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Novel 2-methyl-6-arylpyridines carrying active pharmacophore 4,5-dihydro 2-pyrazolines: synthesis, antidepressant, and anti-tuberculosis evaluation

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Abstract As part of the effort to develop new hybrid molecules for the treatment of depression and tuberculosis, a new series of novel 6-aryl-2-methyl-3-(5-aryl-4,5-dihydro-1*H*-pyrazol-3yl)pyridines **5a–l** were synthesized. These compounds were screened for in vivo antidepressant activity following both modified forced swimming test (FST) and tail suspension test (TST) at a test dose of 100 mg kg^{-1}. Preliminary antidepressant screening of **5a–l** revealed that compound **5b** showed significant decrease in the immobility time when compared to normal control group in both FST and TST and was found to be more active than the standard imipramine (10 mg kg^{-1}, ip). Compounds **5f**, **5h**, **5j**, and **5l** displayed moderate to good antidepressant activity. All synthesized compounds were also screened for their in vitro anti-tuberculosis activity against *Mycobacterium tuberculosis* H37Rv strain. Compounds **5a**, **5c**, and **5i** showed highest anti-tubercular activity with MIC value 12.5 μg mL^{-1} comparable to the standard drugs.

Keywords Aryl pyridine · 2-Pyrazoline · Antidepressant activity · Tail suspension method · Forced swim method · Anti-TB activity

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Introduction

The discovery of new effective drugs for the treatment of depression remains a top priority as depression affects approximately 21% of the world population. The World Health Organization calculates that depression will become the second leading cause of premature death or disability worldwide by the year 2020 [1]. Depression is a common chronic recurrent syndrome, characterised by loss of energy, apathy, slow thinking and activity, as well as profound feelings of gloominess, despair, and suicidal ideation [2]. At present, many antidepressant agents [3] involved in medical therapy, such as tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors (SSRIs), and specific serotonin–noradrenaline reuptake inhibitors (SNRIs). These drugs have proven to be effective in reducing depression, at the expense of some undesirable side effects defeating their therapeutic efficacy. These clinical limitations and adverse effects of currently used antidepressants, along with the sharp rise of depressive cases in today's scenario, necessitate continuous development of novel, efficient, and safe drugs for the treatment of depression.

In recent years, there has been an increased interest in the treatment of one of the most life-threatening diseases tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb). The current frontline therapy for tuberculosis is associated with severe side effects including hepatotoxicity, thrombocytopenia, itching, rashes, fever, drug induced hepatitis [4, 5]. Additionally, the evolution of new virulent forms such as multidrug resistant (MDR), extensively drug resistant (XDR), and the recent cases of total drug resistant (TDR) strains increase the challenges to eliminate TB worldwide [6]. Because of this, synthetic compounds with potential anti-TB activity are receiving increased attention in biological research, medicine, and pharmacology.

In this regard, considerable interest has been focused on the pyrazoline structure, as it has shown to possess a broad spectrum of biological activities such as anti-inflammatory, analgesic, anti-pyretic, psychoanaleptic, monoamine oxidase hypotensive, anti-arrhythmic, muscle relaxant, tranquillizing, anticonvulsant, antibacterial, and antidiabetic activities [7]. Prodrug-based monoamine oxidase (MAO) inhibitors with hydrazide, hydrazine, and amine moiety, such as isocarboxazide [8], phenelzine [9], and meclobemide [10, 11], show prominent antidepressant activity in clinical trials. Moreover, many of the substituted hydrazines and hydrazides have been studied as MAO inhibitors [12, 13]. Pyrazolines are also well known for their antidepressant activity and anti-TB activity. Earlier studies by Soni et al. and Parmar et al. showed that some substituted pyrazolines, such as 1,3,5-triphenyl-2-pyrazolines, which bear a cyclic hydrazine moiety, have monoamine oxidase (MAO) inhibitory properties in the rat brain [14, 15]. Later, Gokhan et al. [16] demonstrated that the antidepressant activity of 1-N-substituted thiocarbamoyl-3-phenyl-5-thienyl-2-pyrazolines was similar to that of MAO inhibitors. Moreover, from the literature it was found that 1-thiocarbamoyl-3,5-diphenyl-2-pyrazolines, 3,5-difenil-2-pyrazolines, 1,3,5-triphenyl-2-pyrazolines, bicyclic pyrazoline 8-thiocarbamoyl-7,8 diazabicyclo[4.3.0]non-6-ene derivatives have antidepressant activity [7, 17–19]. Similarly, numerous

2-pyrazoline derivatives are reported to exhibit potent anti-tuberculosis activity [20–23]. Investigating the chemical structure of the aforementioned compounds, the 2-pyrazolines can be considered as an important heterocyclic scaffold for the development of potential antidepressant and anti-TB activity.

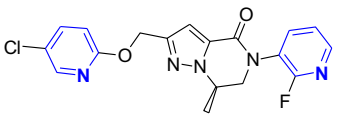
Like 2-pyrazolines, the role of pyridine in medicinal chemistry cannot be ignored due to its diverse biological activities. The pyridine substructure is one of the most prevalent heterocycle found in natural products, pharmaceuticals and functional materials [24–26]. Additionally, non fused pyridines found in a large variety of naturally occurring and synthetic compounds exhibit diverse bioactivities such as insecticidal [27], antitubercular [28], antimicrobial, anticancer [29] and anti-inflammatory [30] activity. On the other hand, several studies have demonstrated that pyridines and pyrazoles conjugated with a range of heterocyclic moieties [18, 31–35] are significant pharmacophores for antidepressant and anti-TB activity (Fig. 1). However, during literature survey, it was found that an aryl pyridine ring has not appeared to be linked with 4,5-dihydro-2-pyrazolines so far. It was hypothesized that if these two pharmacophores were linked, it would generate a new molecular model, which is a prerequisite for antidepressant and anti-TB activity and may prove to be a breakthrough for the development of lead molecule for future antidepressant and anti-TB research. In view of these findings, it was considered of interest to undertake the synthesis of pyridyl pyrazoline derivatives, hoping that these compounds might possess certain antidepressant and anti-TB activity.

Materials and methods

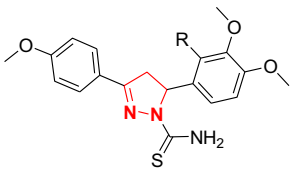
All the chemicals and reagents used for this study were obtained from Merck, S.D. Fine, Sigma-Aldrich Company. The chemicals purchased were of analytical grade and used directly. Melting point of the synthesized compounds was determined in open glass capillaries and is uncorrected. FT (ATR)-IR absorption spectra were recorded on Thermo Nicolet, Avatar 370 in range 4000–400 cm^{-1}. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on Bruker Avance III, 400 MHz and chemical shift values were expressed in parts per million (ppm) downfield from the internal standard, tetramethylsilane. Mass spectra were recorded on Water, synapt G_2 high detection mass spectrometry and are uncorrected. Elemental analysis was carried out using CHNS Elementar Vario EL III. All reactions were monitored by TLC analyses and were performed on silica plates; spots were visualised by UV light, with exposure to iodine vapours. Hexane:ethyl acetate (3:1) and hexane:dichloromethane (2:1) were the adopted solvent systems. Compounds were prepared according to reported procedures.

General synthetic procedure for compounds, 3a–b

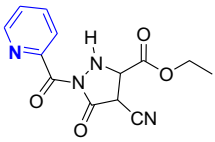
Acetyl acetone (1.1 mmol) and ammonium acetate (6 mmol) were added to the solution of appropriate enaminone **2a–b** (1 mmol) in glacial acetic acid (5 mmol). The reaction mixture was heated under reflux for 3 h. Upon cooling, the solution



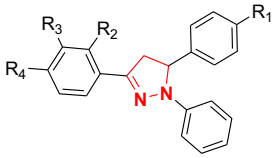
Antidepressant activity
J.Med.Chem., 58, 7485 (2015)



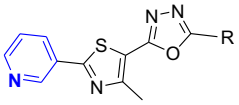
Antidepressant activity
Arch. Pharm. Chem. Life Sci., 341, 209(2008)



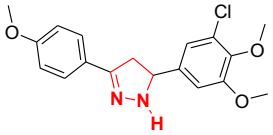
Antidepressant activity
Eur.J.Med.Chem., 44, 3480 (2009)



Anti-TB activity
Bioorg. Med. Chem. Lett., 20, 3169 (2010)



Anti-TB activity
Bioorg.Med.Chem.Lett.,26, 7065 (2016)



Antidepressant activity
Eur.J.Med.Chem., 36, 539-543 (2001)

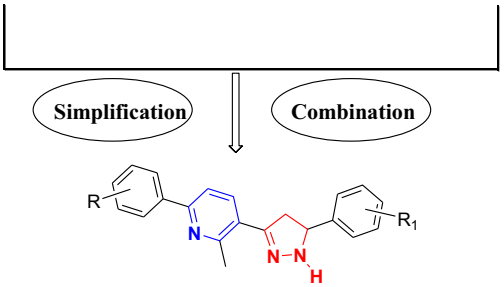


Fig. 1 Literature reports for active pyridine, pyrazole based antidepressant and anti-TB derivatives and strategy employed for designing pyridine based 2-pyrazolines

was poured into ice-cold water, the residue obtained was filtered, and washed with hexane followed by water, recrystallized from ethanol [36].

*1-(6-(3,5-Difluorophenyl)-2-methylpyridine-3-yl)ethanone (**3a**)*

Brown solid (yield 92%), m.p. 50–52 °C; IR (ATR) (cm^{-1}): 1680 (C=O), 1572 (C=N) and 1118 (C–F); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.62 (*s*, 3H, –CO–CH_3), 2.83 (*s*, 3H, CH_3), 6.88 (t, 1H, J = 8.4 Hz, C_4-H of 3,5-$F_2C_6H_3$), 7.62 (*d*, 3H, J = 8.4 Hz, C_2 and C_6-H of 3,5-$F_2C_6H_3$ and C_4-H of pyridine), 8.07 (*d*, 1H, J = 8 Hz, C_5-H of pyridine); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 199.74 (>C=O), 164.6 and 162.2 (1C, *d*, $^1J_{C-F}$ = 247.1 Hz), 158.6, 155.7, 141.6, 138,

131.8, 117.2, 110.2 and 109.9 (2C, *d*, $^2J_{C-F} = 26.1$ Hz), 104.8 (1C, t, $^2J_{C-F} = 25.3$ Hz), 29.3 (–CO–CH_3), 25.1 (–CH_3); EI-MS: *m/z*: (M + 1, 248.2); Anal. calcd for $C_{14}H_{11}NF_2O$: C, 68.01; H, 4.48; N, 5.67. Found: C, 68.02; H, 4.47; N, 5.66.

*1-(6-(4-Bromophenyl)-2-methylpyridine-3-yl)ethanone (**3b**)*

Off-white solid (yield 95%), m.p. 67–68 °C; IR (ATR) (cm^{-1}): 1667 (C=O), 1569 (C=N) and 797 (C–Br); ^{1}H NMR(400 MHz, $CDCl_3$, δ ppm): 2.52 (*s*, 3H, CO–CH_3), 2.78 (*s*, 3H, CH_3), 7.52 (*d*, 2H, $J = 8.7$ Hz, C_3 and C_5-H of 4-BrC_6H_4), 7.71 (*d*, 2H, $J = 8.6$ Hz, C_2 and C_6-H of 4-BrC_6H_4), 7.91 (*d*, 1H, $J = 7.1$ Hz, C_4-H of pyridine), 8.01 (*d*, 1H, $J = 7.2$ Hz, C_5-H of pyridine); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 199.8 (>C=O), 158.2, 157.7 138.2, 137.2, 135.3 131.7, 131.0, 124.4, 119, 29.23 (–CO–CH_3), 23.75 (–CH_3); EI-MS: *m/z* (M + 1, 290.2; M + 2, 292.06); Anal. Calcd for $C_{14}H_{12}NBrO$: C, 57.95; H, 4.17; N, 4.83. Found: C, 57.93; H, 4.18; N, 4.81.

General synthetic procedure for chalcones (4a–l)

A mixture of **3a**–**b** (0.06 mol), appropriate aromatic aldehyde (0.06 mol), and sodium methoxide (0.12 mol) in methanol (50 mL) was stirred at room temperature for about 12 h. It was then poured into ice cold water and neutralized with dil. HCl. The precipitate obtained was collected by filtration, washed with petroleum ether followed by water, dried and recrystallized from suitable solvents to afford **4a**–**l** in 70–90% yield.

*1-(6-(3,5-Difluorophenyl)-2-methylpyridin-3-yl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (**4a**)*

Pale yellow powder (yield 82%), m.p. 124–125 °C; IR (ATR) (cm^{-1}): 1658 (C=O), 1587 (C=C), 1437 (C=N), and 1116 (C–F); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.84 (*s*, 3H, –CH_3), 3.87 (*s*, 6H, –OCH_3), 6.79 (tt, 1H, $^1J = 8.8$ Hz, $^2J = 2.4$ Hz, C_4-H of 3,5-$F_2C_6H_3$), 6.98 (*d*, 1H, $J = 8$ Hz, C_4-H of 3,4-$(OCH_3)_2C_6H_3$), 7.12 (*d*, 1H, $J = 16$ Hz, H_b of $H_bC{=}CH_a$), 7.24 (*d*, 1H, $J = 2$ Hz C_2-H of 3,4-$(OCH_3)_2C_6H_3$), 7.39–7.42 (dd, 1H, $^1J = 8.2$ Hz, $^2J = 2$ Hz, C_6-H of 3,4-$(OCH_3)_2C_6H_3$), 7.46 (*d*, 1H, $J = 8.2$ Hz, C_4-H of pyridine), 7.56 (dd, 2H, $^1J = 8.2$ Hz and $^2J = 2.4$ Hz, C_2 and C_6-H of 3,5-$F_2C_6H_3$), 7.82–7.88 (overlapped dd, 2H, $J = 16$ Hz and 8 Hz, H_a of $H_bC{=}CH_a$ and C_5-H of pyridine); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 193.9 (C=O), 164.6 and 162.2 (1C, *d*, $^1J_{C-F} = 246.2$ Hz), 157.7, 155.3, 149.4, 149.1, 142.3, 141.8, 137, 133.5, 132.4, 125.8, 122.4, 117.1, 112.3, 110.4, 109.6 and 109.3 (2C, *d*, $^2J_{C-F} = 26.6$ Hz), 104.7 (1C, t, $^2J_{C-F} = 25.2$ Hz), 56.2 (–OCH_3), 56.1 (–OCH_3), 24.2 (–CH_3); EI-MS: *m/z* (M + 1, 396.16); Anal. calcd for $C_{23}H_{19}NF_2O_3$: C, 69.87; H, 4.84; N, 3.54. Found: C, 69.74; H, 4.82; N, 3.49.

*1-(6-(3,5-Difluorophenyl)-2-methylpyridin-3-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**4b**)*

Yellow solid (yield 94%), m.p. 141–142 °C; IR (ATR) (cm^{-1}): 1657 (C=O), 1580 (C=C), 1425 (C=N), and 1114 (C–F); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.81 (*s*, 3H, $–CH_3$), 3.76 (*s*, 3H, 4-(OCH_3), 3.79 (*s*, 6H, 3,5-$(OCH_3)_2$, 6.77 (tt, 1H, ^1J = 8.8 Hz, ^2J = 2.4 Hz, C_4-H of 3,5-$F_2C_6H_3$), 6.86 (*s*, 2H, C_2 and C_6-H of 3,4,5-$(OCH_3)_3C_6H_2$), 7.14 (*d*, 1H, J = 16 Hz, H_b of $H_bC{=}CH_a$), 7.43 (*d*, 1H, J = 8.2 Hz, C_4-H of pyridine), 7.54 (dd, 2H, J = 8.4 Hz and 2.2 Hz, C_2 and C_6-H of 3,5-$F_2C_6H_3$), 7.81–7.86 (overlapped dd, 2H, J = 16 Hz and 8 Hz H_a of $H_bC{=}CH_a$ and C_5-H of pyridine); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 193.2 (–C=O), 164.8 and 162.4 (1C, *d*, $^1J_{C–F}$ = 246.9 Hz), 157.7, 155.6, 153.6, 153.4, 149.6, 142.2, 141.6, 137.2, 132.6, 132.1, 125.9, 117.2, 110.1 and 109.8 (2C, *d*, $^2J_{C–F}$ = 25.9 Hz), 104.9 (1C, t, $^2J_{C–F}$ = 25 Hz), 103.2, 64.2 ($–OCH_3$), 58.2 ($–OCH_3$), 24.1($–CH_3$); EI-MS: *m/z* (M + 1, 425.53); Anal. calcd for $C_{24}H_{21}NF_2O_4$: C, 67.76; H, 4.98; N, 3.29. Found: C, 67.74; H, 4.99; N, 3.26.

*3-(2-Chloro-6-fluorophenyl)-1-(6-(3,5-difluorophenyl)-2-methylpyridin-3-yl)prop-2-en-1-one (**4c**)*

Off white solid (yield 89%), m.p. 133–134 °C; IR (ATR) (cm^{-1}): 1648 (C=O), 1592 (C=C), 1415 (C=N), 1110 (C–F) and 874 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.65 (*s*, 3H, $–CH_3$), 6.84 (tt, 1H, ^1J = 8.4 Hz, ^2J = 2.4 Hz, C_4-H of 3,5-$F_2C_6H_3$), 7.11 (*d*, 1H, J = 15.8 Hz, H_b of $H_bC{=}CH_a$), 7.16 (*m*, 1H, C_5-H of 2-Cl-6-F-C_6H_3), 7.25–7.31 (*m*, 3H, C_2, C_6-H of 3,5-$F_2C_6H_3$ and C_3-H of 2-Cl-6-F-C_6H_3), 7.51 (dd, 1H, ^1J = 8.1 Hz, ^2J = 7.3 Hz, C_4-H of 2-Cl-6-F-C_6H_3) 7.64–7.95 (*m*, 3H, H_a of $H_bC{=}CH_a$, C_4 and C_5-H of pyridine); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 192.9 (–C=O), 164.7 and 162.3 (1C, *d*, $^1J_{C–F}$ = 240 Hz), 161.9 and 159.5 (1C, *d*, $^1J_{C–F}$ = 240.4 Hz), 157.2, 155.2, 142.4, 140.9, 137.7, 134.5, 132.6, 132.2, 127.7, 125.9, 125.6, 117.2, 115.4, 110.5 and 110.1 (1C, *d*, $^2J_{C–F}$ = 25.3 Hz), 104.8 (1C, t, $^2J_{C–F}$ = 25.3 Hz), 23.9 ($–CH_3$); EI-MS: *m/z* (M + 1, 387.16; M + 2, 389.16); Anal. calcd for $C_{21}H_{13}NClF_3O$: C, 65.04; H, 3.38; N, 3.61. Found: C, 65.10; H, 3.32; N, 3.51.

*3-(2,3-Dichlorophenyl)-1-(6-(3,5-difluorophenyl)-2-methylpyridin-3-yl)prop-2-en-1-one (**4d**)*

Off-white solid (yield 96%), m.p. 101–102 °C; IR (ATR) (cm^{-1}): 1641 (C=O), 1590 (C=C), 1415 (C=N), 1114 (C–F) and 841 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.76 (*s*, 3H, $–CH_3$), 7.12 (*d*, 1H, J = 16 Hz, H_b of $H_bC{=}CH_a$), 7.91–9.95 (t, 2H, J = 16 Hz and 8 Hz H_a of $H_bC{=}CH_a$, and C_5-H of pyridine), 6.89 (tt, 1H, ^1J = 8.8 Hz, ^2J = 2.4 Hz, C_4-H of 3,5-$F_2C_6H_3$), 7.29 (*d*, 1H, ^1J = 8 Hz, C_6-H of 2,3-$Cl_2C_6H_3$), 7.54 (dd, 1H, ^1J = 8 Hz, ^2J = 1.6 Hz, C_5-H of 2,3-$Cl_2C_6H_3$), 7.59–7.64 (*m*, 4H, C_2, C_6-H of 3,5-$F_2C_6H_3$, C_4-H of pyridine and C_4-H of 2,3-$Cl_2C_6H_3$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 193.9 (–C=O), 164.7 and 162.2 (2C, *d*, $^1J_{C–F}$ = 246.8 Hz), 157.6, 155.6, 142.1, 141.8, 137.1, 134.9, 134.2, 133.5,

132.8, 132, 129.2, 127.5, 125.9, 117.1, 110.2 and 109.9 (2C, *d*, $^{2}J_{C-F} = 26.3$ Hz), 104.8 (1C, t, $^{2}J_{C-F} = 25.4$ Hz), 24.0 (–CH_3); EI-MS: *m/z* (M + 1, 404.13; M + 2, 406.12). Anal. calcd for $C_{21}H_{13}NCl_2F_2O$: C, 62.40; H, 3.24; N, 3.46. Found: C, 62.21; H, 3.32; N, 3.44.

*3-(4-Bromophenyl)-1-(6-(3,5-difluorophenyl)-2-methylpyridin-3-yl)prop-2-en-1-one (**4e**)*

Off-white solid (yield 95%), m.p. 142–143 °C; IR (ATR) (cm^{-1}): 1656 (C=O), 1560 (C=C), 1435 (C=N), 1467 (C=C), 1110 (C–F) and 741 (C–Br); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.67 (*s*, 3H, –CH_3), 6.89 (tt, 1H, $^{1}J = 8.2$ Hz, $^{2}J = 2.2$ Hz, C_4-H of 3,5-$F_2C_6H_3$), 7.14 (*d*, 1H, $J = 16$ Hz, H_b of $H_bC{=}CH_a$), 7.2–7.50 (*m*, 4H, C_2 and C_6-H of 3,5-$F_2C_6H_3$ and C_3 and C_5-H of 4-BrC_6H_4) 7.68–7.96 (*m*, 5H, H_a of $H_bC{=}CH_a$ and C_4, C_5-H of pyridine and C_2, C_6-H of 4-BrC_6H_4); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 193.4 (–C=O), 164.4 and 162.2 (1C, *d*, $^{1}J_{C-F} = 246.2$ Hz), 157.5, 155.4, 142.2, 141.6, 137.3, 132.8, 132.1, 131.9. 128.4, 125.7, 124.1, 117.2, 109.9 and 109.7 (2C, *d*, $^{2}J_{C-F} = 25.3$ Hz), 104.2 (1C, t, $^{2}J_{C-F} = 24.8$ Hz), 24.0 (–CH_3); EI-MS: *m/z* (M + 1, 414.22; M + 2, 415.12). Anal. calcd for $C_{21}H_{14}NBrF_2O$: C, 60.89; H, 3.41; N, 3.38. Found: C, 60.78; H, 3.39; N, 3.48.

*1-(6-(3,5-Difluorophenyl)-2-methylpyridin-3-yl)-3-(4-fluorophenyl)prop-2-en-1-one (**4f**)*

Brown solid (yield 80%), m.p. 93–94 °C; IR (ATR) (cm^{-1}): 1664 (C=O), 1548 (C=C), 1453 (C=N), 1462 (C=C) and 1116 (C–F); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.72 (*s*, 3H, –CH_3), 6.88 (tt, 1H, $^{1}J = 8.8$ Hz, $^{2}J = 2.4$ Hz, C_4-H of 3,5-$F_2C_6H_3$), 7.11 (*d*, 1H, $J = 16$ Hz, H_b of $H_bC{=}CH_a$), 7.26–7.64 (*m*, 5H, C_2 and C_6-H of 3,5-$F_2C_6H_3$, C_3 and C_5-H of 4-FC_6H_4 and C_4-H of pyridine) 7.72–7.98 (*m*, 4H, H_a of $H_bC{=}CH_a$ and C_5-H of pyridine and C_2, C_6-H of 4-FC_6H_4); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 193.2 (–C=O), 164.9 and 162.5 (1C, *d*, $^{1}J_{C-F} = 246.9$ Hz), 164.5 and 162.1 (1C, *d*, $^{1}J_{C-F} = 247.1$ Hz), 157.8, 155.4, 142.4, 141.2, 137.7, 132.8, 130.9, 130.1, 125.6, 117.4, 116.2 and 116.0 (2C, *d*, $^{2}J_{C-F} = 25.3$ Hz), 110.3 and 110.0 (2C, *d*, $^{2}J_{C-F} = 26.3$ Hz), 104.9 (1C, t, $^{2}J_{C-F} = 25.1$ Hz), 23.8 (–CH_3); EI-MS: *m/z* (M + 1, 353.34); Anal. calcd for $C_{21}H_{14}NF_3O$: C, 71.38; H, 3.99; N, 3.96. Found: C, 71.33; H, 3.89; N, 3.89.

*1-(6-(4-Bromophenyl)-2-methylpyridin-3-yl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (**4g**)*

Pale yellow solid (yield 90%), m.p. 120–122 °C; IR (ATR) (cm^{-1}): 1673 (C=O), 1578 (C=C), 1452 (C=N), 1463 (C=C) and 776 (C–Br); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.87 (*s*, 3H, –CH_3), 3.89(*s*, 6H, –OCH_3), 6.79 (*d*, 1H, $J = 16$ Hz, H_b of $H_bC{=}CH_a$), 7.53–7.70 (*m*, 6H, C_4-H of pyridine, C_3 and C_5-H of 4-BrC_6H_4 and 3,4-$(OCH_3)_2C_6\mathbf{H_3}$), 7.64–7.97(*m*, 4H, H_a of $H_bC{=}CH_a$ and C_2, C_6-H of 4-BrC_6H_4 and C_5-H of pyridine); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 193.9

(–C=O), 157.7, 157.2, 149.4, 148.9, 142.0, 137.3, 137.0, 132.2, 128.5, 128.4, 126.8, 125.7, 124.3, 118.6, 116.4, 111.3, 109.8, 56.1 and 56.0 (–OCH_3), 26.5 (–CH_3); EI-MS: *m/z* (M + 1, 438.21; M + 2, 439.21); Anal. calcd for $C_{23}H_{20}NBrO_3$: C, 63.02; H, 4.60; N, 3.20. Found: C, 63.12; H, 4.54; N, 3.24.

*1-(6-(4-Bromophenyl)-2-methylpyridin-3-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**4h**)*

Yellow solid (yield 93%), m.p. 80–82 °C; IR (ATR) (cm^{-1}): 1672 (C=O), 1585 (C=C), 1462 (C=N), 1483 (C=C) and 782 (C–Br); ^{1}H NMR (400 MHz, $CDCl_3$-*d*, δ ppm): 2.82 (*s*, 3H, –CH_3), 3.76 (*s*, 3H, 4-OCH3), 3.79 (*s*, 6H, 3.5-OCH_3), 6.86 (*s*, 2H, C_2 and C_6-H of 3,4,5-$(OCH_3)_3C_6H_2$), 7.12 (*d*, 1H, J = 16 Hz, H_b of $H_bC{=}CH_a$), 7.50–7.70 (*m*, 3H, C_4-H of pyridine and C_3, C_5-H of 4-BrC_6H_4), 7.86–7.97 (*m*, 4H, H_a of $H_bC{=}CH_a$ and C_2, C_6-H of 4-BrC_6H_4 and C_5-H of pyridine); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 193.8 (–C=O), 157.7, 157.4, 153, 152.9, 149.6, 142.2, 137.2, 137, 132.4, 131, 130.2, 129.1, 125.2, 124.2, 116.8, 106.3, 60.5 (–OCH_3), 56.2 (–OCH_3), 24.3 (–CH_3); EI-MS: *m/z* (M + 1, 467.06; M + 2, 469.08); Anal. calcd for $C_{24}H_{22}NBrO_4$: C, 61.55; H, 4.73; N, 2.99. Found: C, 61.49; H, 4.71; N, 2.93.

*1-(6-(4-Bromophenyl)-2-methylpyridin-3-yl)-3-(2-chloro-6-fluorophenyl)prop-2-en-1-one (**4i**)*

Brown solid (yield 82%), m.p. 108–109 °C; IR (ATR) (cm^{-1}): 1654 (C=O), 1575 (C=C), 1458 (C=N), 1473 (C=C), 948 (C–Cl) and 776 (C–Br); ^{1}H NMR (400 MHz, $CDCl_3$-*d*, δ ppm): 2.79 (*s*, 3H, –CH_3) 7.14 (*d*, 1H, J = 16 Hz, H_b of $H_bC{=}CH_a$), 7.18–7.50 (*m*, 5H, C_3, C_5-H of 4-BrC_6H_4 and C_3, C_4 and C_5-H of 3-Cl-6-FC_6H_3), 7.69–7.98 (*m*, 5H, H_a of $H_bC{=}CH_\mathbf{a}$ and C_4, C_5-H of pyridine and C_2, C_6-H of 4-BrC_6H_4); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 193.9 (–C=O), 164.2 and 162.2 (1C, *d*, $^1J_{C-F}$ = 244.9 Hz), 157.7, 157.2, 141.2, 137.4, 137.1, 134.2, 131.9, 131, 130.9, 129.4, 126.1, 125.0, 124.1, 117, 116.4 and 116.1 (1C, *d*, $^2J_{C-F}$ = 25.7 Hz), 26.1 (–CH_3); MS-EI: *m/z* (M + 1, 428.06; M + 2, 430.09; M + 4, 432.05); Anal. calcd for $C_{21}H_{14}NBrClFO$: C, 58.56; H, 3.28; N, 3.25. Found: C, 58.62; H, 3.27; N, 3.30.

*1-(6-(4-Bromophenyl)-2-methylpyridin-3-yl)-3-(2,3-dichlorophenyl)prop-2-en-1-one (**4j**)*

Off-white solid (yield 92%), m.p. 152–153 °C; IR (ATR) (cm^{-1}): 1666 (C=O), 1585 (C=C), 1442 (C=N), 1453 (C=C), 813 (C–Cl) and 778 (C–Br); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.76 (*s*, 3H, –CH_3) 7.12 (*d*, 1H, J = 16 Hz, H_b of $H_bC{=}CH_a$), 7.53–7.70 (*m*, 6H, C_4-H of pyridine, C_3, C_5-H of 4-BrC_6H_4 and 2,3-$Cl_2C_6\mathbf{H_3}$) 7.89–7.97 (*m*, 4H, H_a of $H_bC{=}CH_a$ and C_2, C_6-H of 4-BrC_6H_4 and C_5-H of pyridine); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 193.9 (–C=O), 157.7, 157.1, 141, 137.1, 137, 135.0, 134.2, 133.4, 132, 131, 129, 128.8, 128, 127, 125, 124.2, 116, 24.1 (–CH_3); EI-MS: *m/z* (M + 1, 446.06; M + 2, 448.06; M + 4, 450.05).

Anal. calcd for $C_{21}H_{14}NCl_2O$: C, 56.41; H, 3.16; N, 3.13. Found: C, 56.42; H, 3.12; N, 3.16.

*3-(4-Bromophenyl)-1-(6-(4-bromophenyl)-2-methylpyridin-3-yl)prop-2-en-1-one (**4k**)*

Off-white solid (yield 84%), m.p. 132–133 °C; IR (ATR) (cm^{-1}): 1656 (C=O), 1552 (C=C), 1439 (C=N), 1453 (C=C), and 847 (C–Br); ^{1}H NMR (400 MHz, $CDCl_3$-*d*, δ ppm): 2.87 (*s*, 3H, $–CH_3$) 7.14 (*d*, 1H, $J = 16$ Hz, H_b of $H_bC{=}CH_a$), 7.32–7.48 (*m*, 4H, C_3 and C_5-H of 4-BrC_6H_4), 7.53–7.72 (*m*, 5H, C_2, C_6-H of 4-BrC_6H_4 and C_4-H of pyridine), 7.89–7.95 (*d* (overlapped), 2H, H_a of $H_bC{=}CH_a$ and C_5-H of pyridine); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 193.2 (–C=O), 157.9, 156.9, 141.6, 137.9, 137.7, 137.1, 131.8, 131.5, 131.4, 129.6, 128.9, 124.3, 124.2, 123.2, 116.9, 24.3 ($–CH_3$); EI-MS: *m*/*z* (M + 1, 454.36; M + 2, 456.36; M + 4 458.35); Anal. calcd for $C_{21}H_{15}NBr_2O$: C, 55.17; H, 3.31; N, 3.06. Found: C, 55.27; H, 3.32; N, 3.04.

*1-(6-(4-Bromophenyl)-2-methylpyridin-3-yl)-3-(4-fluorophenyl)prop-2-en-1-one (**4l**)*

Brown powder (yield 77%), m.p. 139–140 °C; IR (ATR) (cm^{-1}): 1647 (C=O), 1568 (C=C), 1442 (C=N), 1463 (C=C), 1108 (C–F) and 867 (C–Br); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.76 (*s*, 3H, $–CH_3$) 7.15 (*d*, 1H, $J = 16$ Hz, H_b of $H_bC{=}CH_a$), 7.23 (*m*, 2H, C_3 and C_5-H of 4-FC_6H_4), 7.47–7.78 (*m*, 5H, 4H'*s* of 4-BrC_6H_4 and C_4-H of pyridine), 7.80–7.98 (*m*, 4H, H_a of $H_bC{=}CH_a$ and C_2, C_6-H of 4-FC_6H_4 and C_5-H of pyridine); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 193.9 (–C=O), 164.4 and 162.2 (1C, *d*, C–F, $^1J = 248.2$ Hz), 157.2, 156.2, 142.2, 137.7, 137.2, 131.8, 131.4, 130.9, 129.9, 128.9, 124.6, 123.3, 116.7, 114.8 and 114.6 (2C, *d*, C–F, $^2J = 25.2$ Hz), 23.9 ($–CH_3$); EI-MS: *m*/*z* (M + 1, 396.21; M + 2, 397.21); Anal.calcd for $C_{21}H_{15}NBrFO$: C, 63.65; H, 3.82; N, 3.53. Found: C, 63.67; H, 3.79; N, 3.51.

General synthetic procedure for 2-pyrazolines (5a–l)

Hydrazine hydrate (98%) (2 mL) was added to a suspension of appropriate chalcone (**4a–l**) (5 mmol) in ethanol and a catalytic amount of dil. HCl. The reaction mixture was heated under reflux condition for 3 h. Completion of the reaction was monitored using TLC. After completion of the reaction, reaction mixture was concentrated to small volume and left at room temperature for 24 h to give solid compound. It was then filtered and recrystallized with ethanol to afford a corresponding yield 6-(aryl)-2-methyl-3-(5-(aryl)-4,5-dihydro-1*H*-pyrazol-3yl)pyridines (**5a–l**) in 57–93%.

*6-(3,5-Difluorophenyl)-2-methyl-3-(5-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-3yl)pyridine (**5a**)*

Off-white solid (yield 92%), m.p. 117–119 °C; IR (ATR) (cm^{-1}): 32,760 (NH-sharp), 1585 (C=N), 1457 (C=C) and 1121 (C–F); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.89 (*s*, 3H, CH_3), 3.08 (dd, 1H, H_B, $H_{BA} = 16$ Hz, $H_{BX} = 9.2$ Hz), 3.48 (dd, 1H, H_A, $H_{AB} = 16.2$ Hz, $H_{AX} = 10.6$ Hz), 3.88 (*s*, 6H, 3,4-$(OCH_3)_2$), 4.90 (t, 1H, H_X, $J = 9.6$ Hz), 6.83–6.86 (*m*, 2H, C_2-H of 3,4-$(OCH_3)_2$ C_6H_2 and C_4-H of 3,5-$F_2C_6H_3$), 6.90–6.92 (dd, 1H, $^1J = 8.4$ Hz, $^2J = 2$ Hz, C_6-H of 3,4-$(OCH_3)_2C_6H_2$), 6.96 (*d*, 1H, $J = 8.2$ Hz, C_5-H of 3,4-$(OCH_3)_2C_6H_2$) 7.55 (*d*, 1H, $J = 8$ Hz, C_4-H of pyridine), 7.60 (*m*, 2H, C_2 and C_6-H of 3,5-$F_2C_6H_3$), 7.70 (*d*, 1H, $J = 8.4$ Hz, C_5-H of pyridine); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 164.6 and 162.2 (2C, *d*, $^1J_{C–F} = 246.2$ Hz), 157.2, 152.4, 149.6, 149.3, 148.8, 142.3, 136.2, 134.6, 127.1, 118.6, 117.3, 111.3, 109.7 and 109.4 (2C, *d*, $^2J_{C–F} = 26.2$ Hz), 109.2, 104 (1C, t, $^2J_{C–F} = 25.5$ Hz) 64.1, 55.9, 43.4, 26.3 (**C**H_3); EI-MS: *m/z* (M + 1, 409.53); Anal.calcd. for $C_{23}H_{21}N_3F_2O_2$: C, 67.47; H, 5.17; N, 10.26. Found: C, 67.42; H, 5.07; N, 10.28.

*6-(3,5-difluorophenyl)-2-methyl-3-(5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl) pyridine (**5b**)*

Off-white solid (yield 90%), m.p. 124–126 °C; IR (ATR) (cm^{-1}): 3290 (NH-sharp), 1587 (C=N), 1453 (C=C) and 1123 (C–F); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.82 (*s*, 3H, CH_3), 3.02 (dd, 1H, H_B, $H_{BA} = 16.2$ Hz, $H_{BX} = 9.8$ Hz), 3.45 (dd, 1H, H_A, $H_{AB} = 16$ Hz, $H_{AX} = 10.4$ Hz), 3.77 (*s*, 3H, 4-OCH_3), 3.79 (*s*, 6H, 3,5-$(OCH_3)_2$, 4.81 (t, 1H, H_X, $J = 10.2$ Hz), 6.56 (*s*, 2H, C_2 and C_6-H of 3,4,5-$(OCH_3)_3C_6H_2$), 6.75 (tt, 1H, $J = 8.4$ Hz and 2.4 Hz, C_4-H of 3,5-$F_2C_6H_3$), 7.47 (*d*, 1H, $J = 8$ Hz, C_4-H of pyridine), 7.52 (*m*, 2H, C_2 and C_6-H of 3,5-$F_2C_6H_3$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 164.6 and 162.2 (2C, *d*, $^1J_{C–F} = 246$ Hz), 157.2, 153.6, 152.4, 149.6, 142.3, 137.7, 136.9 136.2, 127.0, 117.3, 109.7 and 109.4 (2C, *d*, $^2J_{C–F} = 26.2$ Hz), 104.1 (1C, t, $^2J_{C–F} = 25.2$ Hz), 103.2, 64.6, 60.8, 56.1, 43.6, 26.3 (–CH_3); EI-MS: *m/z* (M + 1, 440.27); Anal. calcd for $C_{24}H_{23}N_3F_2O_3$: C, 65.59; H, 5.28; N, 9.56. Found: C, 65.53; H, 5.22; N, 9.59.

*3-(5-(2-chloro-6-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-(3,5-difluorophenyl)-2-methylpyridine (**5c**)*

Brown crystals (yield 69%), m.p. 136–138 °C; IR (ATR) (cm^{-1}): 3293 (NH-sharp), 1581 (C=N), 1453 (C=C), 1123 (C–F) and 743 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.84 (*s*, 3H, CH_3), 3.04 (dd, 1H, H_B, $H_{BA} = 16$ Hz, $H_{BX} = 9.6$ Hz), 3.42 (dd, 1H, H_A, $H_{AB} = 16.2$ Hz, $H_{AX} = 10.6$ Hz), 4.84 (t, 1H, H_X, $J = 10.4$ Hz), 6.73 (tt, 1H, $J = 8.2$ Hz and 2.4 Hz, C_4-H of 3,5-$F_2C_6H_3$), 6.92 (*m*, 1H, C_5-H of 2-Cl-6-F-C_6H_3), 7.13 (dd, 1H, $J = 8.2$ Hz and 1.4 Hz, C_3-H of 2-Cl-6-F-C_6H_3), 7.33–7.37 (*m*, 3H, C_2 and C_6-H of 3,5-$F_2C_6H_3$ and C_4-H of 2-Cl-6-F-C_6H_3), 7.47 (*d*, 1H, $J = 8$ Hz, C_4-H of pyridine), 7.68 (*d*, 1H, $J = 8.4$ Hz, C_5-H of pyridine); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 164.6 and 162.2 (2C, *d*, $^1J_{C–F} = 246$ Hz), 161.8 and

158.1 (1C, *d*, $^{1}J_{C–F} = 240$ Hz) 157.3, 152.6, 149.2, 136.4, 134.6, 133.4, 129.8, 129.2, 127.1, 126.3, 117.3, 114.2 and 113.9 (1C, *d*, $^{2}J_{C–F} = 25.5$ Hz), 109.7, 109.4 and 109.1 (2C, *d*, $^{2}J_{C–F} = 24.6$ Hz), 104.1 (1C, t, $^{2}J_{C–F} = 25.2$ Hz), 64.2, 43.6, 26.2 (–CH_3); MS-EI: *m/z* (M + 1, 401.91; M + 2, 403.91); Anal. calcd for $C_{21}H_{15}N_3ClF_3$: C, 62.77; H, 3.76; N, 10.46. Found: C, 62.57; H, 3.66; N, 10.56.

*3-(5-(2,3-Dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-(3,5-difluorophenyl)-2-methylpyridine (**5d**)*

Off-white crystals (yield 93%), m.p. 125–127 °C; IR (ATR) (cm^{-1}): 3308 (NH-sharp), 1585 (C=N), 1443 (C=C), 1112 (C–F) and 811 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.87 (*s*, 3H, –CH_3), 2.95 (dd, 1H, H_B, $H_{BA} = 16.2$ Hz, $H_{BX} = 9.6$ Hz), 3.74 (dd, 1H, H_A, $H_{AB} = 16.4$ Hz, $H_{AX} = 10.4$ Hz), 5.37 (t, 1H, H_X, $J = 10.2$ Hz), 6.83 (tt, 1H, $^{1}J = 8.8$ Hz, $^{2}J = 2.4$ Hz, C_4-H of 3,5-$F_2C_6H_3$), 7.26 (t, 1H, $J = 4$ Hz C_6-H of 2,3,-($Cl_2C_6H_3$), 7.43 (dd, 1H,^{1} $J = 8$ Hz, $^{2}J = 1.2$ Hz, C_5-H of 2,3-$Cl_2C_6H_3$) 7.51 (*d*, 1H, $J = 8.4$ Hz, C_4-H of pyridine), 7.56–7.59 (*m*, 3H, C_2 and C_6-H of 3,5-$F_2C_6H_3$ and C_4-H of 2,3-$Cl_2C_6H_3$), 7.68 (*d*, 1H, $J = 8.4$ Hz, C_5-H of pyridine); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 164.6 and 162.2 (2C, *d*, $^{1}J_{C–F} = 240$ Hz), 157.3, 152.5, 149.3, 141.9, 137.6, 136.3, 133.4, 130.9, 129.6, 127.8, 126.8, 125.1, 117.3, 109.8 and 109.5 (2C, *d*, $^{2}J_{C–F} = 26.3$ Hz), 109.7 104.1 (1C, t, $^{2}J_{C–F} = 25.3$ Hz), 61.4, 41.7, 26.2 (–CH_3); EI-MS: *m/z* (M + 1 418.16; M + 2 420.16; M + 4 422.16); Anal. calcd for $C_{21}H_{15}N_3Cl_2F_2$: C, 60.30; H, 3.61; N, 10.05. Found: C, 60.29; H, 3.63; N, 10.10.

*3-(5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-(3,5-difluorophenyl)-2-methylpyridine (**5e**)*

Off-white crystals (yield 74%), m.p. 90–92 °C; IR (ATR) (cm^{-1}): 3301 (NH-sharp), 1587 (C=N), 1453 (C=C), 1123 (C–F) and 767 (C–Br); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.89 (*s*, 3H, –CH_3), 3.10 (dd, 1H, H_B, $H_{BA} = 16$ Hz, $H_{BX} = 9.8$ Hz), 3.72 (dd, 1H, H_A, $H_{AB} = 16.2$ Hz, $H_{AX} = 10.2$ Hz), 5.36 (t, 1H, H_X, $J = 10.2$ Hz), 6.86 (tt, 1H, $J = 8.6$ Hz and 2.4 Hz, C_4-H of 3,5-$F_2C_6H_3$), 7.35–7.42 (*m*, 6H, C_2 and C_6-H of 3,5-$F_2C_6H_3$ and 4-BrC_6H_4) 7.52 (*d*, 1H, $J = 8.2$ Hz, C4-H of pyridine), 7.69 (*d*, 1H, $J = 8$ Hz, C_5-H of pyridine); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 164.6 and 162.2 (2C, *d*, $^{1}J_{C–F} = 244$ Hz), 157.2, 154, 149.6, 139.4, 137.7, 136.2, 133.4, 128.8, 127.7, 123.4, 117.4, 109.6 and 109.3 (2C, *d*, $^{2}J_{C–F} = 24.6$ Hz), 104.2 (1C, t, $^{2}J_{C–F} = 25.6$ Hz), 62.1, 43.2, 26.2 (CH_3); EI-MS: *m/z* (M + 1, 427.10; M + 2, 429.10); Anal. calcd for $C_{21}H_{16}N_3BrF_2$: C, 58.89; H, 3.77; N, 9.81. Found: C, 58.86; H, 3.65; N, 9.80.

*6-(3,5-difluorophenyl)-2-methyl-3-(5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)pyridine (**5f**)*

Off-white crystals (yield 93%), m.p. 108–112 °C; IR (ATR) (cm^{-1}): 3299 (NH-sharp), 1578 (C=N), 1453 (C=C) and 1112 (C–F); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.36 (*s*, 3H, –CH_3), 3.02 (dd, 1H, H_B, $H_{BA} = 16.2$ Hz, $H_{BX} = 9.6$ Hz), 3.23

(dd, 1H, H_A, H_{AB} = 16.2 Hz, H_{AX} = 10.6 Hz), 5.32 (t, 1H, H_X, J = 10.6 Hz), 6.79 (tt, 1H, ^1J = 8.8 Hz, ^2J = 2.2 Hz, C_4-H of 3,5-$F_2C_6H_3$), 6.98 (sextet, 2H, ^1J = 8.8 Hz, ^2J = 1.2 Hz, C_3 and C_5-H of 4-FC_6H_4), 7.30 (t, 2H, ^1J = 8 Hz, C_2 and C_6-H of 4-FC_6H_4), 7.47 (*m*, 2H, C_2 and C_6-H of 3,5-$F_2C_6H_3$), 7.68 (*d*, 1H, J = 8.4 Hz, C_4-H of pyridine), 7.98 (*d*, 1H, J = 8.2 Hz, C_5-H of pyridine); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 164.6 and 162.2 (2C, *d*, $^1J_{C-F}$ = 240 Hz), 162.8 and 160.4 (1C, *d*, $^1J_{C-F}$ = 240 Hz), 157.2, 153.1, 149.3, 140.1, 136.6, 132.2, 128.6, 127.2, 117.4, 114.6 and 114.4 (1C, *d*, $^2J_{C-F}$ = 26.2 Hz), 109.8 and 109.5 (2C, *d*, $^2J_{C-F}$ = 25.8 Hz), 104.2 (1C, t, $^2J_{C-F}$ = 25.3 Hz), 63.9, 43, 26.3 (–CH_3); EI-MS: *m*/*z* (M + 1, 367.57); Anal. calcd for $C_{21}H_{16}N_3F_3$: C, 68.66; H, 4.39; N, 11.44. Found: C, 68.56; H, 4.42; N, 11.41.

*6-(4-Bromophenyl)-2-methyl-3-(5-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)pyridine (**5g**)*

Off-white solid (yield 87%), m.p. 116–119 °C; IR (ATR) (cm-1): 3321 (NH-sharp), 1585 (C=N), 1458 (C=C), 1255 (C-O) and 750 (C–Br); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.89 (*s*, 3H, –CH_3), 3.09 (dd, 1H, H_B, H_{BA} = 16 Hz, H_{BX} = 9.6 Hz), 3.50 (dd, 1H, H_A, H_{AB} = 16.2 Hz, H_{AX} = 10.4 Hz), 3.87 (*s*, 6H, –OCH_3), 4.88 (t, 1H, H_X, J = 10 Hz), 6.84 (*d*, 1H, J = 8 Hz, C_5-H of 3,4-$(OCH_3)_2C_6H_3$) 6.89–6.91 (dd, 1H, ^1J = 8 Hz, ^2J = 2 Hz, C_6-H of 3,4-$(OCH_3)_2$-C_6H_3), 6.96 (*d*, 1H, J = 2 Hz, C_2-H of 3,4-$(OCH_3)_2C_6H_3$), 7.53–7.66 (*m*, 4H, 4H's of 4-BrC_6H_4), 7.93 (dd, 1H, J = 6.8 Hz, C_4 and C_5-H of pyridine); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 157.1, 153.9, 149.9, 149.3, 148.7, 137.8, 136.2, 134.7, 131.84, 128.4, 126.3, 123.4, 118.6, 117.0, 111.3, 109.2, 64.1, 56.0, 55.9, 43.5, 26.3 (–CH_3); EI-MS: *m*/*z* (M + 1, 452.19; M + 2, 453.18); Anal. calcd for $C_{23}H_{22}N_3$-BrO_2: C, 61.07; H, 4. 90; N, 9.29. Found: C, 61.12; H, 4. 87; N, 9.31.

*6-(4-Bromophenyl)-2-methyl-3-(5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)pyridine (**5h**)*

Off-white crystals (yield 86%), m.p. 134–136 °C; IR (ATR) (cm^{-1}): 3294 (NH-sharp), 1586 (C=N), 1457 (C=C), 1239 (C-O) and 687 (C–Br); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.87 (*s*, 3H, –CH_3), 2.95 (dd, 1H, H_B, H_{BA} = 16.4 Hz, H_{BX} = 10.2 Hz), 3.42 (dd, 1H, H_A, H_{AB} = 16.2 Hz, H_{AX} = 10.4 Hz), 3.76 (*s*, 3H, 4-OCH_3), 3.78 (*s*, 6H, 3,5-$(OCH_3)_2$, 4.91 (t, 1H, H_X, J = 10.3 Hz), 6.53 (*s*, 2H, C_2 and C_6-H of 3,4,5-(OCH3)$_3C_6H_2$), 7.52–7.58 (*m*, 3H, C_3 and C_5-H of 4-BrC_6H_4 and C_4-H of pyridine), 7.60 (dd, 2H, ^1J = 8.5 Hz, ^2J = 1.4 Hz, C_2 and C_6-H of 4-BrC_6H_4), 7.96 (*d*, 1H, J = 6 Hz, C_5-H of pyridine); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 161.0, 159.8, 152.6, 152.4, 151.1, 138.5, 137.8, 135.3, 132.2, 131.0, 127.8, 123.4, 125.2, 117.2, 64.6, 61.4, 56.2, 42.8, 26.3 (–CH_3); EI-MS: *m*/*z* (M + 1, 482.40; M + 2, 483.16); Anal. calcd for $C_{24}H_{24}N_3BrO_3$: C, 59.76; H, 5.01; N, 8.71. Found: C, 59.56; H, 5.10; N, 8.61.

*6-(4-Bromophenyl)-2-methyl-3-(5-(2-chloro-6-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)pyridine (**5i**)*

Off-white solid (yield 79%), m.p. 140–142 °C; IR(ATR) (cm^{-1}): 3298 (NH-sharp), 1586 (C=N), 1443 (C=C), 1123 (C–F), 997 (C–Cl) and 799 (C–Br); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.36 (*s*, 3H, $–CH_3$), 2.98 (dd, 1H, H_B, H_{BA} = 16.4 Hz, H_{BX} = 10.4 Hz), 3.28 (dd, 1H, H_A, H_{AB} = 16.2 Hz, H_{AX} = 10.2 Hz), 5.32 (t, 1H, H_X, J = 10.3 Hz), 6.92 (dd, 1H, ^1J = 8.3 Hz, ^2J = 1.2 Hz, C_5-H of 2-Cl-6-FC_6H_3), 7.01 (dd, 1H, ^1J = 8.6 Hz, ^2J = 1.2 Hz, C_3-H of 2-Cl-6-FC_6H_3) 7.32 (t, 1H, ^1J = 8.3 Hz, C_4-H of 2-Cl-6-FC_6H_3), 7.52 (*d*, 2H, J = 8.5 Hz, C_3 and C_5-H of 4-BrC_6H_4), 7.62–7.67 (*m*, 3H, C_2 and C_6-H of 4-BrC_6H_4 and C_4-H of pyridine), 7.96 (*d*, 1H, J = 5.7 Hz, C_5-H of pyridine); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 164.1, 162.3, 158.8, 151.6, 137.3, 132.6, 129.8, 127.6, 127.4, 127.0, 124.3, 124.02, 122.5, 121.3, 114.2, 60.0, 43.08, 23.5 ($–CH_3$); EI-MS: *m/z* (M + 1, 443.03; M + 2, 445.03; M + 4, 447.03); Anal. calcd for $C_{21}H_{16}N_3BrClF$: C, 56.71; H, 3.63; N, 9.45. Found: C, 56.70; H, 3.58; N, 9.30.

*6-(4-Bromophenyl)-2-methyl-3-(5-(2,3-dichlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)pyridine (**5j**)*

Off-white solid (yield 80%), m.p. 96–99 °C; IR (ATR) (cm^{-1}): 3279 (NH-sharp), 1576 (C=N), 1453 (C=C), 799 (C–Cl) and 583 (C–Br); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.88 (*s*, 3H, $–CH_3$), 2.94 (dd, 1H, H_B, H_{BA} = 16.4 Hz, H_{BX} = 10.4 Hz), 3.73 (dd, 1H, H_A, H_{AB} = 16.4 Hz, H_{AX} = 10.8 Hz), 5.37 (t, 1H, H_X, J = 10.0 Hz), 7.22–7.24 (dd, 1H, ^1J = 7.8 Hz, ^2J = 1.2 Hz, C_6-H of 2,3-$Cl_2C_6H_3$), 7.40–7.43 (dd, 1H, ^1J = 8 Hz, ^2J = 1.6 Hz, C_5-H of 2,3-$Cl_2C_6H_3$) 7.52–7.65 (*m*, 5H, 4H's of 4-BrC_6H_4 and C_4-H of 2,3-$Cl_2C_6H_3$), 7.92 (dd, 2H, ^1J = 6.8 Hz, ^2J = 1.6 Hz, C_4 and C_5-H of pyridine), ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 157.2, 154.0, 149.6, 142.6, 137.7, 136.2, 133.4, 131.8, 130.9, 129.5, 128.4, 127.7, 125.9, 125.2, 123.4, 117.0, 61.3, 41.7, 26.3 ($–CH_3$); EI-MS: *m/z* (M + 1, 460.04; M + 2, 462.04; M + 4, 464.04); Anal. calcd for $C_{21}H_{16}N_3BrCl_2$: C, 54. 69; H, 3.50; N, 9.11. Found: C, 54. 62; H, 3.51; N, 9.10.

*6-(4-Bromophenyl)-2-methyl-3-(5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)pyridine (**5k**)*

Off-white solid (yield 79%), m.p. 106–108 °C; IR (ATR) (cm^{-1}): 3309 (NH-sharp), 1569 (C=N), 1443 (C=C) and 687 (C–Br); ^{1}H NMR(400 MHz, $CDCl_3$, δ ppm): 2.36 (*s*, 3H, $–CH_3$), 2.98 (dd, 1H, H_B, H_{BA} = 16 Hz, H_{BX} = 10.2 Hz), 3.15 (dd, 1H, H_A, H_{AB} = 16.2 Hz, H_{AX} = 10.6 Hz), 5.27 (t, 1H, H_X, J = 10.0 Hz), 7.32 (*d*, 2H, J = 7.8 Hz, C_2 and C_6-H of 4-BrC_6H_4), 7.40–7.46 (*m*, 4H, C_3, C_5-H and C_3', C_5'-H of 4-BrC_6H_4) 7.50–7.62 (*m*, 3H, C_2, C_6-H of 4-BrC_6H_4 and C_4-H of pyridine), 7.98 (*d*, 1H, J = 8.4 Hz, C_5-H of pyridine); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 157.4, 154, 149.4, 139.2, 137.6, 136.4, 133.3, 131, 129.9, 128.4, 127.6, 123.3, 123.1, 117.1, 61.4, 43.2, 26.4 ($–CH_3$); EI-MS: *m/z* (M+, 468.48; M+ 2, 470.98; M + 4,

472.8); Anal. calcd for $C_{21}H_{17}N_3Br_2$: C, 53.53; H, 3. 64; N, 8.92. Found: C, 53.49; H, 3. 60; N, 8.90.

*6-(4-Bromophenyl)-2-methyl-3-(5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)pyridine (**5l**)*

Off-white solid (yield 57%), m.p. 143–145 °C; IR (cm^{-1}): 3289 (NH-sharp), 1587 (C=N), 1453 (C=C), 1123 (C–F) and 691 (C–Br); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.50 (*s*, 3H, $–CH_3$), 2.98 (dd, 1H, H_B, $H_{BA} = 16.2$ Hz, $H_{BX} = 10.6$ Hz), 3.20 (dd, 1H, H_A, $H_{AB} = 16.2$ Hz, $H_{AX} = 10$ Hz), 5.32 (t, 1H, H_X, $J = 10.3$ Hz), 6.92 (*m*, 2H, C_3 and C_5-H of 4-FC_6H_4), 7.10 (*m*, 2H, C_2 and C_6-H of 4-FC_6H_4), 7.40 (*d*, 2H, $J = 8.5$ Hz, C_3 and C_5-H of 4-BrC_6H_4) 7.52–7.60 (*m*, 3H, C_2, C_6-H of 4-BrC_6H_4 and C_4-H of pyridine), 7.96 (*d*, 1H, $J = 8.2$ Hz, C_5-H of pyridine); ^{13}C NMR(100 MHz, $CDCl_3$, δ ppm): 164.2 and 162.6 (1C, *d*, $^1J_{C–F} = 246.2$ Hz), 157.3, 154.1, 149.2, 142.2, 136.7, 136.2, 131.8, 128.4, 128.2, 127.7, 117.4, 114.4 and 114.2 (2C, *d*, $^2J_{C–F} = 26.2$ Hz), 63.6, 43.2, 26.2 ($–CH_3$); EI-MS: *m/z* (M +, 409.6; M + 2, 411.08); Anal. calcd for $C_{21}H_{17}N_3BrF$: C, 61.48; H, 4. 18; N, 10.24. Found: C, 61.52; H, 4. 16; N, 10.29.

Pharmacological screening

Animals

Male Swiss albino mice weighing 25–30 g were used for the present study. They were maintained under standard environmental conditions of 22 ± 3 °C and 65 ± 55% relative humidity, and during the whole experiment, they were exposed to a 12-h light and dark cycle. Animals were given standard food pellets, and water was supplied ad libitum.

Forced swimming test (FST)

The behavioral despair test (forced swimming test) was used to evaluate the anti-depressant activity of newly synthesized 4,5-dihydropyrazoline derivatives. The tested compounds and reference drug (imipramine) were suspended in 2% aqueous solution of Tween 80. On the testing day, mice were assigned into different groups (n = 6 for each group). All the tested compounds (**5a–l**) (100 mg kg^{-1}) and standard drug Imipramine (10 mg kg^{-1}) were administered orally to mice at a volume of 0.5 mL/body weight. Control animals were similarly treated with 2% aqueous solution of Tween 80. After 1 h, the mice were dropped at a time into flexi glass cylinder (25 cm height), 30 cm diameter containing water to a height of 20 cm at 21–23 °C and left for 6 min. At the end of the first 2 min, the animal showing initial vigorous struggling was considered immobile. The duration of immobility was recorded during the last 4 min to total a 6-min test.

Tail suspension test (TST)

The effectiveness of synthesized compounds was re-evaluated in a tail suspension test (TST). After 1 h of drug application, mice were suspended on the edge of the table, 50 cm above the floor with the help of adhesive tape placed 1 cm from the tip of the tail, for a period of 6 min. Immobility time was measured during the 6-min period. Mice were considered immobile when they did not show any body movement while hanging passively and completely motionless. The percentage decrease in immobility duration (%DID) is considered the indicator of the anti-depressant-like activity [37]. Standard drug used was imipramine at a dose level of 10 mg kg^{-1}.

Statistical analysis

The data obtained by pharmacological studies were analyzed by one-way analysis of variance (ANOVA) followed by Dunnet'*s* test and used to evaluate the results by GraphPad Prism software version 5.0. The experimental results were expressed as mean ± SEM. *n* represents the number of animals. A *p* value of less than 0.05 was considered statistically significant.

Percentage decrease in immobility duration (% DID) for test and standard drugs was calculated using following relation:

$$\%\,\mathrm{DID} = (A - B)A \times 100,$$

where *A* is the duration of immobility (*s*) in the control group, and B is the duration of immobility (*s*) in the test group.

Ethics

All procedures involving animals were carried out as per OECD guidelines, and the animal treatment protocol was approved by Reg. No. SCSCP/IEEC/09/2016-17.

Anti-TB assay

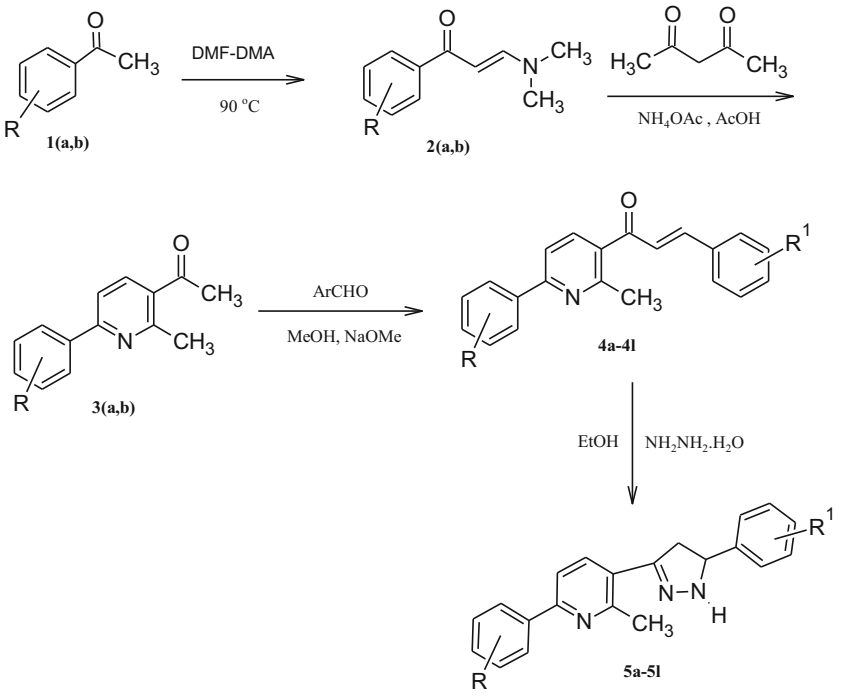
The inhibition effect of the target compounds against *M. tuberculosis* H37Rv was tested using a Microplate Alamar Blue Assay (MABA) technique according to the reported method [38]. This methodology shows good correlation with the BACTEC radiometric method. This technique is nontoxic and uses a thermally stable reagent. Here, Middlebrook 7H9 broth base with Middlebrook OADC growth supplement was used as medium. Two hundred microliters of sterile deionized water was added to all outer perimeter wells of a sterile 96-well plate to minimize evaporation of medium in the test wells during incubation. The 96-well plate received 100 µL of the Middlebrook 7H9 broth. One milligram of each compound was dissolved in 1 mL of DMSO separately, and to make stock solutions of 100 µg mL^{-1} and 10 µg mL^{-1}, it was further diluted with DMSO. Using the stock solution, serial dilutions of 50, 25, 12.5, 6.25, 3.12, 1.6, 0.8, 0.4, and 0.2 µg mL^{-1} were made with

DMSO directly on the 96-well plate. We further inoculated the test organism with a medium containing ciprofloxacin, streptomycin, and isoniazid drugs used as positive control, and a medium containing only DMSO (50 μL) used as negative control. These wells were inoculated with *M. tuberculosis* H37Rv strain. Plates were covered and sealed with Parafilm and incubated at 37 °C for 5 days. After this time, 25 μL of a freshly prepared 1:1 mixture of Alamar Blue reagent and 10% Tween 80 were added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and pink was scored as growth. This colour change of the Alamar Blue from a fully oxidized, non-fluorescent blue to a fully reduced, fluorescent pink is due to the cellular metabolism. MIC was defined as lowest drug concentration, which prevented the color change from blue to pink.

Results and discussion

Chemistry

The reaction sequence involving the synthesis of desired 6-arylpyridine based 4,5-dihydro-2-pyrazlines is depicted in Scheme 1. We initiated our synthetic protocol



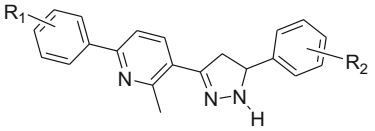
Where R = 3,4-F_2, 4-Br and R^1 = 3,4-$(OMe)_2$, 3,4,5-$(OMe)_3$, 2-Cl-6-F, 2,3-Cl_2, 4-Br, 4-F

Scheme 1 Synthesis of 6-aryl-2-methyl-3-(5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)pyridines **(5a–l)**

with commercially available 3,5-difluroacetophenone and 4-bromoacetophenone **1a–b** refluxing with dimethyl formamide dimethyl acetal in solvent-free condition, which furnished corresponding enaminones **2a–b**. 1-(2-Methyl-6-arylpyridin-3-yl)ethanones **3a–b** were prepared in excellent yields by one-pot, three-component heterocylocondensation of enaminones **2a–b**, diketone and ammonium acetate in glacial acetic acid media. The intermediates, 1-(2-Methyl-6-arylpyridin-3-yl)-3-arylprop-2-en-1-one **(4a–l)** have been synthesized by conventional Claisen-Schmidt condensation reaction of 1-(2-methyl-6-arylpyridin-3-yl)ethanones and appropriate benzaldehydes in presence of base. Finally, preparation of 4,5-dihydro-2-pyrazolines **(5a–l)** was achieved via the reaction of the appropriate chalcone derivatives **(4a–l)** with excess of hydrazine hydrate in refluxed ethanol in the presence of a catalytic amount of dil.HCl. The formation of 6-aryl-2-methyl-3-(5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)pyridines **(5a–l)** (Table 1) are evidenced by their elemental analysis and spectral data.

The formation of intermediates (**4a–l)** from corresponding aromatic aldehydes and ketones were confirmed by the appearance of characteristic stretching vibrations for C=O and C=C groups in FT-IR. The compound **4d**, showed the carbonyl stretching vibration of keto-enol moiety as a strong and sharp absorption band at 1641 cm^{-1}. Another sharp and strong absorbtion band at 1590 cm^{-1} was assigned to C=C of α, β-unsaturated system. The ^1H NMR spectrum showed trans olefinic protons H_α and H_β as two ortho-coupled doublets at 7.12 ($J = 16$ Hz) and 7.93 ($J = 16$ Hz) respectively. The downfield resonance of the H_β as compared to H_α could be attributed to the electron deficient environment of the β carbon in the

Table 1 Physical data of 1-(4-aryl-thiazol-2-yl)-2-((1-(fluoroaryl)-2,5-dimethyl-1*H*-pyrrol-3-yl)pyridines **(5a–l)**



Compound	R_1	R_2	Mol. formula	Mol. weight	M.p. (°C)
5a	3,5-F_2	3,4-$(OCH_3)_2$	$C_{23}H_{21}F_2N_3O_2$	409.43	117–119
5b	3,5-F_2	3,4,5-$(OCH_3)_3$	$C_{24}H_{23}F_2N_3O_3$	439.45	124–126
5c	3,5-F_2	2-Cl-6-F	$C_{21}H_{15}ClF_3N_3$	401.81	136–138
5d	3,5-F_2	2,3-Cl_2	$C_{21}H_{15}Cl_2F_2N_3$	418.27	125–127
5e	3,5-F_2	4-Br	$C_{21}H_{16}BrF_2N_3$	428.27	90–92
5f	3,5-F_2	4-F	$C_{21}H_{16}F_3N_3$	367.37	108–112
5g	4-Br	3,4-$(OCH_3)_2$	$C_{23}H_{22}BrN_3O_2$	452.09	116–119
5h	4-Br	3,4,5-$(OCH_3)_3$	$C_{24}H_{24}BrN_3O_3$	482.37	134–136
5i	4-Br	2-Cl-6-F	$C_{21}H_{16}BrClFN_3$	443.02	140–142
5j	4-Br	2,3-Cl_2	$C_{21}H_{16}BrCl_2N_3$	461.18	96–97
5k	4-Br	4-Br	$C_{21}H_{17}Br_2N_3$	471.19	106–107
5l	4-Br	4-F	$C_{21}H_{17}BrFN_3$	410.28	143–145

enone moiety. The coupling constant value (J) for these olefinic protons was found to be 16 Hz. Similarly, for all other chalcones (**4a–l**), the "J" values were in the range of 15.8–16 Hz, indicating that they are stereoselective and attained trans (E) configuration, whereas the proton of the pyridine unit methyl group appeared as a sharp singlet at 2.76 ppm. The protons belonging to the aromatic ring were observed with expected chemical shifts and integral values. The ^{13}C NMR spectrum of compound **4d** showed signal at 193.9 ppm corresponding to the C=O group of the α-β unsaturated fragment. The signal at 24.0 ppm corresponding to the methyl carbon of the pyridine moiety evidences the formation of compound **4d**. Further confirmation of the structure **4d** was given by mass spectrum that showed M^+ at 404.1 *m*/*z* and isotopic peak M^++2 at 406.1 m/z. The spectral data of other compounds followed similar pattern.

Structures of the synthesized 6-aryl-2-methyl-3-(5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)pyridines **(5a–l)** were elucidated by IR, ^{1}H NMR, ^{13}C NMR, mass spectral data, and elemental analyses. In FT-IR spectra **(5a–l)**, the band corresponding to carbonyl functionality of α, β-unsaturated carbonyl disappeared. Also, new characteristic peaks corresponding to C=N, N–N stretching of the ring and NH stretching vibrations were observed, indicating the formation of cyclized pyrazoline analogues. Additionally, in ^{1}H NMR spectra the signals corresponding to the olefinic protons H_α and H_β of intermediates (**4a–l**) were found to be missing, which clearly demonstrated the involvement of enone moiety during cyclization. In addition to this, the major feature of these spectra was the signals of the pyrazoline ring protons H_x, H_a , and H_b. In all cases, the two methylene protons H_a and H_b of the prochiral carbon atom C-4 and the sterogenic proton H_x in C-5 generates an ABX spin pattern. For the prototype compound **5b**, ^{1}H NMR spectrum reveals that the signals of the pyrazoline ring protons which were found in the region H_x at 4.81 ppm (1H, t, $J = 10.2$ Hz) and H_a, H_b displayed as double-doublets centred at 3.42 ppm ($J_{ab} = 16$ Hz, $J_{ax} = 10.4$ Hz), 2.99 ppm ($J_{ba} = 16.2$ Hz, $J_{bx} = 9.8$ Hz), respectively, which evidenced the presence of two unequivalent geminal protons of the methylene group coupled to each other and in turn with the vicinal methine proton. It has been also noticed that the vicinal coupling constant range is 9.8–10.4 Hz, indicating the presence of trans configuration, in other words, the chiral proton is cis to the aryl group attached to the chiral carbon of the pyrazoline ring. Furthermore, the upfield shifting of prochiral H_a and H_b protons compared with the H_x proton of the pyrazoline ring can be assumed due to its structure. All the other aromatic and aliphatic protons were observed at expected regions. Additionally, the structures of all target compounds were further established by ^{13}C NMR, and the spectrum of **5b** confirmed the formation of a pyrazoline ring by displaying signals at 43.6 ppm and 60.8 ppm due to the sp^3 carbons of C-4 and C-5, respectively. The downfield resonance of C-5 as compared to C-4 can be assumed due to its benzylic nature and proximity to nitrogen that provide an additional support for the formation of the target compound. All of the synthetic compounds gave satisfactory mass spectroscopic data, which were in accordance with their depicted structure. Characteristic M + 2, M + 4 isotope peaks were observed for the compounds having chloro and bromo substituents. The mass spectrum of **5b**

exhibited a molecular ion peak at 440.2 m/z in accordance with its formula and evidences the formation of the compound.

Pharmacological activity

Antidepressant activity

The synthesized compounds **(5a–I)** were evaluated for antidepressant activity via tail suspension test (TST) and forced swim test (FST) [39, 40]. We used a well-known MAO-inhibitor imipramine as standard. Results are summarized in Tables 2 and 3 and expressed as immobility time in seconds and percentage decrease in immobility duration (%DID). The ability of tested compounds to decrease immobility time in both FST and TST was taken as a measure of its antidepressant-like activity. As for activity of the tested compounds showed reduced immobility times when compared to the normal control group at 100 mg kg^{-1} dose level.

In the FST method, the treatment with doses of 100 mg kg^{-1} of **5b** with 3,5-difluorophenyl moiety attached to the pyridine ring and 3,4,5-trimethoxyphenyl ring attached to the other extreme end of the pyrazoline ring was emerged as most active antidepressant compound by exhibiting significantly decreased immobility time 66.67 ± 7.15 s, when compared to normal control group 198.5 ± 4.03 s and was more effective than the standard-treated group 75.50 ± 3.35 s at a test dose of 10 mg kg^{-1}. Meanwhile, replacing the 3,5-difluorophenyl group by 4-bromophenyl

Table 2 Antidepressant activity of the title compounds **(5a–l)** by forced swimming test method

Sl. no.	Compounds[a]	Duration of immobility[b] (s)	% decrease in immobility duration (%DID)
1	**5a**	109.3 ± 15.55	45.05
2	**5b**	**66.67 ± 7.15**	**66.58**
3	**5c**	130.7 ± 13.46	34.24
4	**5d**	156.0 ± 13.68	21.46
5	**5e**	160.3 ± 15.17	19.29
6	**5f**	**79.17 ± 4.50**	**60.01**
7	**5g**	109.2 ± 16.66	44.84
8	**5h**	**94.33 ± 14.96**	**52.47**
9	**5i**	151.3 ± 14.22	23.58
10	**5j**	128.2 ± 11.42	35.25
11	**5k**	137.7 ± 13.22	30.45
12	**5l**	**81.67 ± 4.40**	**58.75**
13	Imipramine	75.50 ± 3.35	62.12
14	Control	198.5 ± 4.03	–

Values represent the mean ± SEM; n = 6

[a] Tested compounds and imipramine were tested at 100 and 10 mg/kg dose level, i.p. respectively

[b] Bold font indicates a significant activity of the compounds

Table 3 Antidepressant activity of the title compounds **(5a–l)** by tail suspension test method

Sl. no.	Compounds[a]	Duration of immobility[b] (s)	% decrease in immobility duration (%DID)
1	**5a**	154.7 ± 25.78	22.06
2	**5b**	**70.33 ± 13.09**	**64.56**
3	**5c**	136.0 ± 36.72	31.48
4	**5d**	178.3 ± 22.58	10.17
5	**5e**	184.7 ± 21.77	6.95
6	**5f**	115.3 ± 23.32	41.91
7	**5g**	161.5 ± 50.0	18.64
8	**5h**	**88.50 ± 22.81**	**55.41**
9	**5i**	156.2 ± 21.20	21.31
10	**5j**	**89.83 ± 12.84**	**54.74**
11	**5k**	110.3 ± 24.88	44.43
12	**5l**	**97.33 ± 14.96**	**50.96**
13	Imipramine	75.50 ± 3.35	61.96
14	Control	198.5 ± 4.03	–

Values represent the mean ± SEM; n = 6

[a] Tested compounds and imipramine were tested at 100 and 10 mg/kg dose level, i.p. respectively

[b] Bold font indicates a significant activity of the compounds

in **5b** led to compound **5h**, whose immobility time was decreased to 94.33 ± 14.96 s, indicating that the 4-bromophenyl substituent was less efficient when compared to the 3,5-difluorophenyl group. Acute treatment with the compound **5f** having 3,5-difluorophenyl substitution at the pyridine ring and 4-flourophenyl substitution at the other end of the pyrazoline ring showed good antidepressant-like activity by exhibiting decreased immobility time 79.17 ± 4.50 s, and this effect was significant ($p < 0.001$) when compared to standard-treated group. Further, replacing 3,5-difluorophenyl substituent in the pyridine ring of **5f** with 4-bromophenyl substituent resulted in compound **5l**, which promoted a decrease in the immobility time, their activity being nearly equal to 92.44% that of the imipramine and emerged as good antidepressant active compound, but when compared to **5f**, the activity was slightly less, which clearly indicates the efficacy of 3,5-difluoro substituent on the antidepressant activity. The results clearly indicated that introducing 4-bromophenyl substituent on the pyridine ring failed to increase significantly the antidepressant activity when compared to 3,5-difluoro phenyl substituent. The other treated compounds **5a**, **5c**, **5d**, **5e**, **5g**, **5i**, **5j,** and **5k**, were least active, that is, the immobility time did not statistically differ from the control-treated group.

On the other hand, compounds exhibit good results in antidepressant screening by the TST method. The screening results are reported in Table 3. Some of the compounds, especially **5b**, **5h**, **5j**, and **5l**, exhibit good antidepressant activity at a tested dose of 100 mg kg^{-1}. Particularly, **5b** with 3,5-difluorophenyl substitution at the pyridine ring and 3,4,5-trimethoxyphenyl substitution at the other end of the

pyrazoline ring emerged as the most potent antidepressant agent by exhibiting reduction in the immobility time to 70.33 ± 13.09 s from 198.5 ± 4.03 s of the control group. Additionally, the animals treated with the compound **5j** having 4-bromophenyl substitution at the pyridine ring and 2-chloro-6-fluorophenyl substitution at the other end of the pyrazoline moiety, which was inactive in FST, showed reduction in immobility time, their activity being nearly equal to 85.31% of that in the standard treated group. In the TST method also, a fluoro substituent on the aryl ring displayed good results by a reduction in immobility time when compared to the normal-treated group, indicating their antidepressant efficacy.

When we correlate the structures of the test samples and their activity, it appears that the halogen substituents on the phenyl ring in either one or the other position of the pyrazoline moiety imparted superior activity when compared to the other substituents. This is because of the lipophilic nature of the halogen atoms especially fluorine atom can increase the rate of cell penetration and transportation of molecules to the active site to create the desired level of biological activity [41], which is reflected on the antidepressant activity of **5b,** being the most potent of all. Further, derivatives with an electron donating group, particularly the methoxy group on the phenyl ring at the 5th position of pyrazoline, considerably enhanced the antidepressant activity when compared to the pyrazolines with other substituents on the phenyl ring.

Anti-TB activity

Antimycobacterial potency of the all newly synthesized compounds (**5a–l**) was checked by performing in vitro antimycobacterial activity by Microplate Alamar Blue Assay (MABA) method against *Mycobacterium tuberculosis* H37Rv using

Table 4 Antimycobacterial activity of selected target molecules against *M. tuberculosis* H37Rv

No.	Compound	MIC (μg mL^{-1})
1	**5a**	**12.5**
2	**5b**	25
3	**5c**	**12.5**
4	**5d**	25
5	**5e**	25
6	**5f**	25
7	**5g**	25
8	**5h**	50
9	**5i**	**12.5**
10	**5j**	25
11	**5k**	50
12	**5l**	50
13	Isoniazid	6.25
14	Streptomycin	6.25
15	Ciprofloxacin	3.125

Bold font indicates a significant activity of compounds against *M. tuberculosis* H37Rv

ciprofloxacin, streptomycin, and isoniazid as standards. The results are reported in Table 4. From the results, it is clear that the tested compounds displayed moderate to good inhibitory activity with MIC value ranging from 12.5 to 50 μg mL^{-1}. Among the series, compound **5a** (R_1 = 3,5-F_2 and R_2 = 3,4-$(OCH_3)_2$), **5c** (R_1 = 3,5-F_2 and R_2 = 2-Cl-6-F) and **5i** (R_1 = 4-Br and R_2 = 2-Cl-6-F) showed highest inhibition with MIC value 12.5 μg mL^{-1}. Meanwhile, introducing the additional methoxy group in compound **5a** led to compound **5b** (R_1 = 3,5-F_2 and R_2 = 3,4,5-$((OCH_3)_3)$, whose activity was reduced with MIC value 25 μg mL^{-1}. Again, replacement of 3,5-difluoro group by 4-Bromo in **5b**, that is compound **5h** (R_1 = 4-Br and R_2 = 3,4,5-$((OCH_3)_3)$, led to significant loss of activity with MIC value 50 μg mL^{-1}. Further, tested compounds **5d**, **5e**, **5f**, **5g,** and **5j** exhibited moderate activity (25 μg mL^{-1}) against *Mycobacterium tuberculosis* H37Rv strain. Further, isosteric replacement of 2,4-difluoro group at phenyl ring attached to the pyridine ring in **5e** and **5f** with a 4-Br group led to compounds **5k** and **5l**, which displayed reduction in inhibition activity with MIC value 50 μg mLo^{-1}, thus it was clear that introduction of a bromo group did not improve their mycobacterial activity. In addition to this, comparison of the activity results of the target compounds demonstrated that the fluoro and chloro substituents in the aryl part are essential for the pronounced activity.

Conclusions

In conclusion, a series of 6-aryl-2-methyl-3-(5-aryl-4,5-dihydro-1*H*-pyrazol-3yl)pyridines **(5a–l)** were prepared in good yield and their antidepressant and anti-TB activity were investigated. Evaluations of the antidepressant activity of the derivatives were carried out by following FST and TST methodologies in mice. Compound **5b** induced amazing antidepressant activity by exhibiting the highest % DID of 66.58 and 64.56 in the FST and TST, respectively, whereas, the reference drug imipramine displayed % DID of 62.12 and 61.91 in the FST and TST, respectively. Therefore, **5b** can be used as lead molecule for further optimization. Further, compounds **5f**, **5h**, **5j**, and **5l** furnished good antidepressant activity compared to the standard drug in both FST and TST model. Antidepressant activity results demonstrated that halogen atoms, particularly fluorine and chlorine on the aryl ring, have made good contribution to potency. In addition to this, in vitro anti-TB activity results reveals that compounds **5a**, **5c**, **5i** displayed good growth inhibitory activity against *Mycobacterium tuberculosis* H37Rv strain with MIC value 12.5 μg mL^{-1}. Consequently, such compounds would signify the fruitful matrix for the development of new class of anti-TB and antidepressant agents.

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References

1. E.S. Lee, H.R. Robert, E.B. Chad, A.H. Zoe, K. Xavier, E.M. Jessica, R.L. Sharon, Am. Soc. Exp. Neuro Ther. **2**, 590 (2005)
2. Z.F. Yu, L.D. Kong, Y. Chen, J. Ethnopharmacol. **83**, 161 (2002)
3. F. Henn, B. Vollmayr, A. Sartorius, Drug Discov Today **1**, 407 (2004)
4. D. Yee, C. Valiquette, M. Pelletier, I. Parison, D. Menzies, Am. J. Respir. Crit. Care Med. **167**, 1472 (2003)
5. L.P. Ormerod, N. Horsifield, Tuber Lung. Dis. **77**, 37 (1996)
6. R.C. Goldman, K.V. Plumley, B.E. Laughon, Infect. Disord. Drug Targets **7**, 73 (2007)
7. E. Palaska, D. Erol, R. Demirdamar, Eur. J. Med. Chem. **31**, 43 (1996)
8. R.L. Shader, D.J. Greenblatt, J. Clin. Psychopharm. **19**, 105 (1999)
9. L.J. Urichuk, K. Allison, A. Holt, A.J. Greenshaw, G.B. Baker, J. Affec. Disord. **58**(135), 9 (2000)
10. A. Fitton, D. Faulds, K. Goa, Drugs **43**, 561 (1992)
11. M. Ferigolo, H.M. Barros, A.R. Marquardt, M. Tanhauser, Pharmacol. Biochem. Behav. **60**, 431 (1998)
12. F. Chimenti, A. Bolasco, D. Secci, P. Chimenti, A. Granese, S. Carradori, M. Yanez, F. Orallo, F. Ortuso, S. Alcaro, Bioorg. Med. Chem. **18**, 5715 (2010)
13. D. Secci, A. Bolasco, S. Carradori, M. D'Ascenzio, R. Nescatelli, M. Yanez, Eur. J. Med. Chem. **58**, 405 (2012)
14. N. Soni, K. Pande, R. Kalsi, T.K. Gupta, S.S. Parmar, J.P. Barthwal, Res. Commun. Cement Pathol. Pharm. **56**, 137 (1987)
15. S.S. Parmar, B.R. Pandey, C. Dwivedi, R.D. Harbison, J. Pharm. Sci. **63**, 1155 (1974)
16. N. Gokhan, A. Yesilada, G. Ucar, K. Erol, A.A. Bilgin, Arch. Pharm. Pharm Med Chem. **336**, 371 (2003)
17. A.A. Bilgin, E. Palaska, R. Sunal, Arzneim-Forsch. Drug Res. **43**, 1044 (1993)
18. E. Palaska, M. Aytemir, I.T. Uzbay, D. Erol, Eur. J. Med. Chem. **36**, 543 (2001)
19. Y.R. Prasad, A.L. Rao, L. Prasoona, K. Murali, P.R. Kumar, Bioorg. Med. Chem. Lett. **15**, 5034 (2005)
20. S.G. Kucukguzel, S. Rollas, Farmaco **57**, 587 (2002)
21. S.G. Kucukguzel, S. Rollas, H. Erdeniz, M. Kiraz, A. Cevdet Ekinci, A. Vidin, Eur. J. Med. Chem. **35**, 771 (2000)
22. D. Nauduri, G.B. Reddy, Chem. Pharm. Bull. **46**, 1260 (1998)
23. M. Radi, V. Bernardo, B. Bechi, D. Castagnolo, Tetrahedron Lett. **50**, 6575 (2009)
24. G. Jones, in *Comprehensive Heterocyclic Chemistry II*, ed by A.R. Katritzky, C.W. Rees, E.F.V. Scriven, A. McKillop (Pergamon: Oxford), **5**, 167 (1996)
25. G.D. Henry, Tetrahedron **60**, 6043 (2004)
26. J.P. Michael, Nat. Prod. Rep. **22**, 627 (2005)
27. Z.B. Yang, D.Y. Hu, S. Zeng, B.A. Song, Bioorg. Med. Chem. Lett. **26**, 1161 (2016)
28. N. Nayak, J. Ramprasad, U. Dalimba, Bioorg. Med. Chem. Lett. **25**, 5540 (2015)
29. S. Prachayasittikul, L. Treeratanapiboon, S. Ruchirawat, V. Prachayasittikul, EXCLI J. **8**, 129 (2009)
30. D. Kumar, G. Khare, S. Beena, A.K. Kidwai, R. Tyagi, D.S.Rawat Singh, Med. Chem. Comm. **6**, 131 (2015)
31. M. Abdel-Azi, G.A. Abuo-Rahma, A.A. Hassan, Eur. J. Med. Chem. **44**, 3480 (2009)
32. J.L. Engers, A.L. Rodriguez, L.C. Konkol, R.D. Morrison, A.D. Thompson, F.W. Byers, A.L. Blobaum, S. Chang, D.F. Venable, M.T. Loch, C.M. Niswender, J.S. Daniels, C.K. Jones, P.J. Conn, C.W. Lindsley, K.A. Emmitte, J. Med. Chem. **58**, 7485 (2015)
33. E. Palaska, F. Aydin, G. Uçar, D. Erol, Arch. Pharm. Chem. Life Sci. **341**, 209 (2008)
34. S.T. Dhumal, A.R. Deshmukh, M.R. Bhosle, V.M. Khedkar, L.U.N. Awale, D. Srkar, R.A. Mane, Bioorg. Med. Chem. Lett. **26**, 3646 (2016)
35. P.M. Sivakumar, S.P. Seenivasan, V. Kumar, M. Doble, Bioorg. Med. Chem. Lett. **20**, 3169 (2010)
36. B. Al-Saleh, M.M. Abdel-Khalik, A.M. Eltoukhy, M.H. Elnagdi, J. Heterocycl. Chem. **39**, 1035 (2002)
37. L. Steru, R. Chermat, B. Thierry, P. Simon, Psychopharmacology **85**, 367 (1985)
38. M.C.S. Lourenço, Marcus V. N. de Souza, Alessandra C. Pinheiro, Marcelle de L. Ferreira, Raoni S. B. Gonçalves, Thais Cristina M. Nogueira, Mônica A. Peraltab, ARKIVOC(XV), 181 (2007)
39. R.D. Porsolt, A. Bertin, M. Jalfre, Arch. Int. Pharmacodyn. Ther. **229**, 327 (1977)

40. R.D. Porsolt, G. Anton, N. Blavet, M. Jalfre, Eur. J. Pharmacol. **47**, 379 (1978)
41. M. Han, X. Ma, Y. Jin, W. Zhou, J. Cao, Y. Wang, S. Zhou, G. Wang, Y. Zhu, Bioorg. Med. Chem. Lett. **24**, 5284 (2014)