Int. J. Pharm. Investigation, 2025; 15(3):1012-1016.
https://www.jpionline.org

Original Article

Assessment of the Anti-Tubercular Efficacy of a Hybrid Compound Using *in vitro* and *in silico* Methods

Akula Sowjanya*, Joshi Meripe, Midde Jeevitha, Udutha Bhanu Prasad, Mallika Shaik, Maipala Uma Mahesh

Department of Pharmacology, Marri Laxman Reddy Institute of Pharmacy, Dundigal, Hyderabad, Telangana, INDIA.

ABSTRACT

Background: Tuberculosis is mostly caused by *Mycobacterium tuberculosis*; however, it can be caused by a variety of mycobacteria. Tuberculosis predominantly impacts the respiratory system, although it may also affect other areas of the body. Approximately 10% of latent infections advance to active disease and if left untreated, this condition has a death risk surpassing 50%. In recent years, numerous research investigations have been conducted to identify and produce anti-tuberculosis medications. Nevertheless, several of these treatments have exhibited undesirable effects and have evolved resistance to multiple therapies. Consequently, there remains a necessity for the advancement of a novel class of anti-tuberculosis pharmaceuticals. **Materials and Methods:** We selected a new ligand for the *in vitro* and *in silico* evaluation of the antitubercular activity of 4-(3-(((1-phenyl-1H-1,2,3-triazol-4-yl) methoxy) methyl) quinolin-2-yl) morpholine using the MABA assay and AutoDock, respectively. This compound demonstrates elevated binding energies for the target proteins 4BAE (Bacterial DNA gyrase) and 2CIG (Bacterial DHFR). **Results:** The MABA assay demonstrated that this compound at 200 µg and 100 µg exhibited inhibitory effects against *M. smegmatis*. The compound exhibits no anti-tubercular activity against Mtb (H37Ra), as the colour transition from blue to pink signifies the proliferation of mycobacteria. According to *in silico* studies, this compound functions by inhibiting bacterial DNA gyrase and topoisomerase IV. This may impede DNA synthesis and ultimately lead to bacterial mortality. **Conclusion:** The selected compound exhibited anti-tubercular activity against the *Mycobacterium smegmatis* strain relative to the conventional rifampicin, but failed to show anti-tubercular activity against the H37ra.

Keywords: Autodock, *In silico*, *In vitro*, MABA, Tuberculosis.

Correspondence:
Dr. Sowjanya Akula
Department of Pharmacology, Marri Laxman Reddy Institute of Pharmacy, Dundigal, Hyderabad, Telangana, INDIA.
Email: akula.sowjuu@gmail.com
ORCID ID: 0000-0002-6560-4505)

Received: 27-09-2024;
Revised: 05-12-2024;
Accepted: 12-03-2025.

INTRODUCTION

Numerous Mycobacterium strains, most notably *Mycobacterium tuberculosis*, are the causative agents of tuberculosis, an infectious bacterial disease. Pulmonary tuberculosis primarily targets the lungs, while extra-pulmonary tuberculosis can affect other areas of the body.[1] Tuberculosis poses a significant health risk to individuals who have HIV and is a prominent contributor to mortality in this population. Tuberculosis is classified as an Opportunistic Infection (OI), indicating that it is more prevalent or more severe in individuals with compromised immune systems. HIV infection is the primary identified risk factor for the development of Tuberculosis illness.[2] This is because HIV compromises the immune system, thereby impairing the body's ability to combat tuberculosis bacteria. HIV/TB co-infection refers to the simultaneous presence of both HIV and TB in an individual, which significantly heightens the likelihood of developing Tuberculosis in people who have advanced HIV. Tuberculosis primarily impacts individuals throughout their peak productive adult years. People of all ages are susceptible to the risk. More than 80% of cases and fatalities transpire in low- and middle-income nations.[3]

In 2022, the World Health Organization (WHO) documented 1.3 million fatalities attributable to Tuberculosis, comprising 167,000 instances among individuals with HIV. Tuberculosis is the second most significant infectious cause of death globally, surpassed only by COVID-19 and it exceeds the mortality rates of HIV and AIDS. In 2022, almost 10.6 million people globally were diagnosed with tuberculosis, including 5.8 million men, 3.5 million women and 1.3 million children.[4] The main goal is to address a critical global health issue, while also making progress in scientific understanding and promoting policies that have the potential to save lives. The field of Tuberculosis (TB) research provides ample opportunities for investigation, with the capacity to greatly influence worldwide health results. By immersing ourselves in this field, we have the chance to create more efficient therapies and preventive measures, thereby enhancing the global battle against TB and advancing public health on a global scale.[5]

DOI: 10.5530/ijpi.20250132

In recent years, numerous research studies and anti-TB medications have been developed; however, many of these drugs exhibit side effects and multi-drug resistance. Consequently, the synthesis of a novel class of anti-tuberculosis agents remains imperative. We selected a novel ligand for the *in vitro* and *in silico* evaluation of the antitubercular activity of 4-(3-(((1-phenyl-1H-1, 2,3-triazol-4-yl) methoxy) methyl) quinolin-2-yl) morpholine.

MATERIALS AND METHODS

Docking Studies

Molegro Virtual Docker was employed for molecular docking, a rapid and versatile program that provides the most likely binding conformation of a ligand to a macromolecule. The docking scoring capability of Mol Dock enhances the Piecewise direct Potential (PLP) by incorporating additional hydrogen bonding and electrostatic components. To further enhance docking precision, a repositioning scoring capability is introduced, which differentiates the most favorable docking configuration from those obtained by the docking algorithm.[6]

Preparation of ligand

The primary focus of the docking study was the A Chain of DHF rase and DNA Gyrase. PDB documents often exhibit inadequate or absent assignments of expressed hydrogens and the design of PDB records does not accommodate bond request data. In this context, legitimate bonds, bond requests, hybridization and charges were allocated using the MVD. The 3D structures of the phytoconstituents were obtained from PubChem chemical databases and rendered using ChemDraw Ultra 8.0 software subsequently saved in mol format following energy minimization.[7]

Microplate Alamar Blue Assay (MABA)

The Microplate Alamar Blue Assay (MABA) is an uncomplicated and reliable technique. This approach utilizes the oxidation, reduction process of alamar blue as a colorimetric indicator to assess the inhibition of Mycobacterium by natural extracts or chemicals and to determine the Minimum Inhibitory Concentration (MIC). This signifies a cost-effective alternative to the other anti-TB assay. The compound's anti-mycobacterial efficacy was evaluated against *Mycobacterium smegmatis* and *Mycobacterium tuberculosis* strains using The Microplate Alamar Blue test (MABA). In summary, 200 µL of sterile deionized water was added to the outside perimeter wells of a sterile 96-well plate to reduce the evaporation of the medium in the test wells during incubation. 100 µL of Middlebrook 7H9 broth was allocated to each well of the 96-well plate and the compounds were diluted sequentially directly on the plate. The drug concentrations assessed in the final trial were 10, 25, 50, 100 and 200 micrograms per milliliter (µg/mL) shown in Table 1. The plates were carefully coated and sealed with parafilm, then placed in an incubator at 37ºC for five days. Following this period, 25 µL of a freshly prepared 1:1 solution of alamar blue reagent and 10% Tween 80 was introduced to the plate and permitted to incubate for 24 hr. The absence of bacterial growth was indicated by a blue hue in the well, while the presence of growth was represented by a pink tint. The Minimum Inhibitory Concentration (MIC) represents the lowest concentration of a medication that effectively prevents the color change from blue to pink.[8]

RESULTS

In silico anti-tubercular activity

Tables 2 and 3 shown the Docking studies of the compound 4-(3-(((1-phenyl-1H-1, 2, 3-triazol- 4-yl) methoxy) methyl) quinolin-2-yl) morpholine reveals its higher binding energies towards the target proteins 4BAE (Bacterial DNA gyrase) and 2CIG (Bacterial DHFR). The compounds 2-(4-(3-bromo-4-chlo ro-5-methyl-1H-pyrrole-2-carboxamido)-3-methoxypiperidin -1-yl)-4-(1-methyl- 1H-1,2,4-triazol-5-yl) thiazole-5-carboxylic acid and (4R)-Isonicotinic-acetyl-nicotinamide- adenine dinucleotide is used as standard for 4BAE and 2CIG respectively. Figure 1 indicates the compound hasn't shown any interactions with H-bond amino acids but interacted with hydrophilic amino acids namely Val 49, Asn52, Ala53, Glu56, Ile84, Pro85, Val123, Val125, Ile 171 while using 4BAE as target protein for docking studies. But when it was tested for docking scores for the target protein 2CIG the compound showed its affinity for both amino acids with H-bond and hydrophobic amino acids. The H-bond amino acids Ala7, Ile14, Ser49 and hydrophobic amino acids Ala7, Ile14, Asp19, Ile20, Asp27, Val46, Leu50, Gly95, Ala 126 interacted with the test compound at 2CIG target site shown in the Figure 2.

MABA Assay

The samples at 200 µg and 100 µg demonstrated inhibition against *M. smegmatis*. Figure 3 showed that the transition from blue to pink signifies the proliferation of mycobacteria; however, at concentrations of 100 and 200 µg/mL, the pink coloration was not observed. Inhibition was observed at concentrations of 100 and 200 µg/mL. The sample demonstrates no anti-tubercular activity against Mtb.H37Ra, since the color change from blue to pink indicates mycobacterial proliferation, signifying a lack of inhibition.

DISCUSSION

Tuberculosis is a prevalent and often lethal bacterial infection caused mostly by *Mycobacterium tuberculosis*. Tuberculosis mostly impacts the pulmonary system, although it can also affect other anatomical areas.[9] Tuberculosis is spread via airborne transmission when individuals with an active infection release respiratory fluids through coughing, sneezing, or other methods. A number of diseases, such as tuberculosis, sometimes manifest asymptomatically, a state referred to as latent tuberculosis.

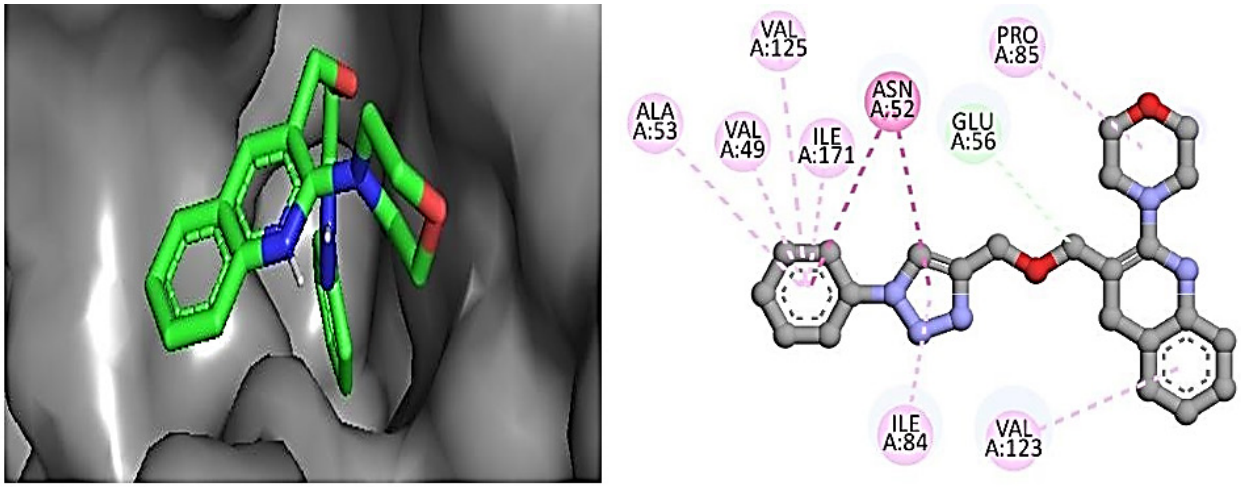

Figure 1: 4-(3-(((1-phenyl-1H-1,2,3-triazol- 4-yl) Methoxy) methyl) Quinoline -2- yl) Morpholine Interaction with 4-BAE.

Table 1: Different concentration levels in 96 well plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	H2O											
B												
C												
D	S	200	100	50	25	10	200	100	50	25	10	
E	S	200	100	50	25	10	200	100	50	25	10	
F												
G												
H		B	B	B	RIF	RIF	RIF	GC	GC	GC		

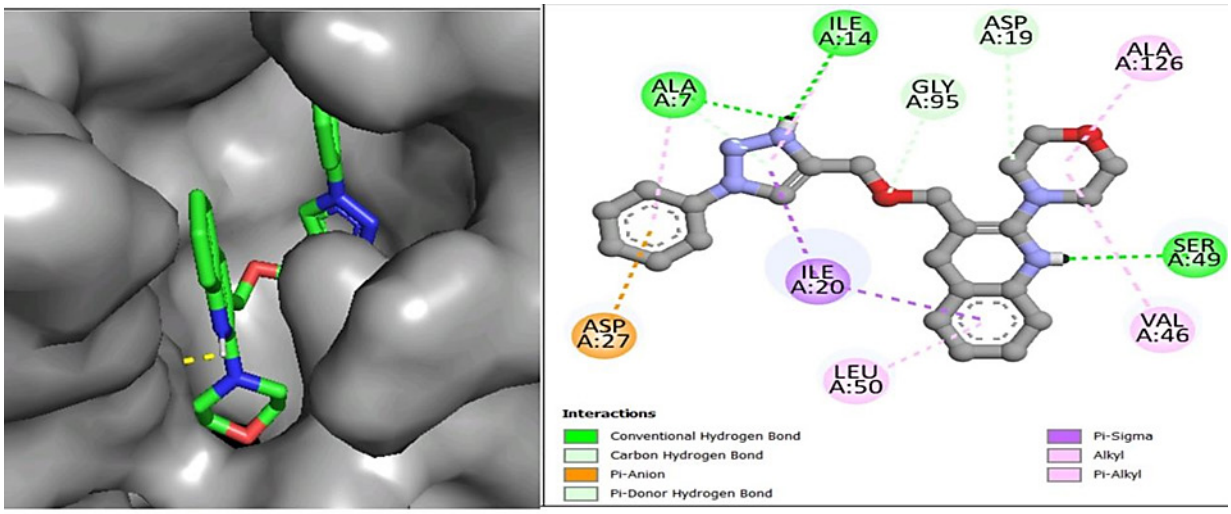

Figure 2: 4-(3-(((1-phenyl-1H-1, 2, 3-triazol-4-yl) methoxy) methyl) quinolin-2- yl) morpholine interact with DHFR.

Roughly 10% of latent infections advance to active disease and if left untreated, this condition has a death risk surpassing 50%. The substance known as 4-(3-(((1-phenyl-1H-1,2,3-triazol-4-yl)-methoxy) methyl) quinolin-2-yl) was selected and assessed for antitubercular efficacy.[10-12]

In silico studies were conducted utilizing 4BAE and 2CIG as target proteins. An *in vitro* study was conducted utilizing the Micro Plate Alamar Bioassay Method (MABA) against two strains of *Mycobacterium tuberculosis* (*H37Ra* and *Mycobacterium smegmatis*).

The *in silico* investigations demonstrated that the synthetic compound displayed superior binding energies for DHFR and DNA gyrase. The compound's mode of action is likely similar to that of traditional anti-tubercular medicines like

Table 2: Scoring profile of ligands with target protein (4BAE).

Ligand name	Target Protein (4BAE) Bacterial DNA gyrase		
	Mol dock score	Interacting amino acids	
		H-bond	Hydrophobic
4-(3-(((1-phenyl-1H-1,2,3-triazol-4-yl) methoxy) methyl) quinolin-2- yl) morpholine.	-8.2	--	Val 49, Asn52, Ala53, Glu56, Ile84, Pro85, Val123,Val125, Ile171.
2-(4-(3-bromo-4-chloro-5-methyl-1H-pyrrole-2- carboxamido)-3-methoxypiperidin-1-yl)-4-(1-methyl-1H-1,2,4-triazol-5-yl) thiazole-5-carboxylic acid.	-7.7	Asp79, Arg82, Gly83, His121	Val123

Table 3: Scoring profile of ligands with target protein (DHFR).

Ligand name	Target Protein (2CIG) Bacterial DHFR		
	Mol dock score	Interacting amino acids	
		H-bond	Hydrophobic
4-(3-(((1-phenyl-1H-1,2,3-triazol-4-yl) methoxy) methyl) quinolin-2- yl) morpholine.	-9.7	Ala7, Ile14, Ser49	Ala7, Ile14, Asp19, Ile20, Asp27,Val46, Leu50, Gly95, Ala126.
(4R)-Isonicotinic-acetyl-nicotinamide-adenine dinucleotide.	-10.0	Asp19, Asp27, Arg44, Gly96, Gly97, Gln98	Trp6, Ala7, Ile20,Arg45, Glu65, Gln68.

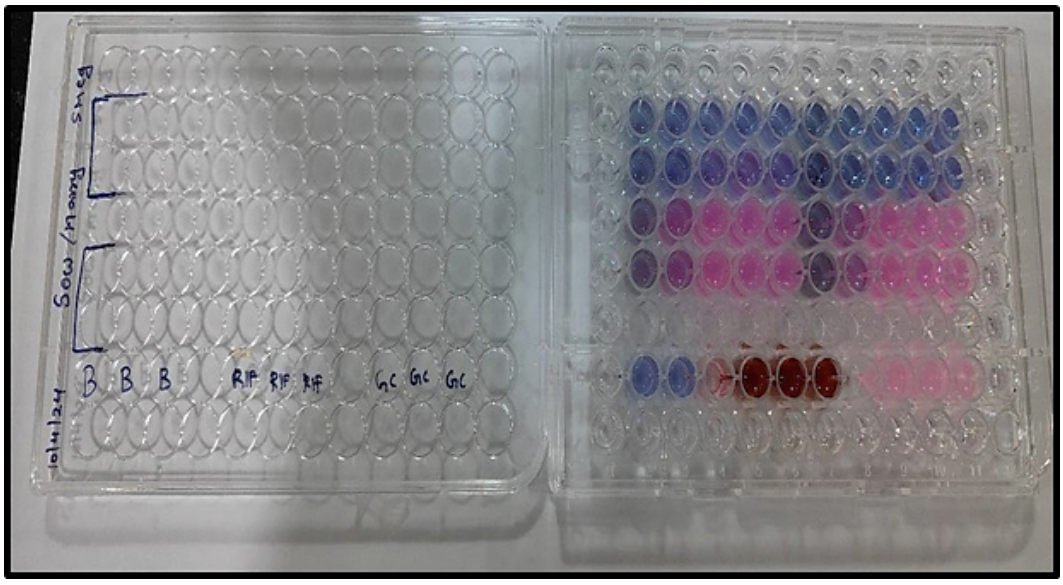

Figure 3: Effect of 4-(3-(((1-phenyl-1H-1, 2, 3-triazol-4-yl) methyl) Quinolin-2yl) morpholine on *Mycobacterium smegmatis* by MABA assay.

fluoroquinolones.[13] These medications operate by blocking bacterial DNA gyrase and topoisomerase IV, enzymes essential for DNA replication and transcription. This inhibition disrupts DNA pathways and ultimately leads to bacterial death. Para-amino salicylic acid functions by inhibiting the enzyme Dihydrofolate Reductase (DHFR).[14,15] DHFR is an enzyme crucial for the synthesis of tetrahydrofolate, a folic acid derivative required for nucleotide synthesis, thereby enabling DNA replication and cell division. The *in vitro* study indicates that the compound exhibited an inhibitory effect particularly against the strain *Mycobacterium smegmatis*, with no effect observed on the H37Ra strain.

CONCLUSION

Anti-tubercular efficacy of a hybrid compound using *in vitro* and *in silico* methods has been clearly demonstrated. *In silico* evaluations were conducted using Autodock software, revealing that the compound exhibited elevated binding energies, indicative of increased absorbance and enhanced potency. *In vitro* tests were conducted utilizing the MABA method against the H37Ra and Mycobacterium smegmatis strains. The compound demonstrated anti-tubercular activity when evaluated against the *Mycobacterium smegmatis* strain in comparison to the standard rifampicin, but did not exhibit anti-tubercular activity against the H37ra strain.

ACKNOWLEDGEMENT

We would like to express our sincere gratitude to Management of Marri Laxman Reddy Institute of Pharmacy for their invaluable support and guidance throughout this research. Their expertise and insights were instrumental in shaping the outcomes of this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DHFR: Dihydrofolate Reductase; **MABA:** Microplate Alamar Blue Assay; **OI:** Opportunistic Infection; **H37Ra:** *Mycobacterium tuberculosis*; **WHO:** World Health Organization; **HIV:** Human immune deficiency virus; **AIDS:** Acquired immunodeficiency virus; **4BAE:** Bacterial DNA gyrase; **TB:** Tuberculosis; **MVD:** Molegro Virtual Docker; **MIC:** Minimum Inhibitory Concentration; **2CIG:** Bacterial DHFR.

REFERENCES

De Souza, M. V. N., Pais, K. C., Kaiser, C. R., Peralta, M. A., de L Ferreira, M. D., & Lourenço, M. C. S. (2009, February 15). Synthesis and *in vitro* antitubercular activity of a series of quinoline derivatives. Bioorganic and Medicinal Chemistry, 17(4), 1474-1480. https://doi.org/10.1016/j.bmc.2009.01.013

Gschwend, D. A., Good, A. C., & Kuntz, I. D. (1996). Molecular docking towards drug discovery. Journal of Molecular Recognition, 9(2), 175-186. https://doi.org/10.1002/(sici)1099-1352(199603)9: 2<175:aid-jmr260>3.0.co;2-d

Kolavi, G., & Hegde, V. (2006, May 1). ahmedKhazi I, Gadad P. Synthesis and evaluation of antitubercular activity of imidazo [2, 1-b][1, 3, 4] thiadiazole derivatives. Bioorganic and Medicinal Chemistry, 14(9), 3069-3080.

Leung, A. N. (1999, February). Pulmonary tuberculosis: The essentials. Radiology, 210(2), 307-322. https://doi.org/10.1148/radiology.210.2.r99ja34307

Lin, C.-H., Lin, C.-J., Kuo, Y.-W., Wang, J.-Y., Hsu, C.-L., Chen, J.-M., Cheng, W.-C., & Lee, L.-N. (2014, December). Tuberculosis mortality: Patient characteristics and causes. BMC Infectious Diseases, 14(1), 1-8. https://doi.org/10.1186/1471-2334-14-5

Ramírez-Lapausa, M., Menéndez-Saldaña, A., & Noguerado-Asensio, A. (2015, June). Extrapulmonary tuberculosis. Revista Espanola de Sanidad Penitenciaria, 17(1), 3-11. https://doi.org/10.4321/S1575-06202015000100002

Sharma, S. K., & Mohan, A. (2004). Multidrug-resistant tuberculosis. The Indian Journal of Medical Research, 120(4), 354-376. https://doi.org/10.5005/jp/books/11539_15

Shingalapur, R. V., Hosamani, K. M., & Keri, R. S. (2009, October 1). Synthesis and evaluation of *in vitro* antimicrobial and anti-tubercular activity of 2-styryl benzimidazoles. European Journal of Medicinal Chemistry, 44(10), 4244-4248. https://doi.org/10.1016/j.ejmech.2009.05.021

Shoichet, B. K., McGovern, S. L., Wei, B., & Irwin, J. J. (2002). Lead discovery using molecular docking. Current Opinion in Chemical Biology, 6(4), 439-446. https://doi.org/10.1016/s1367-5931(02)00339-3

Stewart, P., Patel, S., Comer, A., Muneer, S., Nawaz, U., Quann, V., Bansal, M., & Venketaraman, V. (2023, May 6). Role of B cells in *Mycobacterium tuberculosis* infection. Vaccines, 11(5), 955. https://doi.org/10.3390/vaccines11050955

Stone, M. J., & Joress, M. H. (1924). Concerning the signs and symptoms in early tuberculosis. The Boston Medical and Surgical Journal, 191(23), 1067-1069. https://doi.org/10.1056/NEJM192412041912302

Venkatraju, B., & Prasad, S. (2010, October 30). Beliefs of patients about the causes of tuberculosis in rural Andhra Pradesh. International Journal of Nursing and Midwifery, 2(2), 21-29.

Venugopala, K. N., Chandrashekharappa, S., Pillay, M., Bhandary, S., Kandeel, M., Mahomoodally, F. M., Morsy, M. A., Chopra, D., Aldhubiab, B. E., Attimarad, M., Alwassil, O. I., Harsha, S., Mlisana, K., & Odhav, B. (2019, May 1). Synthesis and structural elucidation of novel benzothiazole derivatives as anti-tubercular agents: In silico screening for possible target identification. Medicinal Chemistry, 15(3), 311-326. https://doi.org/10.2174/1573406414666180703121815

World Health Organization. (2010). Stop TB initiative. World Health Organization. Treatment of tuberculosis: guidelines.

Cite this article: Sowjanya A, Meripe J, Jeevitha M, Prasad UB, Shaik M, Mahesh MU. Assessment of the Anti-Tubercular Efficacy of a Hybrid Compound Using *in vitro* and *in silico* Methods. Int. J. Pharm. Investigation. 2025;15(3):1012-6.