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Research Article

Synthesis and Biological Evaluation of Some Thiadiazole Derivatives as Antitubercular Agents

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The increasing prevalence of multidrug-resistant tuberculosis has created an urgent need for new and effective antitubercular agents. Thiadiazole derivatives are known for their diverse pharmacological properties, particularly antimicrobial activity, making them attractive candidates for tuberculosis drug discovery. In the present study, a series of novel thiadiazole derivatives were synthesized and evaluated for their antitubercular potential. The compounds were prepared using a multistep synthetic pathway and were characterized by FT-IR, ^{1}H-NMR, and mass spectrometry to confirm their chemical structures. The synthesized molecules were screened for in vitro antitubercular activity against Mycobacterium tuberculosis H37Rv strain using standard microbiological techniques. Several derivatives exhibited notable inhibitory activity, indicating promising antitubercular potential. Structure–activity relationship studies suggested that the nature and position of substituents on the thiadiazole ring significantly influenced biological activity. These findings highlight thiadiazole derivatives as valuable lead compounds for the development of new antitubercular drugs.

Keywords: Thiadiazole, Antitubercular activity, Mycobacterium tuberculosis, H37Rv, Drug-resistant TB, Structure–activity relationship.

INTRODUCTION

Tuberculosis (TB) is a long-standing infectious disorder caused by Mycobacterium tuberculosis, a slow-multiplying, aerobic, acid-fast bacterium belonging to the family Mycobacteriaceae. The infection spreads mainly through tiny airborne particles expelled when a person with active TB coughs, sneezes, or talks. When these particles are inhaled, the bacteria enter the lungs and settle in the alveoli, where they are taken up by immune cells known as macrophages. Depending on the strength of the host's immune system, the organisms may be destroyed, persist in a dormant state, or multiply and produce active disease. Although the lungs are most commonly affected, the infection can also spread to other parts of the body, including lymph nodes, bones, joints, kidneys, the gastrointestinal tract, and the central nervous system, resulting in serious complications. Tuberculosis has affected human populations for thousands of years and remains one of the oldest recognized infectious diseases. Despite major advances in medical treatment and public health strategies, TB continues to be a significant global health problem. Although it is both preventable and treatable, it remains a leading cause of death from a single infectious agent worldwide. This continued burden reflects not only the biological nature of the disease but also the influence of social and economic conditions that shape exposure, diagnosis, and treatment outcomes. The distribution of tuberculosis across the world is highly unequal, with the greatest impact seen in low- and middle-income countries. Conditions such as poverty, undernutrition, overcrowding, inadequate sanitation, and poor access to healthcare strongly contribute to ongoing transmission. In many developing regions, late detection, weak healthcare systems, and poor adherence to prolonged drug therapy further worsen disease control. Populations living in urban slums, migrant groups, and socially disadvantaged communities are particularly at risk, emphasizing the

close relationship between tuberculosis and socioeconomic disparity. From a public health standpoint, tuberculosis presents a complex challenge because it requires both effective treatment of active cases and sustained measures to prevent further spread.

MATERIAL AND METHODS

All chemicals employed in the synthesis were procured from Merck (Mumbai), Sigma, Loba Chemie (Mumbai), Rankem (Haryana), and Avera Laboratories (Hyderabad). All solvents, reagents, and catalysts were of analytical grade and were used without further purification. The progress and purity of the synthesized compounds were monitored by thin-layer chromatography (TLC) using silica gel–coated glass plates as the stationary phase, with dichloromethane: methanol (10:1) as the mobile phase. The crude products were purified by recrystallization using suitable solvents. Further purification of the final compounds was achieved by column chromatography employing silica gel (230–400 mesh) packed in a sintered glass column. Melting points were determined by the open capillary method using an Analab scientific melting point apparatus and are reported as uncorrected values. Infrared (IR) spectra were recorded using the KBr pellet technique on an FT-IR 8400S spectrophotometer (Shimadzu, Japan). ^1H-NMR and ^13C-NMR spectra of the synthesized compounds were recorded on a BRUKER AVANCE II 400 spectrometer operating at 400 and 100 MHz, respectively. Mass spectra were obtained using a WATERS Q-TOF MICROMASS (LC-MS) instrument at the Sophisticated Analytical Instrument Facility (SAIF), Panjab University, Chandigarh. Chemical shift values are expressed in δ (ppm). In vitro antitubercular studies were carried out in the Department of Biotechnology, H R Patel Institute of Pharmaceutical Education and Research, Dhule (M.S.) India.

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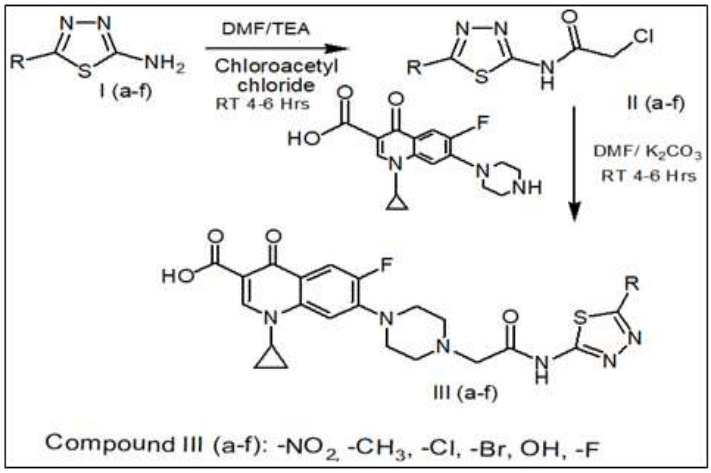
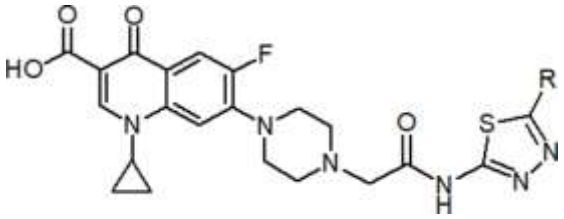
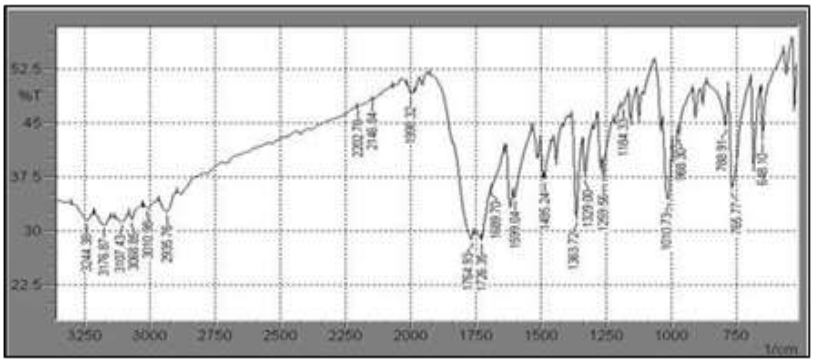


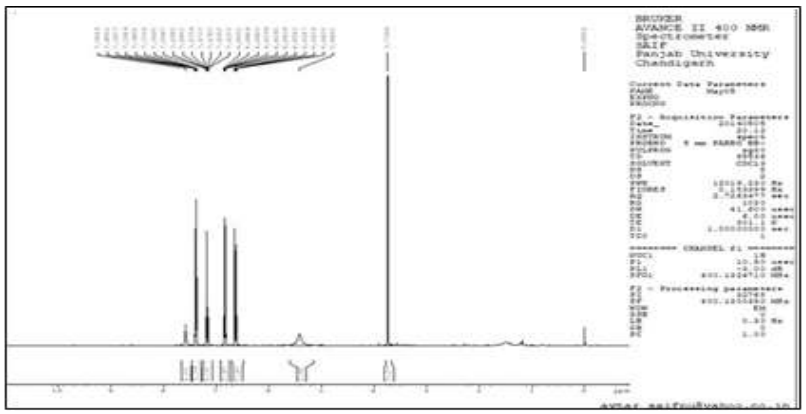
General Procedure for Synthesis of II (a–f): To a stirred solution of 2-amino-5-substituted-1,3,4 thiadiazole (I a–f) (0.01 mol) in dry DMF, chloroacetyl chloride (0.012 mol) was added dropwise at 0–5 °C in the presence of triethylamine (0.02 mol) as a base. The reaction mixture was then stirred at room temperature for 3–4 h. Completion of the reaction was confirmed by TLC. The reaction mixture was poured into ice-cold water, and the resulting solid was filtered, washed thoroughly with water to remove excess base, and dried. The crude product was purified by recrystallization from ethanol to yield chloroacetyl derivatives II (a–f). General procedure for the preparation of III (a-f) A solution of fluoroquinolone piperazine derivative (0.01 mol) in dry DMF was treated with chloroacetylated thiadiazole intermediate II (a–f) (0.01 mol) in the presence of potassium carbonate (0.02 mol). The reaction mixture was stirred at 60–70 °C for 6–8 h. Reaction progress was monitored by TLC. After completion, the mixture was cooled and poured into ice-cold water. The precipitated product was filtered, washed with water, and dried. The crude compounds were purified by recrystallization from methanol/ethanol to afford the final target compounds III (a–f). M.P. (°C) III(a)121-123, III(b)151-153, III (c)139-141, III (d)148-151, III (e)147-149, III (f)151 153; Yield (%) III (a) 48, III (b) 68, III (c) 58, III (d) 51, III (e) 47, III (f)49.

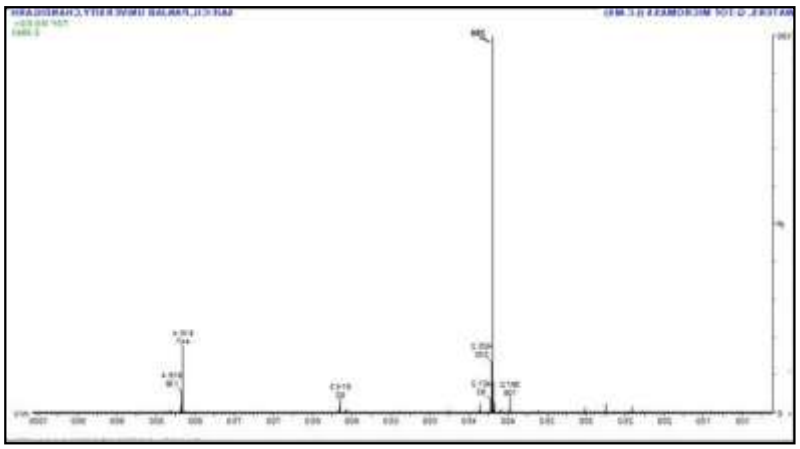
Physical data of title Compound III (a-f)



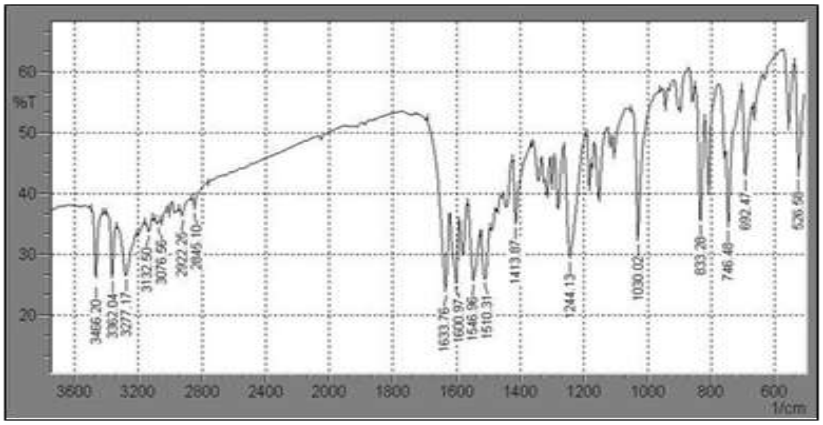
Compound	R	Molecular weight	Molecular formula	Rfvalue
III(a)	-NO2	517	C21H20FN7O6S	0.34
III(b)	-CH3	487	C22H23FN6O4S	0.36
III(c)	-Cl	507	C21H20ClFN6O4S	0.31
III(d)	-Br	551	C21H20BrFN6O4S	0.33
III(e)	-OH	488	C21H21FN6O5S	0.36
III(f)	-F	490	C21H20F2N6O4S	0.39



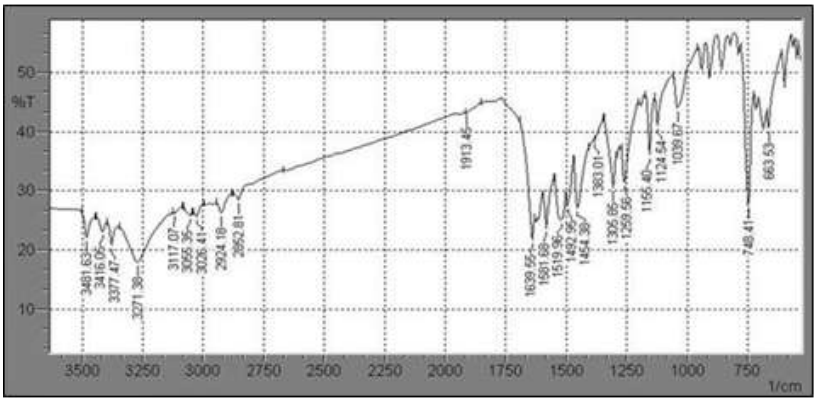




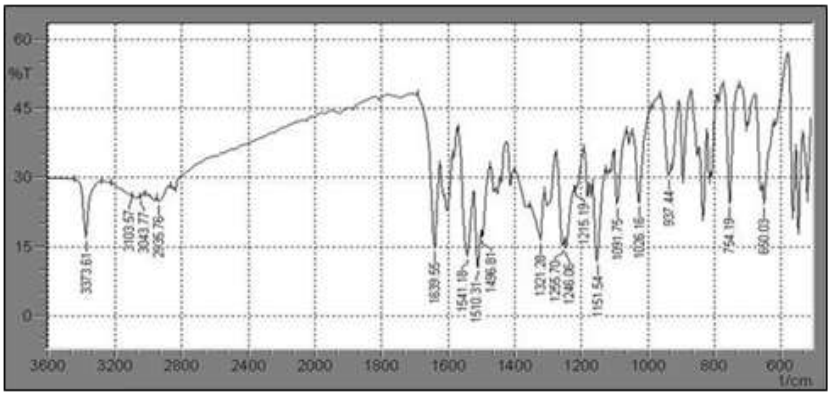
Compound	IIIa	**Melting point:**121-123^{0}C
Molecular weight	517	
FT-IR (cm^{-1})	1599.04(C=Nstr.),3244.38(N-Hstr.),2935.76(Aro.C-Hstr.),3244.38 (AmineN-Hstr.),3010.98(Aro.C=Cstr.),1332,1192(O-H), 1010.73(S-H).	
^{1}HNMRδ (ppm)	2.30(3H, s, methyl-H),3.76(3H, s,methoxy-H),6.90-6.93(4H,m, Arom-H),7.16-7.23(4H, m,Arom-H),7.44-7.62(4H,m,Arom-H), 10.15(1H, s, Amide-H), 10.85(1H, s,-H).	
LC-MS(m/z)	515(M+1)	



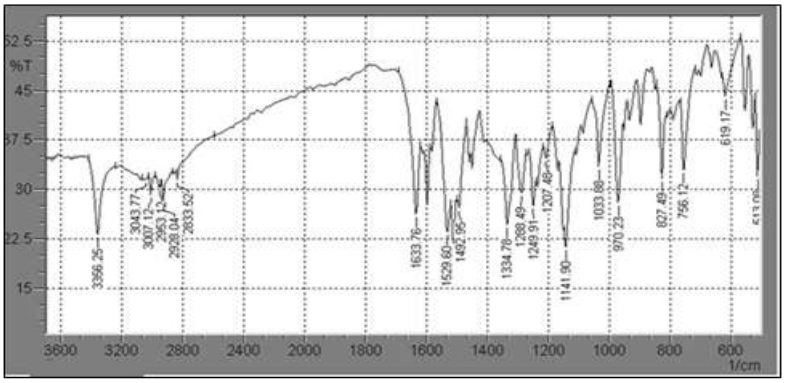
Compound	IIIb	**Melting point:**151-153^{0}C
Molecular weight	487	
FT-IR (cm^{-1})	1633.76(C=Nstr.),3277(AmideN-Hstr.),3466,3362(AmineN-Hstr.),3076(C=Cstr.),2922(Aro.C-Hstr.)	



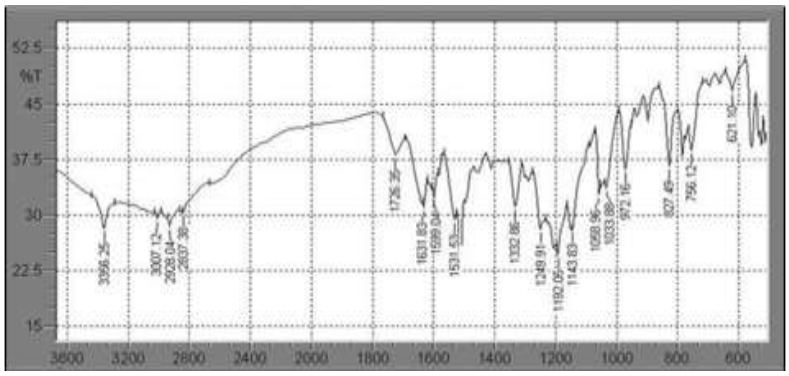
Compound	IVc	**Meltingpoint:**139-141^{0}C
Molecular weight	507	
FT-IR (cm^{-1})	1639(C=Ostr.),3271(AmideN-Hstr.),3481,3371(AmineN-Hstr.), 2924(Aro.C-Hstr.),3026(C=Cstr.).	



Compound	IIId	**Meltingpoint:**148-151^{0}C
Molecular weight	551	
FT-IR (cm^{-1})	1639(C=Ostr.),3373(AmideN-Hstr.),2935(Aro. C-Hstr.),1510(Aro. C=Cbend.),1321,1151(S=Ostr.)	



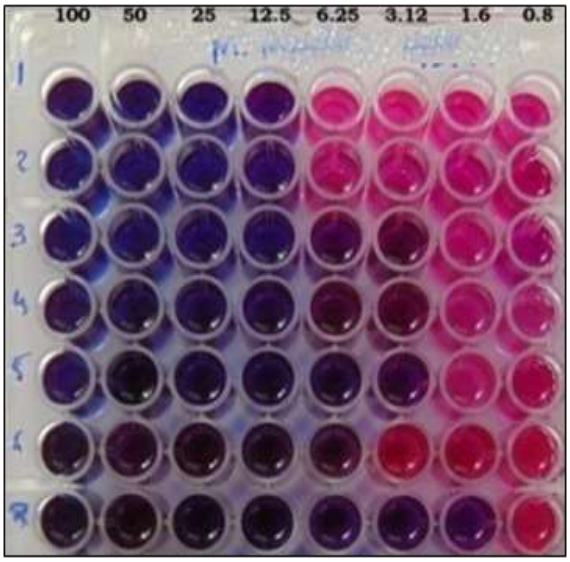
Compound	IIIe	**Meltingpoint:**147-149^{0}C
Molecular weight	488	
FT-IR (cm^{-1})	1633(C=Ostr.),3356(N-Hstr.),2928(Aro.C-Hstr.),3007(C=Cstr.), 1334,1141(S=Ostr.)	



Antitubercular Evaluation: In-vitro Antitubercular Activity of Title Compounds III (a–f) The antitubercular activity of the synthesized compounds III (a–f) was systematically evaluated against the reference strain Mycobacterium tuberculosis H37Rv (ATCC 27294) using the Microplate Alamar Blue Assay (MABA), a rapid, sensitive, and cost-effective colorimetric method widely employed for in-vitro screening of antitubercular agents. All experimental procedures involving live M. tuberculosis cultures were carried out strictly under biosafety level-III (BSL-III) laboratory conditions, in accordance with institutional biosafety regulations and World Health Organization (WHO) guidelines, to ensure containment and safe handling of this pathogenic organism. The MABA method was selected due to its ability to accurately determine the minimum inhibitory concentration (MIC) of test compounds while minimizing the time required compared to conventional solid media-based assays. For cultivation and maintenance of the mycobacterial strain, Middlebrook 7H9 broth was used as the basal medium. This medium was supplemented with 10% OADC enrichment, consisting of oleic acid, albumin, dextrose, and catalase, which provides essential nutrients and protects the bacteria from toxic metabolites, thereby supporting optimal growth of M. tuberculosis. Additionally, 0.05% Tween-80 was incorporated into the medium to prevent clumping of mycobacterial cells, ensuring a uniform bacterial suspension and reliable assay results. The supplemented medium was sterilized by filtration or autoclaving, as appropriate, prior to use in the assay. The synthesized test compounds III (a–f) were initially dissolved in dimethyl sulfoxide (DMSO) to prepare concentrated stock solutions of 1 mg/mL, owing to the good solubilizing ability of DMSO Synthesis and Biological Evaluation of Some Thiadiazole Derivatives as Antitubercular Agents for

a wide range of organic compounds. These stock solutions were further diluted serially with the supplemented Middlebrook 7H9 broth to obtain a series of working concentrations ranging from 0.2 to 100 µg/mL. Care was taken to ensure that the final concentration of DMSO in each assay well did not exceed 1% v/v, as higher concentrations of DMSO are known to exert inhibitory effects on mycobacterial growth and could interfere with the interpretation of results. A solvent control containing an equivalent concentration of DMSO without test compound was included to confirm the absence of solvent-related antimicrobial effects. A freshly grown culture of M. tuberculosis H37Rv was prepared by incubating the organism in Middlebrook 7H9 broth until it reached the logarithmic phase of growth, which is considered the most metabolically active and suitable phase for antimicrobial susceptibility testing. The bacterial suspension was then visually adjusted to match McFarland standard No. 1, corresponding to an approximate density of 1×10^6 CFU/mL, and subsequently diluted with the culture medium to obtain a final inoculum concentration of approximately 1×10^5 CFU/mL. Standardization of the inoculum density is a critical step in antitubercular assays, as variations in bacterial load can significantly influence MIC values and compromise reproducibility. The assay was performed using sterile, flat-bottom 96-well microtiter plates, which allow simultaneous testing of multiple compounds and concentrations in a compact and efficient format. Each well received an appropriate volume of the test compound solution, supplemented culture medium, and standardized bacterial inoculum to achieve the desired final concentrations. Wells containing bacteria without test compound served as the growth control, while wells containing only culture medium without bacteria acted as sterility or blank controls. Standard antitubercular drugs, namely isoniazid, rifampicin, and moxifloxacin, were included as positive controls to validate the assay conditions and enable comparative evaluation of the synthesized compounds against clinically established therapies. Following loading, the microtiter plates were carefully sealed to prevent evaporation and contamination and incubated at 37 ± 1 °C under static conditions for a period of 7 days, which is sufficient for measurable growth of M. tuberculosis in liquid medium. After the initial incubation period, the plates were visually inspected for any signs of contamination or precipitation. Subsequently, Alamar Blue reagent (20 µL) along with 12.5 µL of 20% Tween-80 was added to each well. Alamar Blue is a redox indicator dye that is reduced from its oxidized blue, non-fluorescent form to a pink, fluorescent form by metabolically active cells, thereby serving as an Synthesis and Biological Evaluation of Some Thiadiazole Derivatives as Antitubercular Agents indirect measure of bacterial viability and growth. The addition of Tween-80 at this stage enhances dye penetration and ensures uniform color development. After the addition of the indicator dye, the plates were reincubated at 37 °C for an additional 24 hours to allow sufficient time for color development. At the end of this incubation period, the wells were examined visually for color change. A distinct change from blue to pink indicated active bacterial metabolism and growth, whereas retention of the blue color signified effective inhibition of mycobacterial growth by the test compound at that concentration. In some cases, intermediate purple coloration was interpreted cautiously and confirmed by extended incubation or comparison with controls. Where required, absorbance readings were also taken spectrophotometrically at 570 nm and 600 nm to quantitatively support visual observations. The minimum inhibitory concentration (MIC) for each synthesized compound was defined as the lowest concentration at which no color change from blue to pink was observed, indicating complete inhibition of visible mycobacterial growth.

Sr. No.	Compound Code	R Substituent	MIC(µg/mL)	Activity Classification
1	III(a)	$–NO_2$	1.56	Highly active
2	III(b)	$–CH_3$	12.5	Moderately active
3	III(c)	–Cl	3.12	Highly active
4	III(d)	–Br	6.25	Moderately active
5	III(e)	–OH	25.0	Weakly active
6	III(f)	–F	12.5	Moderately active
7	Rifampicin	Standard	0.20	Highly active
8	Ciprofloxacin	Standard	0.50	Highly active



In-vitro antitubercular activity of synthesized compounds III (a–f) against Mycobacterium tuberculosis H37Rv (ATCC27294) determined by Microplate Alamar Blue Assay (MABA)

RESULT AND DISCUSSION

The synthesized fluoroquinolone–thiadiazole hybrid compounds III (a–f) were evaluated for their in-vitro antitubercular activity against Mycobacterium tuberculosis H37Rv (ATCC 27294) using the Microplate Alamar Blue Assay (MABA). This assay provides a reliable estimation of bacterial viability based on metabolic reduction of the Alamar Blue dye, allowing accurate determination of the minimum inhibitory concentration (MIC). The MIC values obtained for the test compounds, along with those of standard antitubercular drugs, are summarized in Table X. Among the synthesized derivatives, compounds III (a) and III (c) exhibited the most promising antitubercular activity, with MIC values of 1.56 µg/mL and 3.12 µg/mL, respectively. The enhanced activity of compound III (a), bearing a nitro (–NO_2) substituent, can be attributed to the strong electron-withdrawing nature of the nitro group, which may enhance interactions with essential mycobacterial enzymes and improve intracellular penetration. Nitro-containing heterocycles are also known to undergo intracellular bioreductive activation within mycobacteria, leading to the generation of reactive intermediates that interfere with critical metabolic pathways. Similarly, compound III (c), containing a chloro (–Cl) substituent, demonstrated high activity, likely due to an optimal balance between lipophilicity and electronic effects, facilitating better permeation through the lipid-rich mycobacterial cell wall. Compounds III (b) and III (f), substituted with methyl (–CH_3) and methoxy (–F) groups, respectively, exhibited moderate antitubercular activity with MIC values of 12.5 µg/mL. These substituents increase hydrophobicity, which may aid in membrane penetration; however, the absence of strong electron-withdrawing characteristics could limit their binding affinity to intracellular targets. The moderate activity observed for these derivatives suggests that while lipophilicity contributes to antitubercular efficacy, it must be complemented by favorable electronic interactions for optimal activity. Compound III (d), containing a bromo (–Br) substituent, showed moderate activity with an MIC of 6.25 µg/mL. Although bromine is a halogen similar to chlorine, its larger atomic size may introduce steric hindrance, potentially reducing effective interaction with the target site compared to the chloro analogue. Nevertheless, the presence of a halogen substituent still contributed to reasonable activity, supporting the role of halogenation in enhancing antimycobacterial properties. In contrast, compound III (e) bearing a hydroxyl (–OH) group exhibited weak antitubercular activity with an MIC of 25 µg/mL. The reduced activity of this derivative may be attributed to increased Swami Vivekanand College of Pharmacy, Indore Page 43 Synthesis and Biological Evaluation of Some Thiadiazole Derivatives as Antitubercular Agents hydrophilicity and hydrogen-bonding capability, which can limit passive diffusion across the highly lipophilic mycobacterial cell envelope. This observation highlights the importance of

maintaining an appropriate balance between hydrophilicity and lipophilicity for effective antimycobacterial action. The standard drugs isoniazid, rifampicin, and moxifloxacin displayed MIC values of 0.10, 0.20, and 0.50 µg/mL, respectively, confirming the validity and reliability of the assay conditions. Although the synthesized compounds were less potent than the standard drugs, several derivatives, particularly III (a) and III (c), demonstrated noteworthy activity, indicating their potential as lead molecules for further structural optimization. Overall, the antitubercular evaluation revealed a clear structure–activity relationship (SAR), where electron-withdrawing and moderately lipophilic substituents significantly enhanced antimycobacterial activity, while strongly hydrophilic groups diminished efficacy. The incorporation of the thiadiazole moiety into the fluoroquinolone scaffold appears to contribute positively to antitubercular activity, possibly by enhancing target binding and overcoming resistance mechanisms. These findings suggest that further modification of substituents on the thiadiazole ring could lead to the development of more potent antitubercular agents with improved pharmacological profiles. Synthesis and Biological Evaluation of Some Thiadiazole Derivatives as Antitubercular Agents.

CONCLUSION:

The present study successfully designed, synthesized, and evaluated a series of fluoroquinolone thiadiazole hybrid derivatives (III a–f) with the objective of exploring their potential as novel antitubercular agents. The synthesized compounds were systematically screened for in-vitro antitubercular activity against Mycobacterium tuberculosis H37Rv (ATCC 27294) using the Microplate Alamar Blue Assay (MABA) under biosafety level-III conditions, enabling reliable determination of their minimum inhibitory concentrations (MICs). The antitubercular evaluation revealed that several of the synthesized compounds exhibited promising inhibitory activity. In particular, compounds III(a) and III(c) demonstrated the most potent activity, with MIC values of 1.56 µg/mL and 3.12 µg/mL, respectively, indicating the favorable influence of electron-withdrawing substituents such as nitro and chloro groups on antimycobacterial efficacy. Compounds containing moderately lipophilic substituents, including methyl, methoxy, and bromo groups, showed moderate antitubercular activity, while the hydroxyl substituted derivative exhibited comparatively weaker activity. These observations clearly highlight the role of substituent electronics and lipophilicity in governing antimycobacterial activity. The structure–activity relationship analysis suggested that incorporation of the 1,3,4-thiadiazole moiety into the fluoroquinolone scaffold enhances antitubercular potential, possibly by improving interactions with key mycobacterial targets and facilitating penetration through the lipid-rich mycobacterial cell wall. Although the synthesized compounds were less potent than standard drugs such as isoniazid, rifampicin, and moxifloxacin, their appreciable activity indicates that this hybridization strategy is a viable approach for lead optimization. In conclusion, the findings of this study establish fluoroquinolone–thiadiazole hybrids as promising lead candidates for further development as antitubercular agents. Future studies focusing on structural optimization, molecular docking, ADMET profiling, and in-vivo evaluation are warranted to enhance potency and safety, thereby contributing to the ongoing search for effective therapeutics against tuberculosis, particularly in the context of emerging drug resistance.

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