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Synthesis and Biological Evaluation of Some Novel 1,4-Dihydropyridines as Potential AntiTubercular Agents

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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 carbmethoxy and carbethoxy group at C-3 and C-5 of the 1,4-dihydropyridine ring. In addition, 1*H*-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* H_{37}Rv 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-1*H*-pyrazol-4-yl)-2,6-dimethylpyridine-3,5-dicarboxylate 3f, diethyl 1,4-dihydro-4-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-2,6-dimethylpyridine-3,5-dicarbo-xylate 4c and diethyl 1,4-dihydro-4-(3-(4-bromophenyl)-1-phenyl-1*H*-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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Tuberculosis (TB) is a global epidemic caused by various strains of mycobacterium, usually *Mycobacterium tuberculosis*. Tuberculosis has been considered to be a disease of poverty for many years with quite rare occurrence in the developed countries. Unfortunately, recently, more people in the developed world are contracting tuberculosis, because their immune systems are compromised by immunosuppressive drugs, substance abuse or AIDS. Several decades ago, effective anti-TB drugs have been launched, and one could hardly find a TB case to be demonstrated at the medicinal universities. But TB stroke back! (1) The return of tuberculosis was declared by World Health Organization (WHO) as a global emergency compared with a hypothetical third world war with 9 million new TB cases and two million deaths reported each year (2,3); about one-third of the world's population is already infected with *M. tuberculosis* ([a]). The current frontline therapy for treatment of tuberculosis requires a cocktail of three or more drugs such as isoniazide, rifampin, ethambutol and pyrazinamide over an extended period of time (4). Furthermore, in recent times, the occurrence of multidrug-resistant TB (MDR–TB), a form of TB that does not respond to the standard treatments, is more common. It is a shocking revelation that MDR–TB is present in almost all countries as per the recent survey, made by the World Health Organization (WHO) and its partners.

Despite the efforts of agencies such as WHO, the Global Fund for HIV, TB, and Malaria, and the Gates Foundation who have galvanized multilateral support and initiated public–private partnerships to increase resources to combat this disease of poverty, only seven candidate TB drugs from five different clinical classes were undergoing clinical trials till very recently, i.e. fluoroquinolones (gatifloxacin and moxifloxacin), diarylquinoline (TMC207), nitroimidazoles (OPC67683 and PA824), pyrrole (LL3858) and ethylenadiamine (SQ109) (5). All aforementioned facts underscore the importance of the development of new drugs with unique and divergent structure and with a novel mechanism of action for efficacious clinical control of patients with TB using ordinary antimycobacterial drugs.

Recently, studies showed that 3,5-dicarbamoyl derivatives of 1,4-dihydropyridine (DHP) with lipophilic groups have considerable antitubercular activity against *M. tuberculosis* H_{37}Rv (6). It was also observed that esters or substituted isosters of pyridine and pyrazine carboxylic acids (such as tetrazoles) have been more active than the parent acids, especially against resistant strains. These esters are presumably activated by an esterase to parent acid (7–10). Indeed, esters of pyrazinoic acids have been shown to possess activity against pyrazinamide-resistant isolates, which has been attributed to a deficiency of nicotinamidase (7–10). In addition, pyrazoles exhibited significant antitubercular activity (11). Recognizing these facts and in continuation of our work on antitubercular agents (12–16), it appeared of interest to design and synthesize new derivatives of 1,4-dihydropyridines bearing carbmethoxy and

carbethoxy group at C-3 and C-5 of the DHP ring, respectively. It seems that such replacements could effectively overcome the resistant 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 *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 located by iodine. ^{1}H NMR was determined in DMSO-d_6 solution on a Bruker DPX 300 MHz spectrometer (Bruker India, Mumbai, India). ^{13}C NMR (75 and 125 MHz) spectra were registered on a Bruker AC 200, DPX 300 and ARX 500, at 25 °C, in DMSO-d_6. 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-1*H*-pyrazole-4-carbaldehydes (1)*

Synthesis of 3-(aryl)-1-phenyl-1*H*-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-1*H*-pyrazole-4-carbaldehyde (0.01 mole, 1 eq) in methanol (20 mL), ammonia (1 mL) was added dropwise. The reaction mixture was allowed to reflux for 18–20 h. After completion of the reaction, as indicated by TLC (ethyl acetate:hexane 2:3), 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-2,6-dimethyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)pyridine-3,5-dicarboxylate (3a)*

Yield: 64%, mp = 177–179 °C; IR (KBr): 3315, 3010, 2929, 1695, 1596/cm. ^{1}H NMR (DMSO-d_6): δ (ppm) 7.35–7.82 (m, 11H, Ar-H), 5.24 (s, 1H, CH), 5.62 (s, 1H, NH), 3.80 (s, 6H, 2-OCH_3), 2.00 (s, 6H, 2-CH_3). ^{13}C NMR (DMSO-d_6): δ (ppm) 167.4, 149.6, 150.3, 140.2, 133.5, 129.6, 129.8, 128.4, 127.8, 126.6, 122.9, 120.4, 117.5, 104.5, 52.7, 35.7, 16.5. Anal. Calcd. for $C_{26}H_{25}N_3O_4$: C, 70.41; H, 5.68; N, 9.47%. Found: C, 70.32; H, 5.56; N, 9.38.

*Dimethyl 1,4-dihydro-4-(3-(4-hydroxyphenyl)-1-phenyl-1*H*-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): δ (ppm) 6.93–7.79 (m, 10H, Ar-H), 5.27 (s, 1H, CH), 5.67 (s, 1H, NH), 4.73 (s, 1H, OH), 3.84 (s, 6H, 2-OCH_3), 2.03 (s, 6H, 2-CH_3). ^{13}C NMR (DMSO-d_6): δ (ppm) 167.4, 156.7, 150.2, 149.8, 132.5, 133.3, 129.5, 128.4, 127.8, 121.4, 123.0, 117.7, 116.8, 104.4, 52.5, 35.6, 16.3. Anal. Calcd. for $C_{26}H_{25}N_3O_5$: C, 67.96; H, 5.48; N, 9.14%. Found: C, 67.88; H, 5.39; N, 9.17.

*Dimethyl 1,4-dihydro-4-(3-(4-fluorophenyl)-1-phenyl-1*H*-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): δ (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), 2.02 (s, 6H, 2-CH_3). ^{13}C NMR (DMSO-d_6): δ (ppm) 167.7, 161.3, 150.3, 149.5, 136.2, 133.5, 129.3, 128.4, 127.5, 122.8, 121.2, 117.8, 116.6, 104.3, 52.3, 35.7, 16.4. Anal. Calcd. for $C_{26}H_{24}FN_3O_4$: 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-chlorophenyl)-1-phenyl-1*H*-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): δ (ppm) 7.15–7.84 (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): δ (ppm) 167.4, 150.4, 149.6, 137.5, 134.1, 131.6, 129.7, 129.4, 128.7, 127.5, 122.9, 121.4, 117.8, 104.3, 52.2, 35.8, 16.5. Anal. Calcd. for $C_{26}H_{24}ClN_3O_4$: C, 65.34; H, 5.06; N, 8.79%. Found: C, 65.27; H, 4.95; N, 8.69.

*Dimethyl 1,4-dihydro-4-(3-(4-bromophenyl)-1-phenyl-1*H*-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): δ (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): δ (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 $C_{26}H_{24}BrN_3O_4$: C, 59.78; H, 4.63; N, 8.04%. Found: C, 59.67; H, 4.54; N, 7.92.

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

Yield: 59%, mp = 88–90 °C; IR (KBr): 3325, 3018, 2928, 1695, 1590/cm. ^{1}H NMR (DMSO-d_6): δ (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): δ (ppm) 167.6, 150.5, 149.6, 146.1, 145.6, 133.6, 129.5, 128.7, 127.4, 123.1, 121.6, 121.1, 117.7, 104.5, 52.3, 35.6, 16.2. Anal. Calcd. for $C_{26}H_{24}N_4O_6$: C, 63.93; H, 4.95; N, 11.47%. Found: C, 63.84; H, 4.83; N, 11.40.

Dimethyl 1,4-dihydro-4-(3-(4-methylphenyl)-1-phenyl-1H-pyrazol-4-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (3g)

Yield: 64%, mp = 145–147 °C; IR (KBr): 3322, 3015, 2928, 2920, 1694, 1595/cm. ^{1}H NMR (DMSO-d_6): δ (ppm) 7.23–7.82 (m, 10H, Ar-H), 5.22 (s, 1H, CH), 5.64 (s, 1H, NH), 3.81 (s, 6H, 2-OCH_3), 2.35 (s, 3H, CH_3), 2.01 (s, 6H, 2-CH_3). ^{13}C NMR (DMSO-d_6): δ (ppm) 167.5, 150.5, 149.6, 137.1, 136.2, 133.4, 129.5, 129.1, 128.7, 127.5, 122.9, 120.8, 117.7, 104.4, 52.3, 35.6, 24.7, 16.1. Anal. Calcd. for $C_{27}H_{27}N_3O_4$: C, 70.88; H, 5.95; N, 9.18%. Found: C, 70.77; H, 5.88; N, 9.10.

Dimethyl 1,4-dihydro-4-(3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (3h)

Yield: 58%, mp = 132–134 °C; IR (KBr): 3315, 3015, 2928, 1690, 1595/cm. ^{1}H NMR (DMSO-d_6): δ (ppm) 7.05–7.93 (m, 10H, Ar-H), 5.26 (s, 1H, CH), 5.68 (s, 1H, NH), 3.91 (s, 3H, OCH_3), 3.84 (s, 6H, 2-OCH_3), 2.34 (s, 3H, CH_3), 2.04 (s, 6H, 2-CH_3). ^{13}C NMR (DMSO-d_6): δ (ppm) 167.7, 159.1, 150.4, 149.6, 133.3, 132.5, 129.6, 128.7, 127.4, 123.0, 121.5, 117.7, 115.1, 104.3, 55.5, 52.4, 35.5, 16.3. Anal. Calcd. for $C_{27}H_{27}N_3O_5$: C, 68.48; H, 5.75; N, 8.87%. Found: C, 68.39; H, 5.77; N, 8.76.

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, 2855, 1696, 1595/cm. ^{1}H NMR (DMSO-d_6): 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, CH_2CH_3), 3.77–3.84 (q, 2H, CH_2CH_3), 2.23 (s, 6H, 2-CH_3), 1.05–1.14 (t, 6H, 2-CH_3). ^{13}C NMR (DMSO-d_6): 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. Calcd. for $C_{28}H_{29}N_3O_4$: 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-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (4b)

Yield: 61%, mp = 161–163 °C; IR (KBr): 3325, 3013, 2922, 2852, 1696, 1595/cm. ^{1}H NMR (DMSO-d_6): δ (ppm) 7.03–7.94 (m, 11H, Ar-H), 5.89 (s, 1H, NH), 5.65 (s, 1H, CH), 4.72 (s, 1H, OH), 4.02–4.12 (q, 2H, CH_2CH_3), 3.83-3.91 (q, 2H, CH_2CH_3), 2.32 (s, 6H, 2-CH_3), 1.11-1.16 (t, 6H, 2-CH_3). ^{13}C NMR (DMSO-d_6): δ (ppm) 167.3, 158.8, 150.8, 150.4, 140.1, 129.7, 128.8, 126.5, 125.4, 123.3, 117.4, 116.9, 102.3, 61.9, 35.3, 16.6, 14.2. Anal. Calcd. for $C_{28}H_{29}N_3O_5$: C, 68.98; H, 6.00; N, 8.62%. Found: C, 68.86; H, 5.89; N, 8.53.

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. ^{1}H NMR (DMSO-d_6): 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, CH_2CH_3), 3.79–3.86 (q, 2H, CH_2CH_3), 2.26 (s, 6H, 2-CH_3), 1.03-1.11 (t, 6H, 2-CH_3). ^{13}C NMR (DMSO-d_6): 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. Calcd. for $C_{28}H_{28}FN_3O_4$: C, 68.70; H, 5.77; N, 8.58%. Found: C, 68.62; H, 5.68; N, 8.49.

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

Yield: 54%, mp = 128–130 °C; IR (KBr): 3325, 3013, 2932, 2854, 1698, 1595/cm. ^{1}H NMR (DMSO-d_6): δ (ppm) 7.27-7.96 (m, 11H, Ar-H), 5.87 (s, 1H, NH), 5.64 (s, 1H, CH), 3.96–4.06 (q, 2H, CH_2CH_3), 3.83-3.91 (q, 2H, CH_2CH_3), 2.34 (s, 6H, 2-CH_3), 1.11-1.19 (t, 6H, 2-CH_3). ^{13}C NMR (DMSO-d_6): δ (ppm) 167.3, 150.8, 150.1, 139.7, 135.1, 131.4, 129.4, 129.6, 128.7, 126.5, 123.3, 120.1, 117.4, 102.7, 62.1, 35.6, 16.5, 14.3. Anal. Calcd. for $C_{28}H_{28}ClN_3O_4$: C, 66.46; H, 5.58; N, 8.30%. Found: C, 66.37; H, 5.50; N, 8.18.

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

Yield: 59%, mp = 153–155 °C; IR (KBr): 3325, 3015, 2928, 2860, 1698, 1596/cm. ^{1}H NMR (DMSO-d_6) δ ppm: 7.32–8.14 (m, 11H, Ar-H), 5.89 (s, 1H, NH), 5.66 (s, 1H, CH), 4.05-4.11 (q, 2H, CH_2CH_3), 3.88–3.95 (q, 2H, CH_2CH_3), 2.30 (s, 6H, 2-CH_3), 1.12-1.21 (t, 6H, 2-CH_3). ^{13}C NMR (DMSO-d_6): δ (ppm) 167.3, 150.9, 150.5, 140.7, 140.1, 132.3, 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. Calcd. for $C_{28}H_{28}BrN_3O_4$: 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. ^{1}H NMR (DMSO-d_6): 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, CH_2CH_3), 3.84–3.98 (q, 2H, CH_2CH_3), 2.26 (s, 6H, 2-CH_3), 1.08–1.19 (t, 6H, 2-CH_3). ^{13}C NMR (DMSO-d_6): 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. Calcd. for $C_{28}H_{28}N_4O_6$: 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-phenyl-3-p-tolyl-1H-pyrazol-4-yl)pyridine-3,5-dicarboxylate (4g)

Yield: 70%, mp = 145–147 °C; IR (KBr): 3316, 3015, 2930, 1690, 1590/cm. ^{1}H NMR (DMSO-d_6): δ (ppm) 7.16–7.95 (m, 11H, Ar-H), 5.86 (s, 1H, NH), 5.67 (s, 1H, CH), 3.98–4.07 (q, 2H, CH_2CH_3), 3.86–3.94 (q, 2H, CH_2CH_3), 2.26 (s, 6H, 2-CH_3), 2.35 (s, 3H, CH_3), 1.11–1.20 (t, 6H, 2-CH_3). ^{13}C NMR (DMSO-d_6): δ (ppm) 167.2, 151.3, 150.6, 140.5, 138.3, 130.5, 129.6, 129.3, 127.6, 126.2, 123.4, 120.1, 117.4, 102.5, 62.1, 35.6, 16.8, 24.9, 14.3. Anal. Calcd. for $C_{29}H_{31}N_3O_4$: 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}H NMR (DMSO-d_6): 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, CH_2CH_3), 3.86-3.94 (q, 2H, CH_2CH_3), 3.80 (s, 3H, OCH_3), 2.29 (s, 6H, 2-CH_3), 1.05-1.13 (t, 6H, 2-CH_3). ^{13}C NMR (DMSO-d_6): 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, 16.8, 14.3. Anal. Calcd. for $C_{29}H_{31}N_3O_5$: C, 69.44; H, 6.23; N, 8.38%. Found: C, 69.37; H, 6.14; N, 8.27.

Determination of 50% inhibitory concentrations (IC_{50}) in VERO cells

Concurrent with the determination of minimum inhibitory concentrations (MIC's), compounds were tested for cytotoxicity (IC_{50}) in VERO cells at concentrations less than or equal to 62.5 μg/mL or 10 times the MIC for *M. tuberculosis* H_{37}Rv. 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-radioactive Cell Proliferation Assay (Promega, Madison, WI, USA). The selectivity index (SI = IC_{50}/MIC) was also determined; it was considered significant when SI > 10.

Results and Discussion

Chemistry

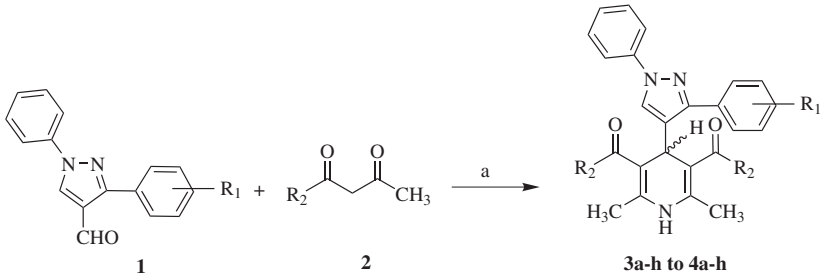
Various 3-(aryl)-1-phenyl-1*H*-pyrazole-4-carbaldehydes (**1a**–**h**) bearing a range of electron-withdrawing and electron-releasing substituents, *viz.*, 4-OH, 4-F; 4-Cl; 4-Br; 4-NO_2; 4-CH_3; 4-OCH_3, were prepared according to the previously reported procedure (17). All the symmetrical 1,4-DHPs (**3a**–**h** to **4a**–**h**) were synthesized by the multi-component Hantzsch reaction involving 3-(aryl)-1-phenyl-1*H*-pyrazole-4-carbaldehydes **1**, ethyl/methyl acetoacetate **2** and ammonia (Scheme 1). Designed series of molecules (**3a**–**h** to **4a**–**h**) were characterized by ^{1}H NMR, ^{13}C NMR, and Mass spectrometry techniques and their purity by elemental analysis. The ^{1}H 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 ^{13}C 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).

Antitubercular activity

All compounds were initially screened for their *in vitro* antimycobacterial activity at 6.25 μg/mL against MTB H_{37}Rv strain by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) in BACTEC 12B medium using the Microplate Alamar Blue Assay (18). Compounds exhibiting ≥90% inhibition in the initial screen were retested at and below 6.25 μg/mL using twofold dilution to determine the actual MIC (Table 1).

In the preliminary screening, six compounds (**3f**, **4a**–**c, 4e** and **4f**) inhibited MTB with 90–100%. In the secondary level, three compounds (**3f**, **4c** and **4e**) inhibited MTB with MIC of <1 μg/mL and two compounds (**4a** and **4b**) with MIC of <2 μg/mL. When compared to isoniazid (MIC: 0.03 μg/mL), three compounds, dimethyl 1,4-dihydro-2,6-dimethyl-4-(3-(4-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl) pyridine-3,5-dicarboxylate **3f**, diethyl 4-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate **4c** and diethyl 4-(3-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate **4e,** were found to be the most active compounds *in vitro* with MIC of 0.02 μg/mL against MTB and were more potent than isoniazid. The preliminary antimycobacterial evaluation results showed that DHP derivatives bearing alkyl ester group at C-3 and C-5 position along with electron-withdrawing groups at the 4th position of 3-aryl substituents on pyrazole nucleus have exhibited comparatively higher antimycobacterial activity probably due to their higher lipophilicity. Extensive structure–activity relation could be derived in future with various other modifications.

Having identified good number of active antimycobacterial dihydropyridines, the next step was to examine the toxicity of the drug candidates. Compounds exhibiting reasonably low MICs (from 0.02 to 3.13 μg/mL) were tested for cytotoxicity (IC_{50}) in VERO cells, and a selectivity index (SI), defined as IC_{50}: MIC, was calculated. The IC_{50} and SI values are shown in Table 1. The compounds **4b** and **4f** were somewhat more toxic than the **3f**, **4a, 4c** and **4e**. Generally, compounds with MIC ≤6.25 μg/mL and SI ≥10 are



3a-h R_1 = H, 4-OH, 4-F, 4-Cl, 4-Br, 4-NO_2, 4-CH_3, 4-OCH_3; R_2 = OCH_3
4a-h R_1 = H, 4-OH, 4-F, 4-Cl, 4-Br, 4-NO_2, 4-CH_3, 4-OCH_3; R_2 = OC_2H_5

Scheme 1: Synthesis of 1,4-dihydropyridines **3a**–**h** to **4a**–**h**.

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

Sr. no.	Molecule no.	R_1	R_2	% Inhibi-tion	MIC[a] μg/mL	IC_{50}[b] VERO cells	SI[c] (SI = IC_{50}/MIC)	*mi* Log *P*[d]
3a	298021	H	OCH_3	12	n.d.	n.d.	n.d.	4.485
3b	298022	4-OH	OCH_3	38	n.d.	n.d.	n.d.	4.006
3c	298023	4-F	OCH_3	71	n.d.	n.d	n.d.	4.649
3d	298024	4-Cl	OCH_3	62	n.d.	n.d.	n.d.	5.163
3e	298025	4-Br	OCH_3	40	n.d.	n.d.	n.d.	5.294
3f	298026	4-NO_2	OCH_3	100	0.02	>10	500	4.444
3g	298027	4- CH_3	OCH_3	71	n.d.	n.d.	n.d.	4.934
3h	298028	4-OCH_3	OCH_3	05	n.d.	n.d.	n.d.	4.542
4a	298003	H	OC_2H_5	95	1.56	>10	6.42	5.237
4b	298004	4-OH	OC_2H_5	97	1.56	4.27	2.73	4.758
4c	298005	4-F	OC_2H_5	100	0.02	>10	500	5.401
4d	298006	4-Cl	OC_2H_5	70	n.d.	n.d.	n.d.	5.915
4e	298007	4-Br	OC_2H_5	100	0.02	>10	500	6.046
4f	298008	4-NO_2	OC_2H_5	93	3.13	8.83	2.82	5.196
4g	298009	4- CH_3	OC_2H_5	84	n.d.	n.d.	n.d.	5.686
4h	298010	4-OCH_3	OC_2H_5	52	n.d.	n.d.	n.d.	5.294
INH	–	–	–	–	0.03	–	–	

n.d., not determined.

[a]Minimum inhibitory concentration against $H_{37}Rv$ strain of *M. tuberculosis* (μg/mL).

[b]Measurement of cytotoxicity in VERO cells: 50% inhibitory concentrations (μg/mL).

[c]Selectivity index (*in vitro*): IC_{50} in VERO cells/MIC against *M tuberculosis*.

[d]*mi* Log *P*: Molinspiration Cheminformatics (http://www.molinspiration.com) calculated Log *P* using online Molinspiration Property Engine v2009.01.

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 carbmethoxy 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 carbethoxy 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.

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Note

[a]http://www.who.int, 2007.

Supporting Information

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

Figure S1. Mass spectrum of compound **4e**.

Figure S2. ^{1}H NMR (300MHz) spectrum of compound **4e**.

Figure S3. ^{13}C NMR (300MHz) spectrum of compound **4e**.

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