Recent studies showed that 1,4-dihydropyridine-3,5-dicarbamoyl derivatives with lipophilic groups have significant antitubercular activity. In this study, we have synthesized new derivatives of 1,4-dihydropyridines bearing carbethoxy and carboxy group at C-3 and C-5 of the 1,4-dihydropyridine ring. In addition, 1H-pyrazole ring is substituted at C-4 position. These analogues were synthesized by multi-component Hantzsch reaction. The in vitro antitubercular activity of compounds against Mycobacterium tuberculosis H37Rv was evaluated. The lowest minimum inhibitory concentration value, 0.02 μg/mL and SI > 500, was found for dimethyl 1,4-dihydro-4-(3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)-2,6-dimethylpyridine-3,5-dicarboxylate 3f, diethyl 1,4-dihydro-4-(3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-2,6-dimethylpyridine-3,5-dicarboxylate 4c and diethyl 1,4-dihydro-4-(3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl)-2,6-dimethyl pyridine-3,5-dicarboxylate 4e, making them more potent than first-line antitubercular drug isoniazid. In addition, these compounds exhibited relatively low cytotoxicity.

Key words: 1,4-dihydropyridines, antimycobacterial activity, Mycobacterium tuberculosis, tuberculosis

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carbethoxy group at C-3 and C-5 of the DHP ring, respectively. It seems that such replacements could effectively overcome the resistent isolates, which have been attributed to a deficiency of amidase or esterase. In addition, pyrazole moiety is substituted at C-4 position of dihydropyridine ring. The antimycobacterial activity of synthesized compounds was evaluated against \textit{M. tuberculosis} H$_{37}$Rv (MTB).

**Methods and Materials**

All of the synthesized compounds were chemically characterized by thin-layer chromatography (TLC), infrared (IR), proton nuclear magnetic resonance ($^1$H NMR) and elemental microanalyses (CHN). Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were recorded on a Bruker AC 200, DPX 300 and ARX 500, at 25 °C. Infrared spectra were recorded on SHIMADZU-FT-IR-8400 using KBr pellets. Elemental analyses of the newly synthesized compounds were carried out on Carlo Erba 1108 analyzer (Carlo Erba, Milan, Italy), and the data were within range of the theoretical values.

**Synthesis of 3-(aryl)-1-phenyl-1H-pyrazole-4-carbaldehydes (1)**

Synthesis of 3-(aryl)-1-phenyl-1H-pyrazole-4-carbaldehydes was achieved by reported method (17).

**General procedure for the synthesis of 1,4-dihydropyridines (3a–h to 4a–h)**

To a stirred solution of the methyl acetoacetate/ethyl acetoacetate (0.02 mole, 2 eq) and an appropriate 3-(aryl)-1-phenyl-1H-pyrazole-4-carbaldehyde (0.01 mole, 1 eq) in methanol (20 mL), ammonia (1 mL) was added dropwise. The reaction mixture was allowed to stand overnight, and the solid product that separated was isolated. The product was washed with 15 mL of cold diethyl ether. Finally, it was purified by silica gel (60–100 mesh) column chromatography using ethyl acetate and hexane (2:3) as the eluents.

**Dimethyl 1,4-dihydro-4-(3-(4-hydroxyphenyl)-1H-pyrazol-4-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (3b)**

Yield: 69%, mp = 163–165 °C; IR (KBr): 3319, 3015, 2924, 1690, 1590/cm. $^1$H NMR (DMSO-$d_6$): $\delta$ (ppm) 7.35–7.92 (m, 10H, Ar-H), 5.25 (s, 1H, CH), 5.68 (s, 1H, NH), 3.82 (s, 6H, 2-OCH$_3$), 2.01 (s, 6H, 2-CH$_3$). $^{13}$C NMR (DMSO-$d_6$): $\delta$ (ppm) 167.4, 150.4, 149.9, 137.5, 131.6, 129.7, 128.7, 127.5, 122.9, 121.4, 117.8, 116.8, 104.3, 52.2, 35.8, 16.5. Anal. Calcd. for C$_{26}$H$_{25}$N$_3$O$_4$: C, 67.87; H, 5.48; N, 9.14%. Found: C, 67.88; H, 5.39; N, 9.17.

**Dimethyl 1,4-dihydro-4-(3-(4-fluorophenyl)-1H-pyrazol-4-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (3c)**

Yield: 54%, mp = 167–169 °C; IR (KBr): 3314, 3015, 2925, 1693, 1595/cm. $^1$H NMR (DMSO-$d_6$): $\delta$ (ppm) 7.03–7.92 (m, 10H, Ar-H), 5.25 (s, 1H, CH), 5.68 (s, 1H, NH), 3.82 (s, 6H, 2-OCH$_3$), 2.01 (s, 6H, 2-CH$_3$). $^{13}$C NMR (DMSO-$d_6$): $\delta$ (ppm) 167.7, 161.3, 150.3, 149.5, 136.2, 133.5, 129.3, 124.8, 127.5, 122.8, 121.2, 117.8, 116.6, 104.3, 52.2, 35.7, 16.4. Anal. Calcd. for C$_{26}$H$_{25}$FN$_3$O$_4$: C, 67.67; H, 5.24; N, 9.11%. Found: C, 67.59; H, 5.15; N, 9.02.

**Dimethyl 1,4-dihydro-4-(3-(4-bromophenyl)-1H-pyrazol-4-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (3d)**

Yield: 65%, mp = 151–153 °C; IR (KBr): 3320, 3013, 2927, 1693, 1596/cm. $^1$H NMR (DMSO-$d_6$): $\delta$ (ppm) 7.15–7.94 (m, 10H, Ar-H), 5.28 (s, 1H, CH), 5.65 (s, 1H, NH), 3.84 (s, 6H, 2-OCH$_3$), 2.01 (s, 6H, 2-CH$_3$). $^{13}$C NMR (DMSO-$d_6$): $\delta$ (ppm) 167.4, 150.4, 149.6, 137.5, 134.1, 131.6, 129.7, 128.7, 127.5, 122.9, 121.4, 117.8, 116.4, 104.3, 52.2, 35.8, 16.4. Anal. Calcd. for C$_{26}$H$_{25}$BrN$_3$O$_4$: C, 59.78; H, 4.95; N, 8.04%. Found: C, 59.67; H, 4.95; N, 7.92.

**Dimethyl 1,4-dihydro-4-(3-(4-chlorophenyl)-1H-pyrazol-4-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (3e)**

Yield: 70%, mp = 126–128 °C; IR (KBr): 3319, 3020, 2932, 1695, 1596/cm. $^1$H NMR (DMSO-$d_6$): $\delta$ (ppm) 7.08–7.89 (m, 10H, Ar-H), 5.23 (s, 1H, CH), 5.68 (s, 1H, NH), 3.82 (s, 6H, 2-OCH$_3$), 2.02 (s, 6H, 2-CH$_3$). $^{13}$C NMR (DMSO-$d_6$): $\delta$ (ppm) 167.6, 150.5, 149.6, 138.8, 133.8, 132.5, 129.5, 128.7, 127.4, 123.1, 122.5, 121.1, 117.8, 104.3, 52.3, 35.7, 16.4. Anal. Calcd. for C$_{26}$H$_{25}$ClN$_3$O$_4$: C, 59.78; H, 4.95; N, 8.04%. Found: C, 59.67; H, 4.54; N, 7.92.

**Dimethyl 1,4-dihydro-4-(3-(4-nitrophenyl)-1H-pyrazol-4-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (3f)**

Yield: 58%, mp = 88–90 °C; IR (KBr): 3325, 3018, 2928, 1695, 1590/cm. $^1$H NMR (DMSO-$d_6$): $\delta$ (ppm) 7.36–8.14 (m, 10H, Ar-H), 5.26 (s, 1H, CH), 5.61 (s, 1H, NH), 3.81 (s, 6H, 2-OCH$_3$), 2.04 (s, 6H, 2-CH$_3$). $^{13}$C NMR (DMSO-$d_6$): $\delta$ (ppm) 167.6, 150.5, 149.6, 146.1, 145.6, 133.6, 129.5, 128.7, 127.4, 123.1, 121.1, 117.7, 104.5, 52.3, 35.6, 16.2. Anal. Calcd. for C$_{26}$H$_{25}$NO$_7$: C, 63.93; H, 4.95; N, 11.47%. Found: C, 63.84; H, 4.83; N, 11.40.

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Diethyl 1,4-dihydro-4-(3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (4d)

Yield: 54%, mp = 128–130 °C; IR (KBr): 3325, 3013, 2922, 2854, 1698, 1595/cm. 1H NMR (DMSO-d6): δ (ppm) 17.16–17.26 (m, 11H, Ar-H), 5.87 (s, 1H, NH), 5.64 (s, 1H, CH), 3.96–4.06 (q, 2H, CH2CH3), 3.83–3.91 (q, 2H, CH2CH3), 2.34 (s, 6H, 2-CH3), 2.04 (s, 6H, 2-CH2). 13C NMR (DMSO-d6): δ (ppm) 167.3, 150.8, 150.1, 147.0, 140.1, 132.4, 130.1, 129.6, 126.2, 123.7, 123.2, 120.4, 117.2, 102.5, 62.0, 35.8, 16.5, 14.4. Anal. Calcld. for C29H29BrN2O6: C, 61.10; H, 5.13; N, 7.63%. Found: C, 60.97; H, 5.04; N, 7.56.

Diethyl 1,4-dihydro-4-(3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (4f)

Yield: 64%, mp = 101–103 °C; IR (KBr): 3316, 3015, 2930, 1690, 1590/cm. 1H NMR (DMSO-d6): δ (ppm) 7.35–8.19 (m, 11H, Ar-H), 5.90 (s, 1H, NH), 5.64 (s, 1H, CH), 4.03–4.14 (q, 2H, CH2CH3), 3.84–3.98 (q, 2H, CH2CH3), 2.26 (s, 6H, 2-CH2). 13C NMR (DMSO-d6): δ (ppm) 167.2, 151.3, 150.6, 148.7, 140.5, 139.3, 129.3, 128.6, 126.4, 123.4, 121.8, 120.4, 117.4, 102.5, 61.9, 35.7, 16.8, 14.3. Anal. Calcld. for C29H29N2O6: C, 65.11; H, 5.46; N, 10.85%. Found: C, 65.01; H, 5.35; N, 10.78.

Diethyl 1,4-dihydro-2,6-dimethyl-4-(1,3-diphenyl-1H-pyrazol-4-yl)pyridine-3,5-dicarboxylate (4a)

Yield: 37%, mp = 195–197 °C; IR (KBr): 3324, 3013, 2928, 2655, 1686, 1595/cm. 1H NMR (DMSO-d6): δ (ppm) 7.25–8.10 (m, 11H, Ar-H), 5.88 (s, 1H, NH), 5.62 (s, 1H, CH), 3.96–4.05 (q, 2H, CH2CH3), 3.77–3.84 (q, 2H, CH2CH3), 2.23 (s, 6H, 2-CH2). 13C NMR (DMSO-d6): δ (ppm) 167.5, 150.6, 150.1, 139.9, 133.1, 130.1, 129.8, 128.5, 127.6, 126.5, 123.3, 120.5, 117.4, 102.7, 62.1, 35.6, 16.5, 14.3. Anal. Calcld. for C28H28ClN3O4: C, 71.32; H, 6.20; N, 8.91%. Found: C, 71.23; H, 6.08; N, 8.82.

Diethyl 1,4-dihydro-4-(3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (4c)

Yield: 65%, mp = 109–111 °C; IR (KBr): 3325, 3013, 2928, 2856, 1695, 1595/cm. 1H NMR (DMSO-d6): δ (ppm) 7.05–7.89 (m, 11H, Ar-H), 5.86 (s, 1H, NH), 5.68 (s, 1H, CH), 3.95–4.06 (q, 2H, CH2CH3), 3.79–3.86 (q, 2H, CH2CH3), 2.26 (s, 6H, 2-CH2). 13C NMR (DMSO-d6): δ (ppm) 167.3, 163.2, 150.9, 150.4, 139.9, 129.9, 129.5, 128.3, 126.4, 120.6, 123.3, 117.4, 116.8, 102.3, 61.9, 35.4, 16.4, 14.3. Anal. Calcld. for C29H29F3N2O6: C, 71.73; H, 6.43; N, 8.65%. Found: C, 71.65; H, 6.45; N, 8.53.
Diethyl 1,4-dihydro-4-(3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (4h)

Yield: 52%, mp = 97–99 °C; IR (KBr): 3320, 3012, 2926, 1695, 1597 cm⁻¹. ¹H NMR (DMSO-d₆): δ (ppm) 7.16–7.95 (m, 11H, Ar-H), 5.88 (s, 1H, NH), 5.67 (s, 1H, CH), 3.98–4.07 (q, 2H, CH₂CH₃), 3.86–3.94 (q, 2H, CH₂CH₃), 3.80 (s, 3H, OCH₃), 2.29 (s, 6H, 2-CH₃), 1.05–1.13 (t, 6H, 2-CH₃). ¹³C NMR (DMSO-d₆): δ (ppm) 167.2, 159.9, 151.1, 150.6, 140.2, 129.7, 129.1, 126.5, 125.5, 123.1, 120.1, 117.4, 114.8, 102.3, 57.1, 62.1, 35.7, 14.3. Anal. Calcd. for C₂₉H₃₁N₃O₅: C, 69.44; H, 6.23; N, 8.38%. Found: C, 69.37; H, 6.14; N, 8.27.

Results and Discussion

Chemistry

Various 3-(aryl)-1-phenyl-1H-pyrazole-4-carbaldehydes (1a–h) bearing a range of electron-withdrawing and electron-releasing substituents, viz., 4-OH, 4-F, 4-Cl, 4-Br, 4-NO₂, 4-CH₃, 4-OCH₃, were prepared according to the previously reported procedure (17). All the symmetrical 1,4-DHPs (3a–h to 4a–h) were synthesized by the multi-component Hantzsch reaction involving 3-(aryl)-1-phenyl-1H-pyrazole-4-carbaldehydes 1, ethyl/methyl acetooacetate 2 and ammonia (Scheme 1). Designed series of molecules (3a–h to 4a–h) were characterized by ¹H NMR, ¹³C NMR, and Mass spectrometry techniques and their purity by elemental analysis. The ¹H NMR spectra of DHPs 3a–h to 4a–h have the typical singlet of methine group lying in the region 5.22–5.68 ppm and multiplet of aromatic part of molecules occurring in region between 6.93 and 8.19 ppm. The ¹³C NMR signal of methine group can be observed at 35.3–35.8 ppm. IR spectra of DHP derivatives were also in agreement with the structures (Figures S1–S3).

Determination of 50% inhibitory concentrations (IC₅₀) in VERO cells

Concurrent with the determination of minimum inhibitory concentrations (MIC’s), compounds were tested for cytotoxicity (IC₅₀) in VERO cells at concentrations less than or equal to 62.5 μg/mL or 10 times the MIC for M. tuberculosis H₃⁷Rv. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 Non-radioassay (Promega, Madison, WI, USA). The selectivity index (SI = IC₅₀/MIC) was also determined; it was considered significant when SI > 10.

Scheme 1: Synthesis of 1,4-dihydropyridines 3a–h to 4a–h.
interesting compounds, and a MIC ≤1 μg/mL in a novel compound class is considered an excellent lead (19), which makes 1,4-dihydropyridines 3f, 4c and 4e very promising antimycobacterial compounds. Further in vitro studies of compounds 3f, 4c and 4e as well as synthesis of analogues of these lead compounds are currently in progress.

**Conclusion**

Comparison of antimycobacterial activities of tested compounds (3a–h to 4a–h) indicated that DHPs 3f, 4c and 4e were the most potent compounds with MIC of 0.02 μg/mL and SI >500. Compound 3f with 4-nitro group at the 3-aryl substituent on pyrazole nucleus along with carboxethoxy group at C-3 and C-5 position of 1,4-dihydropyridine ring was the most potent one among DHPs 3a–h, while compounds 4c and 4e with 4-fluoro and 4-bromo groups, respectively, at the 3-aryl substituent on pyrazole nucleus along with carboxethoxy group at C-3 and C-5 position of 1,4-dihydropyridine ring were the most potent ones among DHPs 4a–h. Therefore, these compounds provide excellent leads for further developments as novel antitubercular molecules.

**Acknowledgments**

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**References**


**Table 1: In vitro antitubercular screening data of dihydropyridines 3a–h to 4a–h**

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<th>Sr. no.</th>
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<th>R₂</th>
<th>% Inhibition</th>
<th>MIC&lt;sup&gt;a&lt;/sup&gt; µg/mL</th>
<th>IC₅₀&lt;sup&gt;b&lt;/sup&gt; VERO cells</th>
<th>SI&lt;sup&gt;c&lt;/sup&gt; (SI = IC₅₀/MIC)</th>
<th>mi Log P&lt;sup&gt;d&lt;/sup&gt;</th>
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n.d., not determined.

<sup>a</sup>Minimum inhibitory concentration against H₃7Rv strain of <i>M. tuberculosis</i> (µg/mL).

<sup>b</sup>Measurement of cytotoxicity in VERO cells: 50% inhibitory concentrations (µg/mL).

<sup>c</sup>Selectivity index (in vitro): IC₅₀ in VERO cells/MIC against <i>M. tuberculosis</i>

<sup>d</sup>mi Log P: Molinspiration Cheminformatics (http://www.molinspiration.com) calculated Log P using online Molinspiration Property Engine v2009.01.
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Note


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Mass spectrum of compound 4e.

Figure S2. 1H NMR (300MHz) spectrum of compound 4e.

Figure S3. 13C NMR (300MHz) spectrum of compound 4e.

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