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Synthesis and anti-tubercular activity of novel pyrazol-5(*H*)-one derivatives

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ABSTRACT

In the present investigation, a series of 1-isonicotinoyl-3-methyl-4-(2-(substituted-phenyl)hydrazono)-1*H*-pyrazol-5(*H*)-ones were synthesized by the reaction between isonicotinohydrazide with substituted ethylacetoacetate derivatives using acetic acid as solvent which yielded substituted pyrazol-5(*H*)-one derivatives. Newly synthesized compounds were tested for their in *vitro* anti-tubercular activity against *Mycobacterium tuberculosis* H37Rv using the BACTEC 460 radiometric system. Among the synthesized compounds, 4-(2-(2,6-dichlorophenyl)hydrazono)-1-isonicotinoyl-3-methyl-1*H*-pyrazol-5(4*H*)-one and 4-(2-(1-isonicotinoyl-3-methyl-5-oxo-1*H*-pyrazol-4(5*H*)-ylidene)hydrazinyl) benzene-sulfonamide were found to be more active agent against *M. tuberculosis* H37Rv with minimum inhibitory concentration of 0.0034, 0.0032 µM at actual MIC 1.66 and 1.64 µg/mL, respectively.

1. Introduction

In 2009 fell the 126th anniversary of Robert Koch's discovery of the bacillus *Mycobacterium tuberculosis* (MTB) [1], the etiological agent of the well-known respiratory disease Tuberculosis (TB). Despite MTB being identified more than a century ago and with many efficient drugs being discovered during that time to eradicate the disease, TB still remains one of the leading causes of worldwide illness and death. Each year, 424,000 people develop multiple drug-resistant (MDR-TB), a form of TB that does not respond to the standard treatment. It emerges when there is mismanagement of drugs and under investment in quality TB control. It can also be spread from one person to another [2]. Moreover the emergence of multiple drug-resistant strains and, more recently, extensively drug-resistant (XDR-TB) strains makes the discovery and the development of new drugs a priority [3-6]. Also, the emergence of AIDS, decline of socioeconomic standards and a reduced emphasis on tuberculosis control programs contribute to the disease's resurgence in industrialized countries [7]. Thus, Resistance of *M. tuberculosis* strains to anti-mycobacterial agents is an increasing problem worldwide [8-10].

Isoniazid (INH), together with rifampicin and pyrazinamide, constitutes the backbone of a good outcome in the treatment of TB. INH has a simple structure, containing a pyridine ring and a hydrazide group, and both molecules are essential for its high activity against *M. tuberculosis* [11-14]. Development of new drugs against TB derived from already known molecules that have been in use for several years and have been proven safe and efficient is an attractive strategy from an economic, pharmaceutical and clinical viewpoint. Since INH is a very important drug in the therapeutic arsenal for TB treatment, efforts are being made toward the development of new INH derivatives with greater activity, lower toxicity and fewer side effects [11,15-23]. Literature survey reveals that when INH molecule incorporated on a pyrazole nucleus, shows activity against strains of *M. tuberculosis* both susceptible and resistant to INH [24,25]. Interestingly, other compounds with a halogen-substituted phenyl group showed even greater activity [26]. We believe that the INH moiety is not the only structure responsible for the anti-mycobacterial activity because pyrazoles with different substituents exhibited very different activities [27-30]. Therefore, it is possible that attaching chemical groups that aid the penetration of INH would make *M. tuberculosis* strains more susceptible to this drug [27,29].

The current work describes the synthesis of the novel pyrazol-5(*H*)-one moiety (Scheme 1) with encouraging anti-mycobacterial activity against *M. tuberculosis* H37Rv.

2. Experimental

2.1. Instrumentation

The entire chemicals were supplied by E. Merck (Germany) and SD Fine Chemicals (India). Melting points were determined by open tube capillary method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G) in the solvent system benzene:ethylacetate (5:1) the spots were located under iodine vapors or UV light. IR spectra were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (KBr pellets). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 400 MHz & 100 MHz

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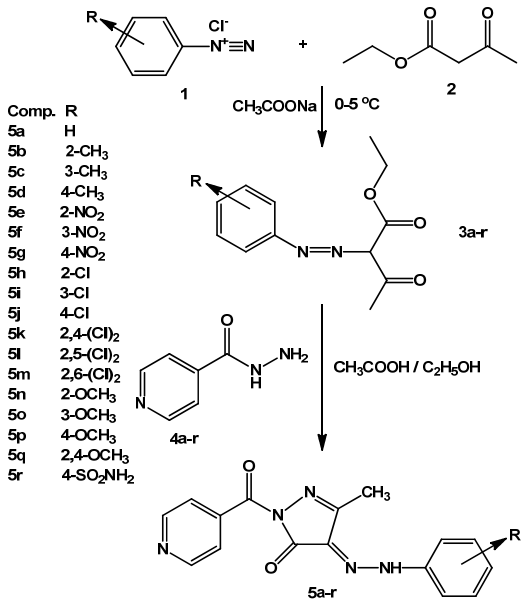
spectrometer using TMS as internal standard in DMSO-d_6/$CDCl_3$, respectively. Mass spectra were recorded on a Bruker Esquire LC-MS using ESI and elemental analyses were performed on a Carlo Erba 1106 elemental analyzer.



Scheme 1

2.2. Synthesis

2.2.1. Ethyl 3-(substituted phenylazo)-2-oxobutanolate (3a)

2-oxobutanolate derivatives were obtained by reacting ethyl acetoacetate (0.01 mol) with appropriate diazonium salt of aromatic amines (0.01 mol) according to the reported method [31].

2.2.2. General procedure (5a-r)

The solution of appropriate 2-oxobutanolate derivative (0.01 mol) and isonicotinohydrazide (0.01 mol) in ethanol (15 mL) and glacial acetic acid (3 mL) were refluxed in a round bottom flask for 7 h. Reaction was monitored by TLC, and after completion the reaction mixture was poured onto crushed ice; the solid mass thus separated, was filtered, washed with water, dried and recrystallized from ethanol to give the desired pyrazolones (**5a**-**r**).

1-isonicotinoyl-3-methyl-4-(2-phenylhydrazono)-1H-pyrazol-5(4H)-one (**5a**): FT-IR (ν, cm^{-1}): 3208-3182 (NH), 1670 (C=O), 1589 (C=N), 3270 (C-H), 2928 (CH_3). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.15 (3H, s, CH_3), 7.45-8.81 (9H, m, aromatic), 11.56 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 12.91 (CH_3), 128.71, 148.11, 162.11 (Ar-C of pyrazol-(5*H*)-one), 117.90-143.07 (Ar-C). MS (EI, m/z): 307 (M+). Anal. Calcd. for $C_{16}H_{13}N_5O_2$ (307.11): C, 62.53; H, 4.26; N, 22.79. Found: C, 62.18; H, 4.08; N, 22.81%.

1-isonicotinoyl-3-methyl-4-(2-o-tolylhydrazono)-1H-pyrazol-5(4H)-one (**5b**): FT-IR (ν, cm^{-1}): 3208-3180 (NH), 1690 (C=O), 1580 (C=N), 3270 (C-H), 2928 (CH_3). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.15 (3H, s, CH_3), 2.50 (3H, s, CH_3), 7.25-8.80 (8H, m, aromatic), 11.50 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 12.11 (CH_3), 21.77 (Ar-CH_3), 127.14, 147.22, 163.72 (Ar-C of pyrazol-(5*H*)-one), 122.50-151.11 (Ar-C). MS (EI, m/z): 322 (M+). Anal. Calcd. for $C_{17}H_{15}N_5O_2$ (321.33): C, 63.54; H, 4.71; N, 21.79. Found: C, 63.11; H, 4.63; N, 21.83%.

1-isonicotinoyl-3-methyl-4-(2-m-tolylhydrazono)-1H-pyrazol-5(4H)-one (**5c**): FT-IR (ν, cm^{-1}): 3208-3180 (NH), 1690 (C=O), 1580 (C=N), 3270 (C-H), 2928 (CH_3). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.15 (3H, s, CH_3), 2.53 (3H, s, CH_3), 7.22-8.80 (8H, m, aromatic), 11.50 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 12.13 (CH_3), 21.99 (Ar-CH_3), 127.59, 148.10, 163.91 (Ar-C of pyrazol-(5H)-one), 112.90-151.11 (Ar-C). MS (EI, m/z): 321 (M+). Anal. Calcd. for $C_{17}H_{15}N_5O_2$ (321.33): C, 63.54; H, 4.71; N, 21.79. Found: C, 63.41; H, 4.73; N, 21.73%.

1-isonicotinoyl-3-methyl-4-(2-p-tolylhydrazono)-1H-pyrazol-5(4H)-one (**5d**): FT-IR (ν, cm^{-1}): 3208-3180 (NH), 1690 (C=O), 1580 (C=N), 3270 (C-H), 2928 (CH_3). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.15 (3H,s, CH_3), 2.54 (3H,s, CH_3), 7.20-8.80 (8H, m, aromatic), 11.50 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d$_6$, δ ppm): 12.1 1(CH_3), 21.99 (Ar-CH_3), 127.75, 148.26, 163.17 (Ar-C of pyrazol-(5*H*)-one), 116.90-150.10 (Ar-C). MS (EI, m/z): 321 (M+). Anal. Calcd. for $C_{17}H_{15}N_5O_2$ (321.33): C, 63.54; H, 4.71; N, 21.79. Found: C, 63.44; H, 4.77; N, 21.91%.

1-isonicotinoyl-3-methyl-4-(2-(2-nitrophenyl)hydrazono)-1H-pyrazol-5(4H)-one (**5e**): FT-IR (ν, cm^{-1}): 3200-3190 (NH), 1680 (C=O), 1582 (C=N), 3270 (C-H), 2928 (CH_3), 1344 and 1560 (NO_2). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.15 (3H, s, CH_3), 7.40-8.89 (8H, m, aromatic), 11.61 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d$_6$, δ ppm): 11.13 (CH_3), 128.72, 148.23, 162.14 (Ar-C of pyrazol-(5*H*)-one), 127.10-149.70 (Ar-C). MS (EI, m/z): 352 (M+). Anal. Calcd. for $C_{16}H_{12}N_6O_4$ (352.30): C, 54.55; H, 3.43; N, 23.85. Found: C, 54.11; H, 3.63; N, 23.73%.

1-isonicotinoyl-3-methyl-4-(2-(3-nitrophenyl)hydrazono)-1H-pyrazol-5(4H)-one (**5f**): FT-IR (ν, cm^{-1}): 3200-3190 (NH), 1680 (C=O), 1582 (C=N), 3270 (C-H), 2928 (CH_3), 1344 and 1560 (NO_2). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.10 (3H, s, CH_3), 7.40-8.89 (8H, m, aromatic), 11.63 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 11.18 (CH_3), 129.81, 149.32, 163.21 (Ar-C of pyrazol-(5*H*)-one), 107.11-148.92 (Ar-C). MS (EI, m/z): 352 (M+). Anal. Calcd. for $C_{16}H_{12}N_6O_4$ (352.30): C, 54.55; H, 3.43; N, 23.85. Found: C, 54.22; H, 3.34; N, 23.77%.

1-isonicotinoyl-3-methyl-4-(2-(4-nitrophenyl)hydrazono)-1H-pyrazol-5(4H)-one (**5g**): FT-IR (ν, cm^{-1}): 3200-3190 (NH), 1680 (C=O), 1582 (C=N), 3270 (C-H), 2928 (CH_3), 1344 and 1560 (NO_2). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.10 (3H, s, CH_3), 7.20-8.80 (8H, m, aromatic), 11.62 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 11.20 (CH_3), 129.71, 149.12, 163.03 (Ar-C of pyrazol-(5*H*)-one), 117.21-147.89 (Ar-C). MS (EI, m/z): 352 (M+). Anal. Calcd. for $C_{16}H_{12}N_6O_4$ (352.30): C, 54.55; H, 3.43; N, 23.85. Found: C, 54.42; H, 3.33; N, 23.63%.

4-(2-(2-chlorophenyl)hydrazono)-1-isonicotinoyl-3-methyl-1H-pyrazol-5(4H)-one (**5h**): FT-IR (ν, cm^{-1}): 3203-3187 (NH), 1680 (C=O), 1586 (C=N), 3270 (C-H), 2928 (CH_3), 782 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.13 (3H, s, CH_3), 6.50-8.71 (8H, m, aromatic), 11.20 (1H, s, NH). ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 11.39 (CH_3), 128.71, 148.12, 162.01 (Ar-C of pyrazol-(5*H*)-one), 127.21-147.78 (Ar-C). MS (EI, m/z): 342 (M+). Anal. Calcd. for $C_{16}H_{12}N_5O_2Cl$ (341.75): C , 56.23; H, 3.54; N, 20.49. Found: C, 56.31; H, 3.67; N, 20.77%.

4-(2-(3-chlorophenyl)hydrazono)-1-isonicotinoyl-3-methyl-1H-pyrazol-5(4H)-one (**5i**): FT-IR (ν, cm^{-1}): 3203-3187 (NH), 1680 (C=O), 1586 (C=N), 3270 (C-H), 2928 (CH_3), 782 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.13 (3H, s, CH_3), 6.80-8.77 (8H, m, aromatic), 11.22 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 11.92 (CH_3), 128.18, 148.21, 162.10 (Ar-C of pyrazol-(5*H*)-one), 117.12-147.87 (Ar-C). MS (EI, m/z): 342 (M+). Anal. Calcd. for $C_{16}H_{12}N_5O_2Cl$ (341.75): C, 56.23; H, 3.54; N, 20.49. Found: C, 56.34; H, 3.69; N, 20.47%.

4-(2-(4-chlorophenyl)hydrazono)-1-isonicotinoyl-3-methyl-1H-pyrazol-5(4H)-one (**5j**): FT-IR (ν, cm^{-1}): 3203-3187 (NH), 1680 (C=O), 1586 (C=N), 3270 (C-H), 2928 (CH_3), 782 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.13 (3H, s, CH_3), 7.10-8.79 (8H, m, aromatic), 11.25 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 11.97(CH_3), 128.17, 148.11, 162.11 (Ar-C of pyrazol-

(5*H*)-one), 117.72-148.17 (Ar-C). MS (EI, m/z): 342 (M+). Anal. Calcd. for $C_{16}H_{12}N_5O_2Cl$ (341.75): C, 56.23; H, 3.54; N, 20.49. Found: C, 56.21; H, 3.69; N, 20.77%.

4-(2-(2,4-dichlorophenyl)hydrazono)-1-isonicotinoyl-3-methyl-1H-pyrazol-5(4H)-one (**5k**): FT-IR (ν, cm^{-1}): 3203-3187 (NH), 1680 (C=O), 1586 (C=N), 3270 (C-H), 2928 (CH_3), 782 (C-Cl). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.30 (3H, s, CH_3), 7.55-7.90 (7H, m, aromatic), 11.09 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 12.18 (CH_3), 127.87, 148.71, 162.91 (Ar-C of pyrazol-(5*H*)-one), 116.92-148.97 (Ar-C). MS (EI, m/z): 377 (M+). Anal. Calcd. for $C_{16}H_{11}N_5O_2Cl_2$ (376.20): C, 51.08; H, 2.95; N, 18.62. Found: C, 51.22; H, 2.89; N, 18.69%.

4-(2-(2,5-dichlorophenyl)hydrazono)-1-isonicotinoyl-3-methyl-1H-pyrazol-5(4H)-one (**5l**): FT-IR (ν, cm^{-1}): 3203-3187 (NH), 1680 (C=O), 1586 (C=N), 3270 (C-H), 2928 (CH_3), 782 (C-Cl). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.33 (3H, s, CH_3), 6.77-7.90 (7H, m, aromatic), 11.09 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 12.11 (CH_3), 127.76, 147.80, 163.80 (Ar-C of pyrazol-(5*H*)-one), 123.72-148.77 (Ar-C). MS (EI, m/z): 377 (M+). Anal. Calcd. for $C_{16}H_{11}N_5O_2Cl_2$ (376.20): C, 51.08; H, 2.95; N, 18.62. Found: C, 51.09; H, 2.99; N, 18.79%.

4-(2-(2,6-dichlorophenyl)hydrazono)-1-isonicotinoyl-3-methyl-1H-pyrazol-5(4H)-one (**5m**): FT-IR (ν, cm^{-1}): 3203-3187 (NH), 1680 (C=O), 1586 (C=N), 3270 (C-H), 2928 (CH_3), 782 (C-Cl). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.31 (3H, s, CH_3), 7.02-7.90 (7H, m, aromatic), 13.18 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 12.18 (CH_3), 127.77, 147.81, 163.83 (Ar-C of pyrazol-(5*H*)-one), 118.77-149.92 (Ar-C). MS (EI, m/z): 377 (M+). Anal. Calcd. for $C_{16}H_{11}N_5O_2Cl_2$ (376.20): C, 51.08; H, 2.95; N, 18.62. Found: C, 51.18; H, 2.91; N, 18.79%.

1-isonicotinoyl-4-(2-(2-methoxyphenyl)hydrazono)-3-methyl-1H-pyrazol-5(4H)-one (**5n**): FT-IR (ν, cm^{-1}): 3206-3190 (NH), 1689 (C=O), 1586 (C=N), 3270 (C-H), 2928 (CH_3), 2830 (OCH_3). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.15 (3H, s, CH_3), 3.92 (3H, s, OCH_3), 6.85-8.10 (8H, m, aromatic), 11.18 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 12.18 (CH_3), 58.69 (OCH_3) 129.96, 147.02, 163.00 (Ar-C of pyrazol-(5*H*)-one), 128.97-148.96 (Ar-C). MS (EI, m/z): 338 (M+). Anal. Calcd. for $C_{17}H_{15}N_5O_3$ (337.12): C, 60.53; H, 4.48; N, 20.76. Found: C, 60.62; H, 4.39; N, 20.72%.

1-isonicotinoyl-4-(2-(3-methoxyphenyl)hydrazono)-3-methyl-1H-pyrazol-5(4H)-one (**5o**): FT-IR (ν, cm^{-1}): 3206-3190 (NH), 1689 (C=O), 1586 (C=N), 3270 (C-H), 2928 (CH_3), 2830 (OCH_3). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.17 (3H, s, CH_3), 3.89 (3H, s, OCH_3), 6.27-7.89 (8H, m, aromatic), 12.11 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 12.18 (CH_3), 59.97 (OCH_3) 129.96, 147.11, 163.19 (Ar-C of pyrazol-(5*H*)-one), 129.91-149.89 (Ar-C). MS (EI, m/z): 338 (M+). Anal. Calcd. for $C_{17}H_{15}N_5O_3$ (337.12): C, 60.53; H, 4.48; N, 20.76. Found: C, 60.74; H, 4.50; N, 20.74%.

1-isonicotinoyl-4-(2-(4-methoxyphenyl)hydrazono)-3-methyl-1H-pyrazol-5(4H)-one (**5p**): FT-IR (ν, cm^{-1}): 3206-3190 (NH), 1689 (C=O), 1586 (C=N), 3270 (C-H), 2928 (CH_3), 2830 (OCH_3). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.11 (3H, s, CH_3), 3.89 (3H, s, OCH_3), 7.02-7.89 (8H, m, aromatic), 12.15 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 12.18 (CH_3), 59.22 (OCH_3) 129.98, 147.02, 163.00 (Ar-C of pyrazol-(5*H*)-one), 128.19-147.97 (Ar-C). MS (EI, m/z): 338 (M+). Anal. Calcd. for $C_{17}H_{15}N_5O_3$ (337.12): C, 60.53; H, 4.48; N, 20.76. Found: C, 60.65; H, 4.48; N, 20.78%.

4-(2-(2,4-dimethoxyphenyl)hydrazono)-1-isonicotinoyl-3-methyl-1H-pyrazol-5(4H)-one (**5q**): FT-IR (ν, cm^{-1}): 3210-3190 (NH), 1690 (C=O), 1586 (C=N), 3270 (C-H), 2928 (CH_3), 2830 (OCH_3). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.10 (3H, s, CH_3), 3.50 (3H, s, CH_3), 3.89 (3H, s, OCH_3), 6.77-8.81 (7H, m, aromatic), 11.11 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 12.20 (CH_3), 57.71 (2xOCH_3), 129.89, 147.11, 164.11 (Ar-C of pyrazol-(5*H*)-one), 121.19-149.77 (Ar-C). MS (EI, m/z): 368 (M+). Anal. Calcd. for $C_{18}H_{17}N_5O_4$ (367.36): C, 58.85, H, 4.66; N, 19.06. Found: C, 58.95; H, 4.78; N, 19.18%.

4-(2-(1-isonicotinoyl-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)hydrazinyl)benzene-sulfonamide (**5r**): FT-IR (ν, cm^{-1}): 3206-3190 (NH), 1689 (C=O), 1586 (C=N), 327 0(C-H), 2928 (CH_3), 1339-1337, 1147-1137 (SO_2), 3440 (NH_2). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.15 (3H, s, CH_3), 6.91-7.89 (8H, m, aromatic), 13.11 (1H, s, NH), 8.83 (2H, s, SO_2NH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 12.29 (CH_3), 127.77, 147.21, 165.09 (Ar-C of pyrazol-(5*H*)-one), 129.30-148.19 (Ar-C). MS (EI, m/z): 387 (M+). Anal. Calcd. for $C_{16}H_{14}N_6O_4S$ (386.39): C, 49.74; H, 3.65; N, 21.75. Found: C, 49.98; H, 3.79; N, 21.79%.

2.3. Biology

The primary screening was conducted at a concentration of 6.25 μg/mL (or molar equivalent of highest molecular weight compound in a series of congeners) against *M. tuberculosis* H37Rv (ATCC27294) in BACTEC 12B medium using the BACTEC 460 radiometric system [32,33]. Compounds demonstrating at least 90% inhibition in the primary screen were re-examined at lower concentration (MIC) in broth micro-dilution assay with Alamar Blue. The MIC was defined as the lowest concentration inhibiting 99% of the inoculum. Concurrent with the determination of MICs, compounds were tested for cytotoxicity (IC50) in VERO at concentration equal to and greater than the MIC for *M. tuberculosis* H37Rv after 72 h of exposure, viability is assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 Non-radioactive Cell proliferation assay.

3. Results and discussion

3.1. Chemistry

1-isonicotinoyl-3-methyl-4-(2-(substituted phenyl)hydra zono)-1*H*-pyrazol-5(*H*)-ones (**5a-r**) described in this study are shown in (Table 1) and a reaction sequence for the preparation is outlined in (Scheme 1). The ethyl 3-(substituted phenyl)azo-2-oxobutanolate (**3a-r**) were prepared by reacting ethylacetoacetate with appropriate diazonium salt of aromatic amines in the presence of ethanol. Reaction between this newly synthesized 2-oxobutanolate derivatives and isonicotino hydrazide in acetic acid (reaction mediator) and ethanol as solvent led to the synthesis of novel pyrazolones (**5a-r**) in 60-92% yield after recrystallization with ethanol. The purity of the compounds was checked by TLC and elemental analyses. Both analytical and spectral data (^1H and ^{13}C NMR, IR and MS) of all the synthesized compounds were in full agreement with the proposed structures. Final compounds in general, in the infrared spectra (IR), revealed NH, C=O, C=N, C-N, and CH_3 peaks at 3220, 1680, 1590, 1320, 2928 cm^{-1}, respectively.

In the nuclear magnetic resonance spectra (^1H and ^{13}C NMR) the signals of the respective protons of the prepared compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. The spectra showed ^1H NMR singlet at δ range 2.10-2.33 ppm corresponding to methyl group; multiplet at, δ range 6.50-8.80 ppm for aromatic protons; singlet at δ range 11.09-13.18 ppm for NH proton. Similarly, ^{13}C NMR signals at δ range 11.18-12.91 ppm (C14 of CH_3), 127.14-129.96 ppm (C13), 147.02-149.32 ppm (C12) and 162.11-165.09 ppm (C11) of pyrazolone ring and signals at δ range 107.11-151.11 ppm of aromatic carbons, respectively. The elemental analysis results were within $\pm$0.4% of the theoretical values.

3.2. In-vitro Anti-mycobacterial activity

Among the ring substituted pyrazolone derivatives (**5a-r**) were tested for their anti-mycobacterial activity in vitro against *M. tuberculosis* H37Rv using the BACTEC 460 radiometric system.

Table 1. Characterization data of the synthesized compounds, **5a-r**.

Compound	R	Yield (%)	Melting Point (°C)	Mol. Formula	Mol. wt. (g)	UV, λ_{max} (CH_3OH) (nm)
5a	H	80	198-200	$C_{16}H_{13}N_5O_2$	307.31	238.4
5b	2-CH_3	70	240-241	$C_{17}H_{15}N_5O_2$	321.33	369.3
5c	3-CH_3	60	170-173	$C_{17}H_{15}N_5O_2$	321.33	373.7
5d	4-CH_3	65	105-107	$C_{17}H_{15}N_5O_2$	321.33	379.9
5e	2-NO_2	75	185-186	$C_{16}H_{12}N_6O_4$	352.30	367.6
5f	3-NO_2	68	250-252	$C_{16}H_{12}N_6O_4$	352.30	384.8
5g	4-NO_2	78	210-212	$C_{16}H_{12}N_6O_4$	352.30	389.9
5h	2-Cl	72	130-132	$C_{16}H_{12}ClN_5O_2$	341.75	363.2
5i	3-Cl	79	125-126	$C_{16}H_{12}ClN_5O_2$	341.75	377.6
5j	4-Cl	75	190-192	$C_{16}H_{12}ClN_5O_2$	341.75	378.8
5k	2,4-Cl_2	77	187-189	$C_{16}H_{11}C_{l2}N_5O_2$	376.20	376.7
5l	2,5-Cl_2	79	189-191	$C_{16}H_{11}C_{l2}N_5O_2$	376.20	375.2
5m	2,6-Cl_2	82	192-194	$C_{16}H_{11}C_{l2}N_5O_2$	376.20	376.9
5n	2-OCH_3	72	144-147	$C_{17}H_{15}N_5O_3$	337.33	355.9
5o	3-OCH_3	58	149-150	$C_{17}H_{15}N_5O_3$	337.33	359.7
5p	4-OCH_3	69	152-154	$C_{17}H_{15}N_5O_3$	337.33	357.8
5q	2,4-OCH_3	72	167-169	$C_{18}H_{17}N_5O_4$	367.36	483.4
5r	4-SO_2NH_2	77	177-179	$C_{16}H_{14}N_6O_4S$	386.89	478.4

Table 2. Anti-mycobacterial activity of the synthesized compounds against *M. tuberculosis* H37Rv.

Compound	Primary Screen (6.25 µg/mL)	% Inhibition	Concentration, µM	Actual MIC, µg/mL
5a	>6.25	64	0.0354	-
5b	>6.25	12	0.1140	6.25
5c	>6.25	32	0.1706	-
5d	>6.25	28	0.1135	6.25
5e	>6.25	44	0.0167	-
5f	>6.25	14	0.0132	6.25
5g	>6.25	26	0.0142	6.25
5h	>6.25	63	0.0130	6.25
5i	6.25	62	0.0138	6.25
5j	>6.25	64	0.0133	6.25
5k	>6.25	69	0.0118	5.67
5l	6.25	96	0.0084	5.77
5m	6.25	98	0.0034	1.66
5n	>6.25	94	0.0092	4.12
5o	>6.25	88	0.0108	6.25
5p	>6.25	86	0.0103	6.25
5q	>6.25	89	0.0107	6.25
5r	6.25	98	0.0032	1.64
Isoniazid (0.025 - 0.05 µg/mL).				

The results are summarized in Table 2 with INH, a standard used for comparison. Among the 18 newly synthesized compounds, **5l**, **5m**, **5n** and **5r** produced highest efficacy and exhibited >90% inhibition at a concentration of 0.0084, 0.0034, 0.0092 and 0.0032 µM followed by **5o**, **5p** and **5q** which showed moderate to good inhibitory activity with 0.0108 µM, 0.0103 µM and 0.0107 µM, respectively. Thus, the 2,6-dichloro and 4-SO_2NH_2 groups substitution derivatives displayed relatively higher inhibitory activity in general. However, the electron rich groups such as, 2,5-chloro, 2-methoxy, 3-methoxy, 4-methoxy, and 2,4-dimethoxy substituted analogue compounds produced significant increase in inhibitory activity against *M. tuberculosis* H37Rv. On the other hand, pyrazolone analogues with methyl group substitution **5b**, **5c**, **5d** and hydrogen substitution **5a** showed relatively low inhibitory activity against *M. tuberculosis* H37Rv. Instead (CH_3) group and (NO_2) group substitution at phenyl ring in pyrazolone analogue worsens the anti-mycobacterial activity.

All the newly synthesized compounds (**5a-r**) were tested for cytotoxicity (IC50) in VERO cells at concentrations of 62.5 µg/mL. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 Non-radioactive Cell proliferation method. All the active compounds were found to be non-toxic till 62.5 µg/mL.

4. Conclusion

Among the newer derivatives, it is conceivable that derivatives showing anti-mycobacterial activity can be further modified to exhibit better potency than the standard drugs. The pyrazolone derivatives discovered in this study may provide valuable therapeutic intervention for the treatment of anti-tubercular diseases.

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