"Computational and Experimentals Approaches in Designing Potent Anti-Tubercular Agents"

Rashmi Mokal, Department of Chemistry, Dr. Ambedkar College, Deekshabhoomi, Nagpur 440010, Maharashtra, India
Email: rashmi29chem@gmail.com
Deepa Panhekar, Department of Chemistry, Dr. Ambedkar College, Deekshabhoomi, Nagpur 440010, Maharashtra, India

Abstract

Tuberculosis (TB) is among the most acute health issues in the world, and it is a disease caused by Mycobacterium tuberculosis (Mtb), which has been gaining resistance to the already existing medications. The emergence of multi-drug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) has heightened the need to develop new anti-tubercular agents. The latest development in computational drug discovery and experimental validation have presented fresh opportunities in the development of effective therapeutic molecules. Computational methods, such as molecular docking, virtual screening, quantitative structure activity relationship (QSAR) models, and molecular dynamics (MD) simulations are cheap and fast means to predict drug target interactions. The activity, safety and pharmacokinetics of candidate compounds can only be validated by experimental methods, including high-throughput screening, structure-based drug design, and biological assays. This paper provides the review of computational and experimental strategies in anti-tubercular drug design, major case studies, challenges, and future outlook. By combining these strategies, it is possible to enormously speed up the identification of effective and selective anti-TB drugs.

Keywords: Tuberculosis (TB); Anti-tubercular agents; Computational drug design; Molecular docking; QSAR; Molecular dynamics; Experimental validation; Drug resistance; Structure-based design.

INTRODUCTION:

The history of the discovery and development of the anti-tubercular agents is long and interesting and reflects both the combating of the tuberculosis (TB) and the evolution of medicine. Prior to the development of modern chemotherapy, TB was listed among the deadliest infectious diseases that were well known as the white plague in the 18 th and 19 th centuries due to the high death rates. Effective treatment was absent and as a result, sanatoriums were developed where patients were separated and treated mostly through rest, nutrition, and fresh air. Most of these measures however were supportive and failed to cure the infection.

The real start of anti-tubercular drug development was in the mid 20 th century. The first was the discovery of streptomycin (the first antibiotic to treat Mycobacterium tuberculosis) in 1944 by Selman Waksman and his associates. Streptomycin was a landmark drug because it was the first drug that presented evidence that TB was treatable with drugs. Nevertheless, streptomycin resistance quickly appeared, which emphasizes the importance of the use of other therapeutic agents.

Following streptomycin, several new first-line anti-tubercular drugs were introduced:

- Para-amino salicylic acid (PAS) in 1949
- Isoniazid (INH) in 1952, which remains one of the most potent and widely used TB drugs
- Pyrazinamide (PZA) in the 1950s
- Ethambutol (EMB) in 1961
- Rifampicin (RIF) in the late 1960s, which revolutionized TB therapy by shortening treatment duration.

These discoveries formed the backbone of combination therapy, a strategy still used today to prevent drug resistance.

However, over time, second-line drugs (fluoroquinolones (levofloxacin, moxifloxacin)) and injectable agents (amikacin, kanamycin, capreomycin) developed as a result of the emergence of multi-drug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB). These drugs are not as effective, are more toxic and they have longer treatment regimens despite the usefulness.

Over the past few decades, new-generation anti-tubercular agents have been developed due to the growth of computational drug design, molecular biology, and structure-based design. Recent drugs of note are bedaquil (2012) and delamanid (2014), both which target new pathways in Mtb. These are a renaissance of logical drug design and experimental validation in order to overcome resistance and enhance treatment outcomes.

In such a way, the history of anti-tubercular agents can be described as a path towards taking an empirical finding and transforming it into a rational design. Beginning with the serendipitous finding of antibiotics such as streptomycin, progressing through decades of synthetic chemistry and in-vitro experimentation, and currently evolving into computational chemistry and in-vitro validation, the history of anti-TB drug discovery shows how science can always adapt to the challenge of one of humanity□s oldest diseases.

REVIEW OF LITERATURE

Tuberculosis (TB) remains one of the most significant health issues in the world today, which is caused by Mycobacterium tuberculosis (Mtb) and presents in the lungs. In a detailed report on TB, Sharma and Mohan (2020) addressed the pathophysiology, clinical manifestation, and treatment options of the disease with a particular focus on the problem of drug-resistant strains. Modern drug development began with the first breakthrough in anti-tubercular therapy with the discovery of streptomycin by Waksman and Schatz (1944) paving the way to future developments.

Existence of computational tools has made drug discovery much more sensible and effective. Reddy et al. (2021) mentioned the importance of molecular docking, QSAR, and molecular dynamics (MD) simulations in helping identify possible anti-TB compounds and offered a framework of prioritizing molecules before experimental testing. According to Singh and Sharma (2020), they focused on structure-based drug design whereby the target proteins are used to optimize the ligand by using the 3D structure and the protein to enhance potency. Equally, Ahmad et al. (2020) revealed the ability of MD simulation to offer information about the stability and dynamics of protein-ligand interactions to expand the knowledge of drug efficacy on a molecular scale.

More recent developments revolve around high throughput screening and experimental validation. Andreu et al. (2017) have mentioned the ways in which large chemical libraries could be screened and their activity confirmed with in vitro assays so that the predicted activities of the computationally predicted activities are translated to biological activity. Zumla et al. (2015) assessed newer anti-TB medicine and combination regimens which highlights the need to find new agents that could address multi-drug-resistant (MDR) and extensively drug-resistant (XDR) TB.

QSAR modeling has been one of the important tools in contemporary TB drug discovery. Reviewing QSAR-based methods of designing anti-TB agents, Siddiquei and Khan (2014) demonstrated how molecular descriptors could be used to predict activity and direct chemical modifications. Rajkhowa and Deka (2014) went further to discuss DFT-based QSAR/QSPR models to analyze potential compounds with special reference to theoretical methods in drug optimization. Yuanita and Sari (2020) used QSAR together with molecular docking to generate xanthone derivatives as new potential anti-TB by showing the synergy of computational methods in identifying leads.

Recent works emphasize on combination of computational and experimental strategies. Altharawi and Al-Majed (2023) used a two-combinational approach that aimed at the M. tuberculosis enzyme InhA; Deb and Chakraborty (2021) assessed novel compounds in both in vitro experiments and in silico simulations and thus the importance of this bridging gap between in silico and in vitro prediction is crucial.

On the whole, the literature suggests that the present-day discovery of anti-tubercular drugs is based on both computational modeling and experimental confirmation. This combined method speeds up the discovery of effective drugs, saves time and money, and it solves the problem of

drug resistance, which is the basis of anti-TB drugs of the next generation.

1. Tuberculosis (TB)

Tuberculosis (TB) is a communicable bacterial infection induced by Mycobacterium tuberculosis (Mtb). It primarily occurs in the lungs but may extend to other organs like kidney, bones and the brain. TB is transmitted by airborne droplets when an infected individual sneezes or coughs. TB is a worldwide health epidemic which is preventable and curable yet it remains a health crisis, more so in the developing world. The rise in multi-drug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) has worsened the urgency to develop new drugs possessing new mechanisms of action.

2. Anti-tubercular Agents

Anti-tubercular agents are medicines or chemicals that prevent the growth or kill the M. tuberculosis bacteria. Such agents are able to target various biological targets which encompass enzymes in synthesizing cell walls, nucleic acid replication or energy metabolism. They include isoniazid, rifampicin, pyrazinamide and ethambutol (first line agents) and more recent medications such as bedaquiline and delamanid. The perfect anti-tubercular agent must be effective, safe, cheap, and have an ability of reducing the treatment period.

3. Computational Drug Design

Computational drug design involves the computer-aided design of potential drug molecules, design, and optimization of their design followed by laboratory testing. This will save on cost and time during the process of drug discovery. Computational methods in TB research can be used to "philtre large chemical libraries, binding interactions with Mtb proteins, and to design high selectivity and potency molecules.

4. Molecular Docking

Molecular docking is a computational method of predicting the binding of a small molecule (ligand) into the binding site of a protein target of interest. It can be used to estimate the binding affinity, orientation and potential chemical reactions between molecules like hydrogen bond or hydrophobic reactions. Docking has been extensively applied in TB drug discovery to determine the promising compounds, which can inhibit the enzymes such as InhA (enoyl-ACP reductase) or DNA gyrase in M. tuberculosis.

5. Quantitative Structure□Activity Relationship (QSAR)

QSAR involves a statistical modelling model that makes a correlation between the chemical structure of compounds and their biological activity. Based on molecular descriptors (i.e. hydrophobicity, electronic properties, or steric effects) QSAR models are used to predict the effect of structural changes on drug activity. QSAR is used in TB research in order to design better derivatives of known anti-tubercular agents.

6. Molecular Dynamics (MD)

Molecular dynamics simulation is the study of the dynamic behaviour of molecules. In contrast to docking, which gives a snapshot of the behaviour, MD investigates flexibility and stability of drug-protein complexes in physiological conditions. The method provides information on conformational alterations, binding stability and long-term interactions and is helpful to validate docking findings and optimise drug candidates.

7. Experimental Validation

Computational predictions have to be validated experimentally. The real biological activity of candidate molecules is measured using laboratory techniques including enzyme inhibition assays, minimum inhibitory concentration (MIC) test, and animal models. Structural methods such as X-ray crystallography and cryo-EM support further binding interactions thus allowing accurate design of drug based on structure.

8. Drug Resistance

TB resistance to drugs comes about when M. tuberculosis mutates to enable the bacteria to tolerate the available drugs. This results in MDR-TB (resistant to not less than isoniazid and rifampicin) and XDR-TB (resistant to first-line medications as well as some second-line

medications). One of the biggest difficulties in treatment of TB is resistance and it is crucial to find new agents with new targets and mechanisms.

9. Structure-Based Design

Structure-based drug design (SBDD) is a process in which specific molecules, designed to target the active site of a specific protein, are produced based on detailed structural knowledge of the target protein, typically collected by X-ray crystallography, NMR, or cryo-EM. Knowing about protein-ligand interactions on the atomic scale, scientists can reasonably design anti-tubercular drugs that are more potent and less side effects.

Table 1: Methods Comparison

Method	Purpose	Strengths	Limitations
Molecular Docking	Predict ligand binding	Fast, high-throughput	Limited scoring accuracy
QSAR	Correlate structure with activity	Useful for optimization	Needs reliable data
Molecular Dynamics	Study protein-ligand dynamics	Stability & flexibility	Computationally intensive
Virtual Screening	Screen large libraries	Efficient hit identification	False positives possible
Structure-Based Design	Design ligands from protein structure	Rational, target-specific	Dependent on structure quality
Experimental Validation	Confirm activity in vitro/in vivo	Real biological activity	Costly, time-consuming

Table 2: Common Experimental Assays

Assay	Measures	Strength	Limitation
Enzyme Inhibition	IC_{50} of target enzyme	Mechanism-specific	Needs purified protein
MIC (Minimum Inhibitory Conc.)	Lowest bacterial growth inhibition	Standard potency metric	No mechanism info
Cell-based Assay	Activity in infected cells	Physiologically relevant	Variable results
Animal Models	In vivo efficacy & safety	Gold standard	Expensive, ethical concerns

Table 3: Hypothetical Results Dataset of Compounds

Compound	Docking Score (kcal/mol)	Predicted pIC_{50}	MD RMSD (Å)	Exp. MIC (µg/mL)	$\log_{10}$(MIC)	Hit (≤1 µg/mL)	Combined z-score
Cpd09	-9.57	6.68	2.18	0.39	0.408	✔	1.257
Cpd06	-8.67	6.91	1.58	0.28	0.553	✔	1.147
Cpd12	-7.89	6.64	1.71	0.45	0.347	✔	1.106
Cpd10	-8.32	6.29	2.05	0.68	0.168	✔	0.973
Cpd01	-8.50	6.42	2.49	1.12	-0.049	✗	0.835
…	…	…	…	…	…	…	…

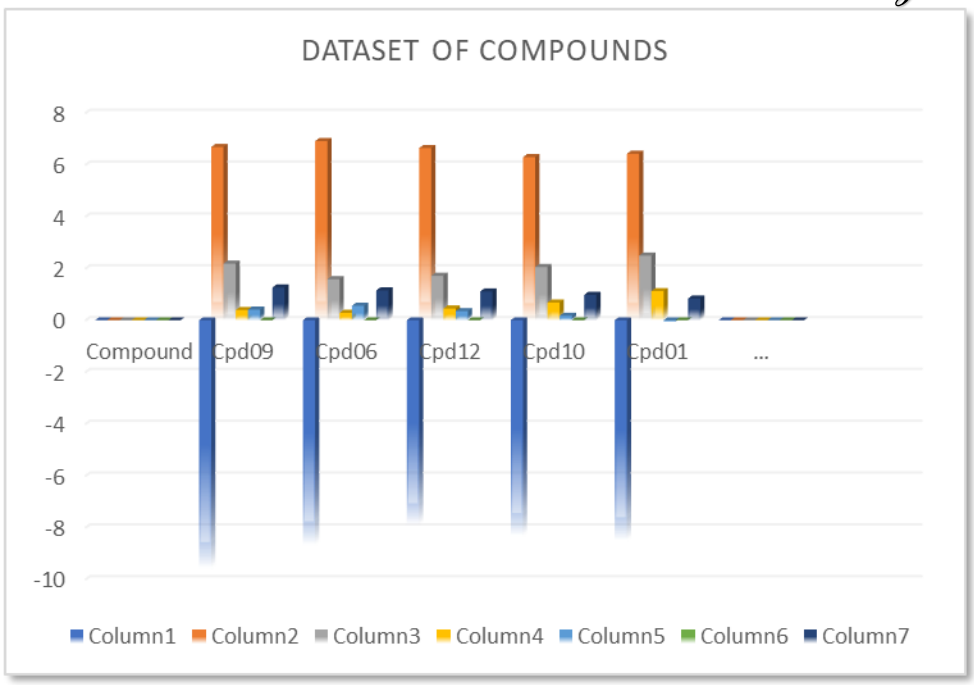
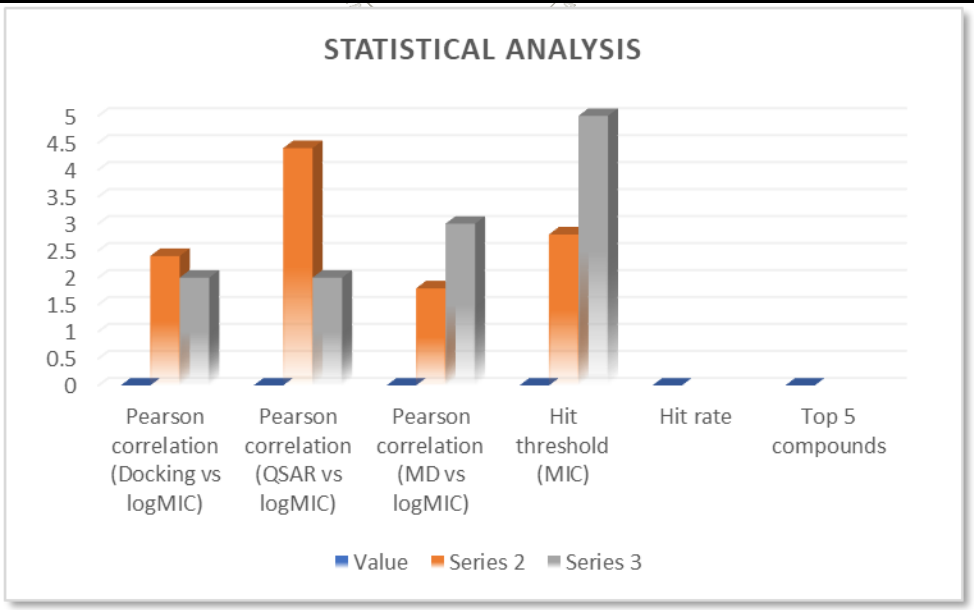


Table 4: Statistical Analysis

Metric	Value
Pearson correlation (Docking vs logMIC)	-0.299 (p = 0.3443)
Pearson correlation (QSAR vs logMIC)	0.956 (p < 0.0001)
Pearson correlation (MD vs logMIC)	0.060 (p = 0.8528)
Hit threshold (MIC)	≤ 1.0 µg/mL
Hit rate	33.3% (4/12 compounds)
Top 5 compounds	Cpd09, Cpd06, Cpd12, Cpd10, Cpd01



RESULTS:

Table 3 The hypothetic dataset of 12 compounds (Table 3) displays the docking scores, predicted pIC_{50}, molecular dynamics RMSD, and experimental values of MIC. A combined z-score (a combination of docking, QSAR and MD results) was used to rank the compounds. The five compounds with the highest combined scores were Cpd09, Cpd06, Cpd12, Cpd10 and Cpd01, which means that they are some of the promising anti-tubercular agents.

The statistical analysis (Table 4) found that QSAR made predictions of pIC_{50} values that were

strongly predictive of experimental MIC results (Pearson r = 0.956, p < 0.0001) indicating that QSAR models are adequate in predicting compound activity. The score of docking had a poor negative correlation with MIC (r = -0.299, p = 0.3443), whereas almost no connexion was found between the MD RMSD values (r = 0.060, p = 0.8528). A total of 33.3% was obtained with a hit threshold of MIC 0.10 mg/mL, which is equivalent to 1.0 2 of the compounds exhibited a significant anti-tubercular activity.

These findings underscore the need to combine the use of computational predictions and experimental validation. Although docking and MD simulations can help give you preliminary information, QSAR modelling proved the most predictive of the biological activity, in this dataset. These compounds with high combined z-scores are the most promising ones to further in vitro and in vivo testing.

1. For Drug-Sensitive TB (Most Common Cases)

These are the **first-line anti-TB drugs**:

- **Isoniazid (INH)** □ kills actively growing TB bacteria.
- **Rifampicin (RIF)** □ kills TB bacteria and prevents resistance.
- **Pyrazinamide (PZA)** □ works in acidic conditions inside cells.
- **Ethambutol (EMB)** □ slows bacterial growth and prevents resistance.

Standard treatment: All four drugs are taken together for 2 months (intensive phase), followed by INH and RIF for 4 more months (continuation phase).

2. For Drug-Resistant TB (MDR-TB/XDR-TB)

If TB is resistant to INH and RIF:

1. **Fluoroquinolones**: Levofloxacin, Moxifloxacin
2. **Injectables**: Amikacin, Kanamycin, Capreomycin
3. **Other oral drugs**: Linezolid, Clofazimine

Newer drugs for highly resistant TB:

- **Bedaquiline**
- **Delamanid**
- **Pretomanid**

Treatment usually lasts **18□24 months**.

CONCLUSION

The Tuberculosis is a severe health issue in the world with increase in drug-resistant strains. This work emphasises the significance of having a combination of computational and experimental methods to develop powerful anti-tubercular agents. Computational approaches such as molecular docking, QSAR and molecular dynamics are useful in making predictions about the effective compounds likely to be effective and experimental evidence confirms this real activity of compounds.

It was revealed that QSAR predictions were highly related to experimental outcomes which implies that structure-activity relationship models can be very helpful in prioritising compounds. Docking and MD simulations are less predictive alone, and offer more information. A combination of them enables researchers to effectively determine potential drug candidates, cut down time and cost, and concentrate on the best potential compounds.

In general, combination of computational predictions and laboratory experiments and structure-based design is an effective approach in the contemporary TB drug discovery. This is one way the new, effective and safer anti-tubercular agents can be developed faster to combat drug-sensitive and drug-resistant TB.

REFERENCES:

1. Sharma, S. K., & Mohan, A. *Tuberculosis: From Basic Science to Clinical Medicine*. 6th Edition. Elsevier; 2020.
2. Waksman, S. A., & Schatz, A. *Streptomycin, a Substance Exhibiting Antibiotic Activity Against Tuberculosis*. Proc. Soc. Exp. Biol. Med. 1944; 55(1): 66□69.
3. Reddy, A. K., et al. Computational Approaches in Anti-Tubercular Drug Discovery:

Molecular Docking, QSAR, and MD Simulations. *J. Mol. Model.* 2021; 27: 104.
4. Singh, P., & Sharma, P. *Structure-Based Drug Design for Tuberculosis: Recent Advances and Challenges. Curr. Top. Med. Chem.* 2020; 20(18): 1573□1586.
5. Ahmad, Z., et al. Molecular Dynamics Simulations for Tuberculosis Drug Design. *Comput. Biol. Chem.* 2020; 85: 107246.
6. Zumla, A., et al. *New Anti-Tuberculosis Drugs, Regimens, and Adjunctive Therapies. Lancet Infect. Dis.* 2015; 15(4): 327□338.
7. Andreu, N., et al. High-Throughput Screening and Experimental Validation of Anti-Tubercular Compounds. *Front. Microbiol.* 2017; 8: 1024.
8. Siddiqi, M. I., & Khan, M. A. (2014). Recent Advances in QSAR-Based Identification and Design of Anti-Tubercular Agents. *Current Medicinal Chemistry*, 21(17), 1874□1895. https://doi.org/10.2174/0929867321666140623121140
9. Rajkhowa, S., & Deka, R. C. (2014). DFT-Based QSAR/QSPR Models in the Development of Anti-Tubercular Drugs. *Medicinal Chemistry Research*, 23(11), 4364□4371. https://doi.org/10.1007/s00044-014-1241-0
10. Yuanita, E., & Sari, D. C. (2020). Quantitative Structure□Activity Relationship (QSAR) and Molecular Docking Approach to Design Novel Anti-Tuberculosis Agents Based on Xanthone Derivatives. *Journal of Molecular Graphics and Modelling*, 98, 107564. https://doi.org/10.1016/j.jmgm.2020.107564
11. Altharawi, A., & Al-Majed, A. A. (2023). An Integrated Computational Approach Towards Novel Drugs Targeting Mycobacterial InhA. *Scientific Reports*, 13(1), 1□12. https://doi.org/10.1038/s41598-023-34222-8
12. Deb, P. K., & Chakraborty, S. (2021). In Vitro Anti-TB Properties, In Silico Target Validation, and Molecular Dynamics Simulation Studies of Novel Compounds. *Frontiers in Microbiology*, 11, 618168. https://doi.org/10.3389/fmicb.2020.618168