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Synthesis of some Novel *N*-Aryl-1,4-Dihydropyridines Derivatives and Their Biological Evaluation as Potential Antimycobaceterial Agents

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ABSTRACT

Tuberculosis, due to its unyielding nature, is now a chief civic health threat. The associated renaissance of TB with the MDR or XDR-TB and HIV/AIDS epidemic has exposed the infirmities of the current drug armatorium. Recent studies showed that 1,4-dihydropyridine-3,5-dicarbamoyl derivatives with lipophilic groups have significant antitubercular activity. We have synthesized new N-aryl-1,4-dihydropyridines bearing carbethoxy and acetyl group at C-3 and C-5 of the DHP ring. In addition, 1H-pyrazole ring is substitutes at C-4 position. The in vitro antitubercular activity of compounds against Mycobacterium tuberculosis H37Rv was evaluated. The lowest minimum inhibitory concentration value, 0.02 μg/mL, was found for diethyl 1-(2-chlorophenyl)-1,4-dihydro-2,6-dimethyl-4-(1,3-diphenyl-1H-pyrazol-4-yl)pyridine-3,5-dicarboxylate 4e making it more potent than first line antitubercular drug isoniazid. In addition, this compound exhibited relatively low cytotoxicity.

Keywords : 1, 4-Dihydropyridines, Antitubercular Activity, Antimycobacterial Activity, Mycobacterium Tuberculosis.

I. INTRODUCTION

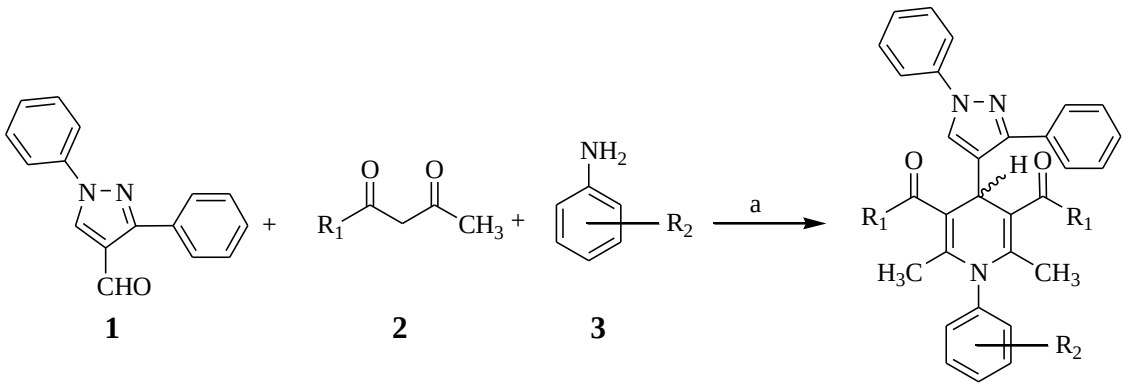
Tuberculosis (TB) is a worldwide pandemic caused by different species of mycobacteria. The latest statistics reveals that around two million people throughout the world die annually from tuberculosis and there are around 8 million new cases each year, out of which developing countries show major share [1]. Among HIV-infected people with weakened immune system, TB is a leading killer epidemic. Every year about 2 million people living with HIV/AIDS die from TB [2]. Furthermore, in recent times the appearance 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. A recent estimation by WHO has revealed that within next 20 years approximately 30 million people will be infected with the bacillus [3]. Keeping in view of the above statistics, WHO declared TB as a global health emergency and aimed at saving 14 million lives between 2006 and 2015 [4]. Despite the efforts of the pharmaceutical companies engaged in the design, synthesis and assays of new potent and selective antitubercular agents, the current therapeutic outlook is poor. Only a few derivatives were found endowed with some antimycobacterial activity, including fluoroquinolones (gatifloxacin and moxifloxacin) [5-8], diarylquinoline (TMC207) [9], nitroimidazoles (OPC67683 and PA824) [10-11], pyrrole (LL3858) [12] and 1,2-diamine (SQ109) [13]. All the above facts reveal that there is an urgent need for development of new drugs with divergent and unique structure and

with a mechanism of action possibly different from that of existing drugs.

Recently, studies showed that 3,5-dicarbamoyl derivatives of 1,4-dihydropyridine (DHP) with lipophilic groups have considerable antitubercular activity against *M. tuberculosis* $H_{37}Rv$ [14]. It was also observed that esters or substituted isosters of pyridine and pyrazinecarboxylic 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 [15-18]. Indeed, esters of pyrazinoic acids have been shown to possess activity against pyrazinamide-resistant isolates which has been attributed to a deficiency of nicotinamidase [15-18]. In addition, pyrazoles exhibited significant antitubercular activity [19]. In view of this, it appeared of interest to design and synthesize new derivatives of *N*-substituted 1,4-dihydropyridines bearing carbethoxy and acetyl 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 *Mycobacterium tuberculosis* $H_{37}Rv$ (MTB).

II. CHEMISTRY

The quest for the synthesis of *N*-aryl-1,4-dihydropyridines **4a-j** and **5a–j** was carried out by heating the 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde **1** (1 eq.), ethyl acetoacetate/acetyl acetone **2** (2 eq.) and substituted anilines **3** (1 eq.), on a steam bath for 2–3 h. After elimination of water, methanol (25 mL) was added directly to the reaction mixture; refluxing for an appropriate time culminated in the synthesis of the molecules albeit in moderate yields (32-45%, Scheme 1). Preparation of 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde **1** is described [20]. Designed series of molecules **4a-j** and **5a–j** (Table 1) 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 **4a-j** and **5a–j** have the typical singlet of methine group lying in the region 5.42–5.47 ppm and multiplet of aromatic part of molecules occurring in region between 6.21 and 7.93 ppm. The ^{13}C NMR signal of methine group can be observed at 32.0-36.2 ppm. IR spectra of DHP derivatives were also in agreement with the structures.



1 **2** **3** **4a-j** and **5a-j**

4a-j $R_1 = OC_2H_5$; R_2 = H, 2-CH_3, 3-CH_3, 4-CH_3, 2-Cl, 3-Cl, 4-Cl, 2-OCH_3, 3-OCH_3, 4-OCH_3
5a-j $R_1 = CH_3$; R_2 = H, 2-CH_3, 3-CH_3, 4-CH_3, 2-Cl, 3-Cl, 4-Cl, 2-OCH_3, 3-OCH_3, 4-OCH_3

Scheme 1. Synthesis of *N*-aryl-1,4-dihydropyridines **4a-j** and **5a-j**. Reagents and conditions: (a) MeOH, reflux.

III. BIOLOGY

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 & Coordinating Facility (TAACF) in BACTEC 12B medium using the Microplate Alamar Blue Assay [21]. Compounds exhibiting ≥90% inhibition in the initial screen were retested at and below 6.25 µg/mL using 2-fold dilution to determine the actual MIC.

IV. RESULTS AND DISCUSSION

In the preliminary screening, seven compounds **4a-c**, **4e, 4f, 5b** and **5c** inhibited MTB with 90–100%. In the secondary level, one compound **4e** inhibited MTB with MIC of <1 µg/mL and three compounds **4a**, **4c** and **4f** with MIC of <2 µg/mL. When compared to isoniazid (MIC: 0.03 µg/mL), diethyl 1-(2-chlorophenyl)-1,4-dihydro-2,6-dimethyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)pyridine-3,5-dicarboxylate **4e** was found to be the most active compound in vitro with MIC of 0.02 µg/mL against MTB and was more potent than isoniazid. The preliminary antimycobacterial evaluation results showed that compounds with carbethoxy substituents at C-3 and C-5 position of DHP ring exhibited higher antimycobacterial activity than the compounds with acetyl substituents at C-3 and C-5 position, probably due to their comparatively higher lipophilicity. In addition, compound **4e** bearing carbethoxy group along with chloro substituent at the 2nd position of *N*-aryl substituent on DHP skeleton. Extensive structure-activity relation could be derived in future with various other modifications.

Table 1. In vitro antitubercular screening data of Dihydropyridines 4a-j and 5a-j

Sr. No.	**R_1**	**R_2**	**% Inhibition**	**MIC µg/mL**	**IC_{50} VERO cells**	**SI (SI =IC_{50}/ MIC)**	***mi* Log *P*[a]**
4a	OC_2H_5	H	96	1.56	>10	6.4	7.179
4b	OC_2H_5	2-CH_3	95	3.13	>10	>3.2	7.580
4c	OC_2H_5	3-CH_3	98	1.56	>10	6.4	7.604
4d	OC_2H_5	4-CH_3	48	48	n.d.	n.d.	7.628
4e	OC_2H_5	2-Cl	100	0.02	>10	500	7.809
4f	OC_2H_5	3-Cl	97	1.56	7.08	4.5	7.833
4g	OC_2H_5	4-Cl	74	n.d	n.d	n.d	7.857
4h	OC_2H_5	2-OCH_3	39	n.d	n.d	n.d	7.188
4i	OC_2H_5	3-OCH_3	55	n.d	n.d	n.d	7.212
4j	OC_2H_5	4-OCH_3	33	n.d	n.d	n.d	7.236

5a	CH_3	H	48	n.d	n.d	n.d	5.794
5b	CH_3	2-CH_3	97	3.13	>10	>3.2	6.194
5c	CH_3	3-CH_3	98	6.25	>10	1.6	6.218
5d	CH_3	4-CH_3	34	n.d	n.d	n.d	6.242
5e	CH_3	2-Cl	59	n.d	n.d	n.d	6.424
5f	CH_3	3-Cl	76	n.d	n.d	n.d	6.448
5g	CH_3	4-Cl	29	n.d	n.d	n.d	6.472
5h	CH_3	2-OCH_3	25	n.d	n.d	n.d	5.803
5i	CH_3	3-OCH_3	77	n.d	n.d	n.d	5.827
5j	CH_3	4-OCH_3	69	n.d	n.d	n.d	5.851
INH	-	-	-	0.03	-	-	

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 6.25 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 compound **4f** was somewhat more toxic than the **4a-c**, **4e, 5b** and **5c**. Generally, compounds with an MIC ≤6.25 μg/mL and an SI ≥10 are interesting compounds, and an MIC ≤1 μg/mL in a novel compound class is considered an excellent lead [22], which makes the diethyl 1-(2-chlorophenyl)-1,4-dihydro-2,6-dimethyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)pyridine-3,5-dicarboxylate **4e** very promising antimycobacterial compound. Further in vitro studies of compound **4e** as well as synthesis of analogues of this lead compounds are currently in progress.

V. CONCLUSION

Comparison of the activities of tested compounds **4a-j** and **5a-j** indicated that compound **4e** with 2-chloro substituent at the *N*-aryl ring and carbethoxy group at C-3 and C-5 positions of the 1,4-dihydropyridine ring was the most potent one among the tested compounds. The results indicate that alkyl ester with optimal lipophilicity could be a suitable candidate against *M. tuberculosis.* In vivo antimycobacterial evaluation is needed for further optimization. In addition, in vitro evaluation of designed compounds against resistant strain of *M. tuberculosis* could be valuable.

VI. EXPERIMENTAL

6.1. Chemistry

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. ^{13}C-NMR (75 and 125 MHz) spectra were registered on a Bruker AC 200, DPX 300 and ARX 500, at 25 ºC, in DMSO-d_6.

Infrared (IR) 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 and the data were within range of the theoretical values.

1.2. Synthesis of 1,3-diphenyl-1H-pyrazole-4 carbaldehyde (**1**) Synthesis of 1,3-diphenyl-1H-pyrazole-4-carbaldehyde was achieved by reported method [21].

1.3. General procedure for the synthesis of N-aryl-1,4-dihydropyridines (**4a-j**)

A mixture of substituted benzaldehyde (1 eq.), ethyl acetoacetate (2 eq.) and substituted aniline (1 eq.) were heated without solvent on a steam bath for 2–3 h. After elimination of water, methanol (25 mL) was added directly to the reaction mixture and refluxed for an appropriate time. After the completion of the reaction, the reaction mixture was poured into water and extracted twice with ethyl acetate (15 mL). The combined ethyl acetate layer was washed with brine (20 mL) and dried over anhydrous sodium sulphate. Ethyl acetate was removed in vacuo to leave the crude product. Finally, it was purified by silica gel (60–100 mesh) column chromatography using ethyl acetate and hexane (4:1) as the eluents.

6.3.1. Diethyl 1,4-dihydro-2,6-dimethyl-1-phenyl-4-(1,3-diphenyl-1H-pyrazol-4-yl)pyridine-3,5-dicarboxylate (**4a**). Yield: 37%, m.p. 68 °C; IR (KBr) cm^{-1}: 3010, 2929, 1695, 1646, 1199, 1080, 1050; ^1H NMR (DMSO-d_6) δ ppm: 1.06-1.10 (t, 6H, 2-CH_2CH_3), 2.00 (s, 6H, 2-CH_3), 3.74 (q, 2H, CH_2CH_3), 3.79 (q, 2H, CH_2CH_3), 5.44 (s, 1H, CH), 6.96-7.83 (m, 16H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 167.6, 154.7, 149.8, 141.0, 139.7, 133.0, 129.9, 129.4, 129.1, 128.3, 127.2, 126.2, 123.0, 119.8, 118.6, 117.0, 116.1, 102.3, 61.9, 36.0, 14.1, 13.8. Anal. Calcd. for $C_{34}H_{33}N_3O_4$: C, 74.57%; H, 6.07%; N, 7.67%. Found: C, 74.49%; H, 5.99%; N, 7.58%.

6.3.2. Diethyl 1,4-dihydro-2,6-dimethyl-4-(1,3-diphenyl-1H-pyrazol-4-yl)-1-o-tolylpyridine-3,5-dicarboxylate (**4b**). Yield: 41%, m.p. 118 °C; IR (KBr) cm^{-1}: 3015, 2919, 1690, 1642, 1190, 1085, 1055; ^1H NMR (DMSO-d_6) δ ppm: 1.07-1.10 (t, 6H, 2-CH_2CH_3), 2.02 (s, 6H, 2-CH_3), 2.38 (s, 3H, CH_3), 3.73 (q, 2H, CH_2CH_3), 3.80 (q, 2H, CH_2CH_3), 5.45 (s, 1H, CH), 6.75-7.85 (m, 15H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 167.8, 154.8, 149.8, 144.3, 139.6, 132.8, 130.1, 129.4, 129.0, 128.8, 128.5, 127.0, 126.7, 126.3, 122.8, 119.9, 118.2, 117.0, 116.3, 102.2, 62.1, 36.0, 16.1, 14.2, 13.7. Anal. Calcd. for $C_{35}H_{35}N_3O_4$: C, 74.84%; H, 6.28%; N, 7.48%. Found: C, 74.74%; H, 6.19%; N, 7.38%.

6.3.3. Diethyl 1,4-dihydro-2,6-dimethyl-4-(1,3-diphenyl-1H-pyrazol-4-yl)-1-m-tolylpyridine-3,5-dicarboxylate (**4c**). Yield: 32%, m.p. 157 °C; IR (KBr) cm^{-1}: 3015, 2915, 1693, 1645, 1195, 1083, 1052; ^1H NMR (DMSO-d_6) δ ppm: 1.05-1.09 (t, 6H, 2-CH_2CH_3), 1.99 (s, 6H, 2-CH_3), 2.37 (s, 3H, CH_3), 3.75 (q, 2H, CH_2CH_3), 3.81 (q, 2H, CH_2CH_3), 5.43 (s, 1H, CH), 6.61-7.83 (m, 15H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 167.9, 154.6, 149.7, 141.3, 139.8, 139.1, 132.8, 129.7, 129.5, 129.2, 128.4, 127.0, 126.4, 122.9, 120.0, 118.9, 117.2, 115.8, 113.1, 102.3, 62.0, 36.2, 24.1, 14.3, 13.9. Anal. Calcd. for $C_{35}H_{35}N_3O_4$: C, 74.84%; H, 6.28%; N, 7.48%. Found: C, 74.70%; H, 6.16%; N, 7.39%.

6.3.4. Diethyl 1,4-dihydro-2,6-dimethyl-4-(1,3-diphenyl-1H-pyrazol-4-yl)-1-p-tolylpyridine-3,5-dicarboxylate (**4d**). Yield: 40%, m.p. 90 °C; IR (KBr) cm^{-1}: 3008, 2918, 1692, 1642, 1189, 1088, 1049; ^1H NMR (DMSO-d_6) δ ppm: 1.06-1.09 (t, 6H, 2-CH_2CH_3), 2.01 (s, 6H, 2-CH_3), 2.40 (s, 3H, CH_3), 3.76 (q, 2H, CH_2CH_3), 3.82 (q, 2H, CH_2CH_3), 5.44 (s, 1H, CH), 6.70-7.87 (m, 15H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 168.0, 154.4, 149.9, 139.9, 138.1, 132.9, 129.9, 129.4, 129.0, 128.6, 128.3, 127.1, 126.6, 123.0, 120.1, 117.2, 116.0, 102.1, 62.2, 36.1, 24.2, 14.4, 13.8. Anal. Calcd. for $C_{35}H_{35}N_3O_4$: C, 74.84%; H, 6.28%; N, 7.48%. Found: C, 74.71%; H, 6.16%; N, 7.40%.

6.3.5. Diethyl 1-(2-chlorophenyl)-1,4-dihydro-2,6-dimethyl-4-(1,3-diphenyl-1H-pyrazol-4-yl) pyridine-

3,5-dicarboxylate (**4e**). Yield: 39%, m.p. 146 °C; IR (KBr) cm^{-1}: 3013, 2916, 1696, 1644, 1194, 1092, 1054, 785; ^1H NMR (DMSO-d_6) δ ppm: 1.05-1.08 (t, 6H, 2-CH_2CH_3), 2.00 (s, 6H, 2-CH_3), 3.77 (q, 2H, CH_2CH_3), 3.84 (q, 2H, CH_2CH_3), 5.45 (s, 1H, CH), 6.86-7.86 (m, 15H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 167.6, 154.6, 149.6, 144.5, 139.7, 132.9, 130.0, 129.5, 129.0, 128.4, 127.9, 127.1, 126.1, 125.0, 122.9, 120.3, 119.9, 117.6, 117.0, 102.0, 62.2, 36.2, 14.1, 13.7. Anal. Calcd. for $C_{34}H_{32}ClN_3O_4$: C, 70.15%; H, 5.54%; N, 7.22%. Found: C, 70.03%; H, 5.42%; N, 7.11%.

6.3.6. Diethyl 1-(3-chlorophenyl)-1,4-dihydro-2,6-dimethyl-4-(1,3-diphenyl-1H-pyrazol-4-yl) pyridine-3,5-dicarboxylate (**4f**). Yield: 45%, m.p. 80 °C; IR (KBr) cm^{-1}: 3015, 2910, 1695, 1640, 1190, 1095, 1050, 780; ^1H NMR (DMSO-d_6) δ ppm: 1.06-1.08 (t, 6H, 2-CH_2CH_3), 2.02 (s, 6H, 2-CH_3), 3.74 (q, 2H, CH_2CH_3), 3.83 (q, 2H, CH_2CH_3), 5.44 (s, 1H, CH), 6.81-7.88 (m, 15H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 167.7, 154.5, 149.7, 142.5, 139.9, 135.3, 133.0, 131.2, 129.4, 129.1, 128.5, 127.4, 126.0, 123.0, 120.5, 118.7, 117.3, 116.5, 114.6, 102.0, 62.2, 36.2, 14.1, 13.8. Anal. Calcd. for $C_{34}H_{32}ClN_3O_4$: C, 70.15%; H, 5.54%; N, 7.22%. Found: C, 70.06%; H, 5.43%; N, 7.13%.

6.3.7. Diethyl 1-(4-chlorophenyl)-1,4-dihydro-2,6-dimethyl-4-(1,3-diphenyl-1H-pyrazol-4-yl) pyridine-3,5-dicarboxylate (**4g**). Yield: 44%, m.p. 133 °C; IR (KBr) cm^{-1}: 3010, 2915, 1693, 1643, 1194, 1089, 1051, 789; ^1H NMR (DMSO-d_6) δ ppm: 1.05-1.09 (t, 6H, 2-CH_2CH_3), 2.01 (s, 6H, 2-CH_3), 3.75 (q, 2H, CH_2CH_3), 3.80 (q, 2H, CH_2CH_3), 5.45 (s, 1H, CH), 6.90-7.84 (m, 15H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 167.5, 154.3, 150.2, 140.0, 139.5, 133.1, 129.9, 129.6, 129.3, 128.9, 127.6, 126.0, 124.5, 123.1, 120.7, 117.8, 117.1, 102.1, 62.2, 36.1, 14.3, 13.9. Anal. Calcd. for $C_{34}H_{32}ClN_3O_4$: C, 70.15%; H, 5.54%; N, 7.22%. Found: C, 70.03%; H, 5.42%; N, 7.14%.

6.3.8. Diethyl 1,4-dihydro-1-(2-methoxyphenyl)-2,6-dimethyl-4-(1,3-diphenyl-1H-pyrazol-4-yl) pyridine-3,5-dicarboxylate (**4h**). Yield: 36%, m.p. 95 °C; IR (KBr) cm^{-1}: 3013, 2912, 1689, 1645, 1197, 1092, 1053; ^1H NMR (DMSO-d_6) δ ppm: 1.06-1.09 (t, 6H, 2-CH_2CH_3), 1.99 (s, 6H, 2-CH_3), 3.65 (s, 3H, OCH_3), 3.73 (q, 2H, CH_2CH_3), 3.79 (q, 2H, CH_2CH_3), 5.45 (s, 1H, CH), 6.62-7.86 (m, 15H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 167.6, 154.7, 150.0, 147.8, 139.4, 132.7, 129.6, 129.2, 128.7, 128.4, 127.1, 126.4, 122.9, 121.8, 120.1, 119.6, 117.4, 117.0, 115.3, 102.3, 62.1, 56.1, 35.9, 14.1, 13.7. Anal. Calcd. for $C_{35}H_{35}N_3O_5$: C, 72.77%; H, 6.11%; N, 7.27%. Found: C, 72.65%; H, 6.03%; N, 7.18%.

6.3.9. Diethyl 1,4-dihydro-1-(3-methoxyphenyl)-2,6-dimethyl-4-(1,3-diphenyl-1H-pyrazol-4-yl) pyridine-3,5-dicarboxylate (**4i**). Yield: 38%, m.p. 99 °C; IR (KBr) cm^{-1}: 3020, 2917, 1693, 1648, 1190, 1089, 1055; ^1H NMR (DMSO-d_6) δ ppm: 1.07-1.11 (t, 6H, 2-CH_2CH_3), 1.98 (s, 6H, 2-CH_3), 3.63 (s, 3H, OCH_3), 3.73 (q, 2H, CH_2CH_3), 3.78 (q, 2H, CH_2CH_3), 5.44 (s, 1H, CH), 6.21-7.89 (m, 15H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 167.8, 161.6, 154.5, 149.8, 142.6, 139.5, 132.5, 130.9, 129.7, 129.3, 128.5, 126.9, 126.6, 123.0, 120.2, 117.1, 108.5, 104.1, 102.1, 99.8, 61.9, 56.0, 36.1, 14.3, 13.8. Anal. Calcd. for $C_{35}H_{35}N_3O_5$: C, 72.77%; H, 6.11%; N, 7.27%. Found: C, 72.63%; H, 6.01%; N, 7.16%.

6.3.10. Diethyl 1,4-dihydro-1-(4-methoxyphenyl)-2,6-dimethyl-4-(1,3-diphenyl-1H-pyrazol-4-yl) pyridine-3,5-dicarboxylate (**4j**). Yield: 44%, m.p. 112 °C; IR (KBr) cm^{-1}: 3018, 2910, 1690, 1645, 1191, 1092, 1050; ^1H NMR (DMSO-d_6) δ ppm: 1.07-1.10 (t, 6H, 2-CH_2CH_3), 2.00 (s, 6H, 2-CH_3), 3.65 (s, 3H, OCH_3), 3.75 (q, 2H, CH_2CH_3), 3.80 (q, 2H, CH_2CH_3), 5.43 (s, 1H, CH), 6.58-7.90 (m, 15H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 167.9, 154.3, 150.9, 149.7, 140.1, 133.4, 132.7, 129.6, 129.2, 128.4, 127.0, 126.5, 123.5, 120.0, 117.5, 117.1, 114.8, 102.5, 62.1, 55.9, 36.0, 14.1, 13.8. Anal. Calcd. for $C_{35}H_{35}N_3O_5$: C, 72.77%; H, 6.11%; N, 7.27%. Found: C, 72.66%; H, 6.03%; N, 7.17%.

1.4. General procedure for the synthesis of N-aryl-1,4-dihydropyridines (**5a-j**)

A mixture of the substituted benzaldehyde (1 eq.), acetyl acetone (2 eq.) and substituted aniline (1 eq.) were heated without solvent on a steam bath for 2–3 h. After elimination of water, methanol (25 mL) was added directly to the reaction mixture and refluxed for an appropriate time. After the completion of the reaction, the reaction mixture was poured into water and extracted twice with ethyl acetate (15 mL). The combined ethyl acetate layer was washed with brine (20 mL) and dried over anhydrous sodium sulphate. Ethyl acetate was removed in vacuo to leave the crude product. Finally, it was purified by silica gel (60–100 mesh) column chromatography using ethyl acetate and hexane (4:1) as the eluents.

6.4.1. 2,6-dimethyl-1-phenyl-4-(1,3-diphenyl-pyrazol-4-yl)-3,5-diacetyl-1,4-dihydro-pyridine (**5a**). Yield: 42%, m.p. 168 °C; IR (KBr) cm^{-1}: 3018, 2927, 1660, 1595, 1190; ^1H NMR (DMSO-d_6) δ ppm: 2.03 (s, 6H, 2-CH_3), 3.31 (s, 6H, 2-$COCH_3$), 5.46 (s, 1H, CH), 6.89-7.91 (m, 16H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 196.2, 152.5, 150.2, 141.1, 139.6, 132.9, 130.0, 129.6, 129.4, 128.7, 127.1, 126.5, 123.5, 120.0, 118.9, 117.0, 116.5, 113.7, 32.1, 27.6, 13.7. Anal. Calcd. for $C_{32}H_{29}N_3O_2$: C, 78.82%; H, 5.99%; N, 8.62%. Found: C, 78.71%; H, 5.90%; N, 8.54%.

6.4.2. 2,6-dimethyl-1-(2-methylphenyl)-4-(1,3-diphenyl-pyrazol-4-yl)-3,5-diacetyl-1,4-dihydro-pyridine (**5b**). Yield: 39%, m.p. 172 °C; IR (KBr) cm^{-1}: 3020, 2925, 1655, 1590, 1195; ^1H NMR (DMSO-d_6) δ ppm: 2.00 (s, 6H, 2-CH_3), 2.47 (s, 3H, CH_3), 3.30 (s, 6H, 2-$COCH_3$), 5.47 (s, 1H, CH), 6.80-7.90 (m, 15H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 196.4, 152.7, 150.3, 143.7, 139.5, 133.1, 130.3, 129.5, 129.2, 129.0, 128.5, 127.3, 126.6, 126.3, 123.7, 120.1, 119.0, 117.2, 116.0, 113.7, 32.2, 27.5, 16.1, 13.8. Anal. Calcd. for $C_{33}H_{31}N_3O_2$: C, 79.01%; H, 6.23%; N, 8.38%. Found: C, 78.90%; H, 6.14%; N, 8.29%.

6.4.3. 2,6-dimethyl-1-(3-methylphenyl)-4-(1,3-diphenyl-pyrazol-4-yl)-3,5-diacetyl-1,4-dihydro-pyridine (**5c**). Yield: 43%, m.p. 188 °C; IR (KBr) cm^{-1}: 3023, 2923, 1657, 1593, 1193; ^1H NMR (DMSO-d_6) δ ppm: 2.01 (s, 6H, 2-CH_3), 2.45 (s, 3H, CH_3), 3.31 (s, 6H, 2-$COCH_3$), 5.45 (s, 1H, CH), 6.61-7.84 (m, 15H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 196.3, 152.8, 150.1, 141.0, 139.4, 139.1, 133.0, 129.8, 129.4, 129.1, 128.6, 127.2, 126.6, 123.2, 120.2, 119.2, 117.1, 116.2, 113.8, 113.1, 32.0, 27.7, 24.5, 13.7. Anal. Calcd. for $C_{33}H_{31}N_3O_2$: C, 79.01%; H, 6.23%; N, 8.38%. Found: C, 78.92%; H, 6.15%; N, 8.30%.

6.4.4. 2,6-dimethyl-1-(4-methylphenyl)-4-(1,3-diphenyl-pyrazol-4-yl)-3,5-diacetyl-1,4-dihydro-pyridine (**5d**). Yield: 40%, m.p. 224 °C; IR (KBr) cm^{-1}: 3019, 2922, 1659, 1591, 1198; ^1H NMR (DMSO-d_6) δ ppm: 2.00 (s, 6H, 2-CH_3), 2.45 (s, 3H, CH_3), 3.33 (s, 6H, 2-$COCH_3$), 5.46 (s, 1H, CH), 6.75-7.86 (m, 15H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 196.4, 152.6, 150.5, 139.6, 138.5, 133.1, 130.2, 129.7, 129.2, 128.8, 128.2, 127.0, 126.4, 123.4, 120.0, 117.0, 116.1, 113.6, 32.1, 27.6, 24.4, 13.9. Anal. Calcd. for $C_{33}H_{31}N_3O_2$: C, 79.01%; H, 6.23%; N, 8.38%. Found: C, 78.89%; H, 6.14%; N, 8.27%.

6.4.5. 2,6-dimethyl-1-(2-chlorophenyl)-4-(1,3-diphenyl-pyrazol-4-yl)-3,5-diacetyl-1,4-dihydro-pyridine (**5e**). Yield: 41%, m.p. 198 °C; IR (KBr) cm^{-1}: 3017, 2924, 1654, 1590, 1191, 785; ^1H NMR (DMSO-d_6) δ ppm: 2.04 (s, 6H, 2-CH_3), 2.47 (s, 3H, CH_3), 3.31 (s, 6H, 2-$COCH_3$), 5.46 (s, 1H, CH), 6.81-7.90 (m, 15H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 196.5, 152.5, 150.2, 144.0, 139.4, 133.3, 129.9, 129.6, 129.1, 128.4, 127.8, 127.4, 126.2, 125.0, 123.6, 120.4, 120.1, 117.9, 117.1, 113.8, 32.0, 27.7, 13.7. Anal. Calcd. for $C_{32}H_{28}ClN_3O_2$: C, 73.62%; H, 5.41%; N, 8.05%. Found: C, 73.51%; H, 5.32%; N, 7.98%.

6.4.6. 2,6-dimethyl-1-(3-chlorophenyl)-4-(1,3-diphenyl-pyrazol-4-yl)-3,5-diacetyl-1,4-dihydro-pyridine (**5f**). Yield: 37%, m.p. 248 °C; IR (KBr) cm^{-1}: 3020, 2920, 1650, 1595, 1195, 780; ^1H NMR (DMSO-d_6) δ ppm: 2.04 (s, 6H, 2-CH_3), 2.47 (s, 3H, CH_3), 3.31 (s, 6H, 2-$COCH_3$), 5.46 (s, 1H, CH), 6.71-7.89 (m, 15H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 196.6, 152.3, 150.1, 142.9, 139.5, 135.4, 133.4, 131.3, 129.5, 129.2, 128.3, 127.7, 126.0, 123.7, 120.3, 118.6, 117.0, 116.5,

114.1, 113.6, 32.1, 27.9, 13.8. Anal. Calcd. for $C_{32}H_{28}ClN_3O_2$: C, 73.62%; H, 5.41%; N, 8.05%. Found: C, 73.50%; H, 5.32%; N, 7.96%.

6.4.7. 2,6-dimethyl-1-(4-chlorophenyl)-4-(1,3-diphenyl-pyrazol-4-yl)-3,5-diacetyl-1,4-dihydro-pyridine (**5g**). Yield: 41%, m.p. 254 °C; IR (KBr) cm^{-1}: 3023, 2924, 1653, 1594, 1191, 783; ^1H NMR (DMSO-d_6) δ ppm: 2.04 (s, 6H, 2-CH_3), 2.47 (s, 3H, CH_3), 3.31 (s, 6H, 2-$COCH_3$), 5.46 (s, 1H, CH), 6.85-7.90 (m, 15H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 196.5, 152.4, 150.2, 139.8, 139.5, 133.5, 129.9, 129.6, 129.1, 128.3, 127.6, 126.1, 124.7, 123.6, 120.1, 117.8, 117.1, 113.5, 32.2, 27.8, 13.7. Anal. Calcd. for $C_{32}H_{28}ClN_3O_2$: C, 73.62%; H, 5.41%; N, 8.05%. Found: C, 73.52%; H, 5.33%; N, 7.98%.

6.4.8. 2,6-dimethyl-1-(2-methoxyphenyl)-4-(1,3-diphenyl-pyrazol-4-yl)-3,5-diacetyl-1,4-dihydro-pyridine (**5h**). Yield: 44%, m.p. 178 °C; IR (KBr) cm^{-1}: 3026, 2927, 1657, 1597 1196, 1195, 1050; ^1H NMR (DMSO-d_6) δ ppm: 2.04 (s, 6H, 2-CH_3), 2.47 (s, 3H, CH_3), 3.31 (s, 6H, 2-$COCH_3$), 3.62 (s, 3H, OCH_3), 5.46 (s, 1H, CH), 6.75-7.91 (m, 15H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 196.4, 152.6, 150.1, 147.7, 139.3, 133.2, 129.7, 129.2, 128.6, 128.3, 127.4, 126.2, 123.8, 122.2, 120.2, 119.7, 117.7, 117.0, 115.0, 113.6, 55.6, 32.1, 27.6, 13.8. Anal. Calcd. for $C_{33}H_{31}N_3O_3$: C, 76.57 %; H, 6.04 %; N, 8.12 %. Found: C, 76.48 %; H, 5.96 %; N, 8.05 %.

6.4.9. 2,6-dimethyl-1-(3-methoxyphenyl)-4-(1,3-diphenyl-pyrazol-4-yl)-3,5-diacetyl-1,4-dihydro-pyridine (**5i**). Yield: 45%, m.p. 186 °C; IR (KBr) cm^{-1}: 3022, 2923, 1654, 1594, 1193, 1191, 1053; ^1H NMR (DMSO-d_6) δ ppm: 2.04 (s, 6H, 2-CH_3), 2.47 (s, 3H, CH_3), 3.31 (s, 6H, 2-$COCH_3$), 3.63 (s, 3H, OCH_3), 5.46 (s, 1H, CH), 6.24-7.87 (m, 15H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 196.4, 161.3, 152.5, 150.0, 142.0, 139.1, 133.1, 130.3, 129.6, 129.3, 128.7, 127.3, 126.1, 123.9, 120.0, 117.2, 113.7, 108.3, 104.1, 99.3, 55.7, 32.0, 27.5, 13.7. Anal. Calcd. for $C_{33}H_{31}N_3O_3$: C, 76.57 %; H, 6.04 %; N, 8.12 %. Found: C, 76.45 %; H, 5.97 %; N, 8.06 %.

6.4.10. 2,6-dimethyl-1-(4-methoxyphenyl)-4-(1,3-diphenyl-pyrazol-4-yl)-3,5-diacetyl-1,4-dihydropyridine (**5j**). Yield: 38%, m.p. 194 °C; IR (KBr) cm^{-1}: 3025, 2926, 1656, 1597, 1196, 1194, 1051; ^1H NMR (DMSO-d_6) δ ppm: 2.04 (s, 6H, 2-CH_3), 2.47 (s, 3H, CH_3), 3.31 (s, 6H, 2-$COCH_3$), 3.65 (s, 3H, OCH_3), 5.46 (s, 1H, CH), 6.72-7.93 (m, 15H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 196.5, 152.4, 150.9, 150.2, 139.2, 133.8, 133.0, 129.5, 129.1, 128.6, 127.5, 126.0, 123.7, 120.1, 117.6, 117.1, 115.5, 113.8, 55.6, 32.1, 27.6, 13.8. Anal. Calcd. for $C_{33}H_{31}N_3O_3$: C, 76.57 %; H, 6.04 %; N, 8.12 %. Found: C, 76.46 %; H, 5.93 %; N, 8.04%.

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