independent variable and biological activity as dependent parameter [19].

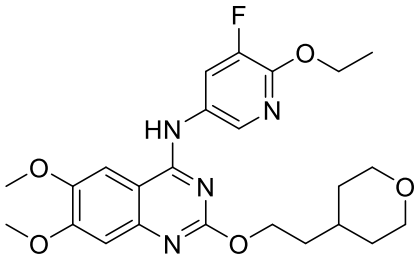
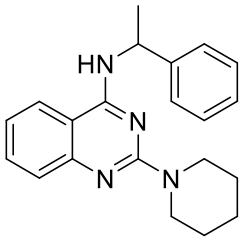
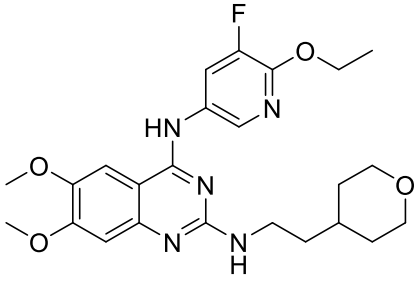
In a quest to find novel chemical entities as antitubercular agents, the methodology was applied to a dataset comprising of 4-substituted amino sulphonyl-2-methyl-7-chloroquinolines [20], bisquinolines [21], imidazo[1,2-a] pyridine ethers, and squaramides [22] which resulted in a model comprising of AARs as independent variables [23,24]. In this paper, we present the validation of the best developed model in a different set of molecules to ascertain the robustness of the model which may help in the identification of NCEs against TB.

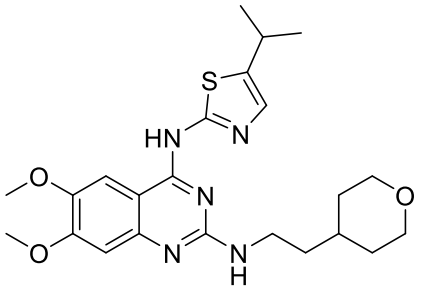
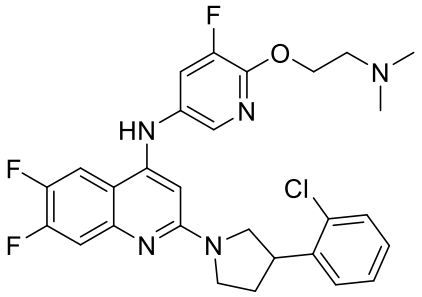
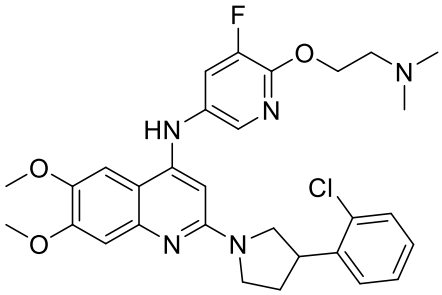
2. MATERIALS AND METHODS

2.1 Dataset

The molecules for the validation of the model were taken from the literature [25] and comprised of 2,4-Diaminoquinolines and aminopyrazolopyrimidines as ATP synthase inhibitors. The molecules were selected rationally by firstly arranging the molecules in the increasing order of activity. This was followed by keeping the most and the least active molecules in the dataset. Additionally, every 4th molecule was also included in the dataset which suitability represented the structural and activity variation. The structure and the biological activity of the molecules of the dataset are given in Table 1.

Table 1. The structure and the biological activity of the molecules of the dataset.

S.No	***Structure***	***Mycobacterial ATP synthase activity IC_{50} (µM)***	***Mycobacterial ATP synthase activity pIC_{50} (µM)***	***Predicted ATP synthase activity pIC_{50} (µM)***
1		25	-1.39794	0.546
2		2.1	-0.32222	0.1332
3		0.6	0.221849	1.1472

4		0.1	1	1.2404
5		0.1	1	1.1968
6		0.04	1.39794	1.4178

2.2 Ligand preparation

The structures of the molecules were drawn using Chemdraw. The drawn molecules were then imported into the LigPrep module of the Maestro in the Schrödinger software [26] using OPLS forcefield and keeping all the other parameters as default which minimizes the ligand and converts the drawn 2D structure into a 3D structure.

2.3 Protein preparation

The protein structure with pdb id 4V1F [27] was downloaded for docking studies which is a crystal structure of rotor ring of mycobacterial ATP synthase complexed with Bedaquiline (BDQ). The protein was further prepared in the Protein preparation wizard module [28] of the Schrödinger software. Since, the co-crystallized ligand BDQ showed interaction with amino acid residues of both and B chains and one water molecule (HOH2018) hence all the three chains as well as the water molecules was retained while other water molecules as well as other heteroatoms were removed before protein minimization which was carried out using default parameters.

2.4 Grid generation

The grid for defining the binding site for docking was generated using the Receptor grid generation module. For the purpose, the co-crystal BDQ was used around which a grid was developed using the default parameters.

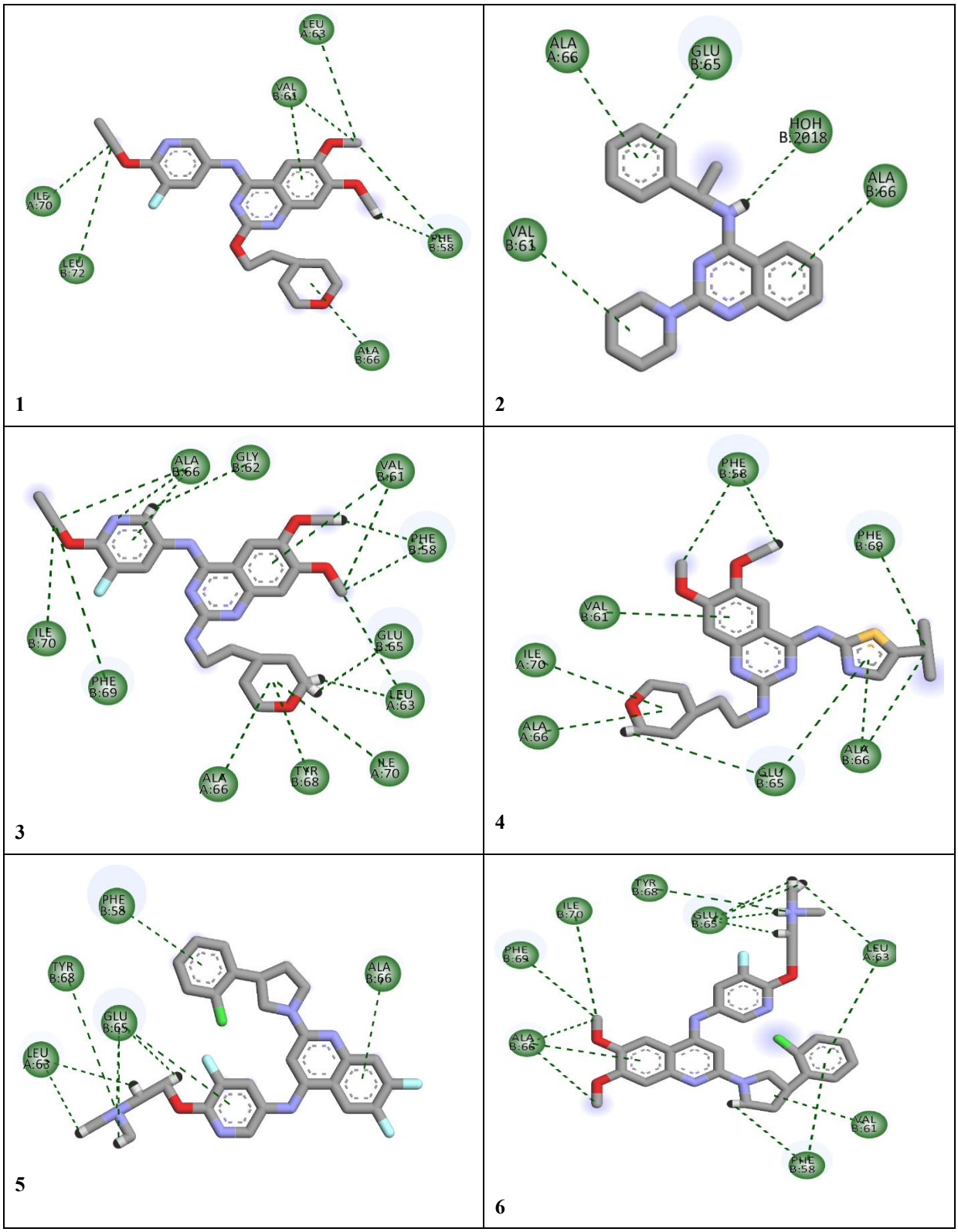
2.5 Docking studies

The docking simulations were performed using the Glide module of the Schrödinger software [29]. Both the generated grid file as well as the ligands were defined. The ligands were docked using the (extra precision) XP protocol of the Glide using the default parameters. The best ranked pose was extracted to the Discovery Studio Visualizer [30] for identifying the interacting AARs.

3. RESULTS AND DISCUSSION

The validation of the docking protocol has already been reported in our previous paper [18]. The 2D structure of the docked molecules have been given in Table 2.

Table 2: The 2D structures of the docked molecules of the dataset



A data matrix for the purpose of validation was prepared where in the combined interactions between the amino acid residues and the molecule was carefully described. Since, only the amino acid residues present in the model (Eq.1) [31] were required for prediction, only these have been displayed in the interaction table (Table 3) The indicator variable (I) was used to impart weightage for the presence '1' and absence '0' of the quinoline nucleus.

pIC_{50}=0.259 + 0.3802(±0.0655) Glu65b -0.793(±0.191) HOHb+ 0.287(±0.176) Ala66b- 0.353(±0.125) Phe69b-

0.870(±0.348) I**…(Eq.1)**

Table 3. Interaction data of the molecules of the data set

	Combined interactions				
S.No	**Glu65b**	**HOHb**	**Ala66b**	**Phe69b**	**I**
1	0	0	1	0	0
2	1	1	1	0	0
3	1	0	3	1	0
4	2	0	2	1	0
5	4	0	1	0	1
6	4	0	3	1	1

The predicted activities derived from the equation 1 have been given in Table 2. A good correlation (R=0.79) was observed between the observed and the predicted biological activity (Figure 3). It was also observed that compound 2 was an outlier, which when removed yielded an excellent correlation (R=0.98). The reason which makes the compound 2 an outlier may lie in the structure of the compound 2 itself which is the only compound in the dataset with a stereocenter. This structural feature makes for a difficult analysis as it is not possible to ascertain which stereoisomer binds in the active site since the activity of the racemic has been provided in the literature.

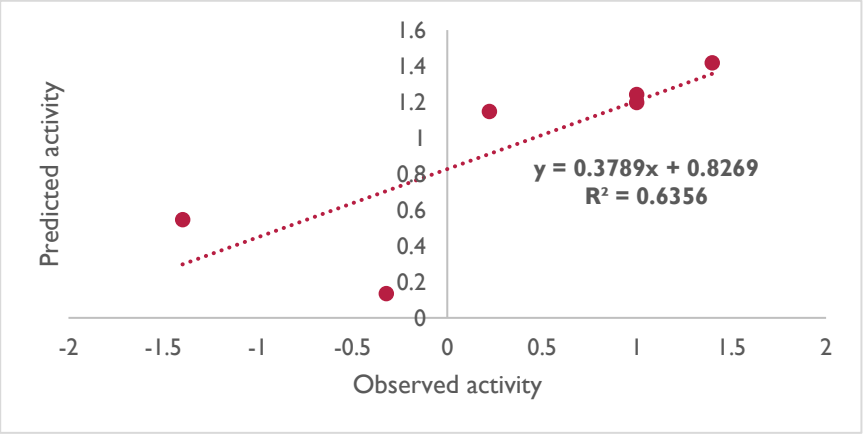


Figure 3. Correlation between the observed and the predicted activity.

Moreover, as suggested previously that Glu65 is the most important amino acid residue, it was also found true in this study too, as the most active molecules 5 and 6 showed four interactions each with the Glu65 whereas the least active molecule 1 did not show any interaction with the Glu65 residue.

4. CONCLUSIONS

The results obtained in the study in the form of correlation (R=0.79) between the observed and the predicted activity are indicative of the robustness of the developed model (Eq.1). The model very well predicted compounds of a diverse set and activity. The studies indicate that stereochemistry of the molecules plays an important role in the binding with the target. The studies also reiterate that Glu65 is the most important amino acid interaction for binding with the receptor and subsequent activity. The results in the form of developed model which has been validated through these studies may help in identifying novel chemical entities as anti-tubercular agents.

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