Conventional and Microwave-assisted Synthesis, Antitubercular Activity and Molecular Docking Studies of Pyrazole and Oxadiazole Hybrids

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Supporting Information

1. Biological assay

**Antimycobacterial assay**

“Microbial strains such as *M. tuberculosis* H37Ra (ATCC 25177) and *M. bovis* BCG (ATCC 35734) were obtained from AstraZeneca, India. The stock culture was maintained at -80 ºC and subcultured once in a liquid medium before inoculation into an experimental culture. Cultures were grown in Dubos media (enrichment media). For the antimycobacterial assay, *M. pheli* medium (minimal essential medium) was used. It contains 0.5 g KH₂PO₄, 0.25 g trisodium citrate, 60 mg MgSO₄, 0.5 g asparagine, and 2 mL glycerol in distilled water (100 mL) followed by pH adjustment to 6.6. All bacterial stock cultures were first grown in Dubos media at 37 ºC at 150 RPM. It takes at least 8-10 days for OD 1 at 620 nm. The antimycobacterial assay was performed in 96-well plates for active as well as dormant stages.
The titled compounds screened against *M. tuberculosis* H$_{37}$Ra (MTB) and *M. bovis* BCG (BCG) in active and dormant stage by established XTT Reduction Menadione Assay (XRMA) and Nitrate Reductase (NR) method respectively, both of the methods were developed earlier in our lab. Briefly, 0.1 OD$_{620}$ culture of MTB/ BCG was treated with synthesized compounds at three different concentrations (30, 10 and 3 µg/mL) and incubated for 8 (active) and 12 (dormant) days at 37 °C. The XRMA and NR were then carried out to estimate viable cells present in different wells of the assay plate. The optical density was read on a microplate reader (SpectraMax Plus 384 plate reader, Molecular Devices Inc.) at 470 nm filter for XTT and 540 nm filter for NR against a blank prepared from cell-free wells. Absorbance given by cells treated with the vehicle alone was taken as 100% cell growth. Initially, primary screening was done at 30, 10, and 3 µg/mL. Compounds showing 90% inhibition of bacilli at 30 µg/mL were selected for the further dose-response curve. MIC and IC$_{50}$ values of the selected compound were calculated from their dose-response curves by using Origin 6 software.”$^{1,2}$

2. Docking Results

![Figure S1. Binding mode of 4f into the active site of sterol mycobacterial enoyl reductase (InhA). (The π-π stacking interaction has been represented using green lines).](image)
**Figure S2.** Binding mode of 4g into the active site of mycobacterial enoyl reductase (InhA).
(The π-π stacking interaction has been represented using green lines).

**Figure S3.** Binding mode of 4p into the active site of mycobacterial enoyl reductase (InhA).
(The π-π stacking interaction has been represented using green lines).
Figure S4. Binding mode of 4s into the active site of mycobacterial enoyl reductase (InhA).
(The π-π stacking interaction has been represented using green lines).

3. Characteristic spectra

Figure S5. IR spectra of Compound 4c.
Figure S6. $^1$H NMR Spectrum of 4c.

Figure S7. $^{13}$C NMR Spectrum of 4c.
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Figure S8. Mass Spectrum of 4c.

Figure S9. $^1$H NMR Spectrum of 4e.
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Figure S10. $^{13}$C NMR Spectrum of 4e.

Figure S11. Mass Spectrum of 4e.
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Figure S12 $^1$H NMR Spectrum of 4f.

Figure S13 $^{13}$C NMR Spectrum of 4f.
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Figure S14. Mass Spectrum of 4f.

Figure S15. $^1$H NMR Spectrum of 4g.
Figure S16. $^{13}$C NMR Spectrum of 4g.

Figure S17. Mass Spectrum of 4g.
Figure S18. $^1$H NMR Spectrum of 4h.

Figure S19. $^{13}$C NMR Spectrum of 4h.
Figure S20. Mass Spectrum of 4h.

Figure S21. $^1$H NMR Spectrum of 4i.
Figure S22. $^{13}$C NMR Spectrum of 4i.
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Figure S23. Mass Spectrum of 4i.

Figure S24. IR spectra of Compound 4j.
Figure S25. $^1$H NMR Spectrum of 4j.

Figure S26. $^{13}$C NMR Spectrum of 4j.
Figure S27. Mass Spectrum of 4j.

Figure S28. $^1$H NMR Spectrum of 4p.
Figure S29. $^{13}$C NMR Spectrum of 4p.

Figure S30. Mass Spectrum of 4p.
Figure S31. $^1$H NMR Spectrum of 4q.

Figure S32. $^{13}$C NMR Spectrum of 4q.
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Figure S33. Mass Spectrum of 4q.

Figure S34. $^1$H NMR Spectrum of 4s.
Figure S35. $^{13}$C NMR Spectrum of 4s.

Figure S36. Mass Spectrum of 4s.
References:

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2. Tambe, M. S.; Choudhari, A.; Sarkar, D.; Sangshetti, J.; Patil, R.; Gholap, S. S. Design, Synthesis and Biological Screening of Novel 1,3,4-Oxadiazoles as Antitubercular Agents, *ChemistrySelect* **2018**, *3*, 13304-13310.