© 2017 IJSRST | Volume 3 | Issue 1 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
Themed Section: Science and Technology

A Versatile Approach for Synthesis of Various Pyrimidine Derivatives Clubbed with Pyrazolone Structure and Their Biological Activities

Amish P. Khamar
Arts, Science & R. A. Patel Commerce College, Bhadran, Gujarat, India

ABSTRACT

Different pyrimidine derivatives clubbed with pyrazolone structure possessing a diversity of substituents in the 6-position have been synthesized and evaluated for Mycobacterium tuberculosis activities. Several compounds demonstrated $IC_{90} \leq 10$ µg/mL and were found to be active against Mycobacterium tuberculosis strain Compound 8d was found to be most active compound in vitro with IC_{90} of 1.53.

Keywords: Pyrazolopyrimidines, Antitubercular Activity, Antimycobacterial Activity, MABA Assay.

I. INTRODUCTION

Tuberculosis (TB) and HIV have been closely linked since the emergence of AIDS. Worldwide, TB is the most common opportunistic infection affecting HIV-seropositive individuals, and it remains the most common cause of death in patients with AIDS [1]. HIV infection has contributed to a significant increase in the worldwide incidence of TB. By producing a progressive decline in cell-mediated immunity, HIV alters the pathogenesis of TB, greatly increasing the risk of disease from TB in HIV-coinfected individuals and leading to more frequent extrapulmonary involvement, atypical radiographic manifestations, and paucibacillary disease, which can impede timely diagnosis. Although HIV-related TB is both treatable and preventable, incidence continues to climb in developing nations wherein HIV infection and TB are endemic and resources are limited. Interactions between HIV and TB medications, overlapping medication toxicities, and immune reconstitution inflammatory syndrome (IRIS) complicate the cotreatment of HIV and TB. This chapter will review the epidemiology, pathogenesis, management, and prevention of TB in the setting of HIV infection.

Currently, there are 10 drugs approved by the U.S. Food and Drug Administration (FDA) for the treatment of TB disease. In addition, the fluoroquinolones (levofloxacin, moxifloxacin, and gatifloxacin), although not approved by the FDA for TB disease, are commonly used to treat TB disease caused by drug-resistant organisms or for patients who are intolerant of some first-line drugs. Rifabutin, approved for use in preventing Mycobacterium avium complex disease in patients with HIV infection but not approved for TB disease, is useful for treating TB disease in patients concurrently taking drugs that interact with rifampin (e.g., certain antiretroviral drugs). Amikacin and kanamycin, nearly identical aminoglycoside drugs used in treating patients with TB disease caused by drug-resistant organisms, are not approved by the FDA for treatment of TB. Of the approved drugs, isoniazid (INH), rifampin (RIF), ethambutol (EMB), and pyrazinamide (PZA) are considered first-line anti-TB drugs and form the core of standard treatment regimens. Rifabutin (RBT) and rifapentine (RPT) may also be considered firstline drugs under certain circumstances. RBT is used as a substitute for RIF in the treatment of all forms of TB caused by organisms that are known or presumed to be susceptible to this agent. RBT is generally reserved for patients for whom drug-drug interactions preclude the use of rifampin. Streptomycin (SM) was formerly considered to be a first-line drug and, in some instances, is still used in the initial treatment regimen. However, an increasing prevalence of resistance to SM in many parts of the world has decreased its overall usefulness. The remaining drugs are reserved for special situations such as drug intolerance or resistance [3].

One additional difficulty associated with the presently available treatment regimens is the potential for drug-drug interactions, primarily those between rifampin and many of the antiretroviral drugs used for the treatment of

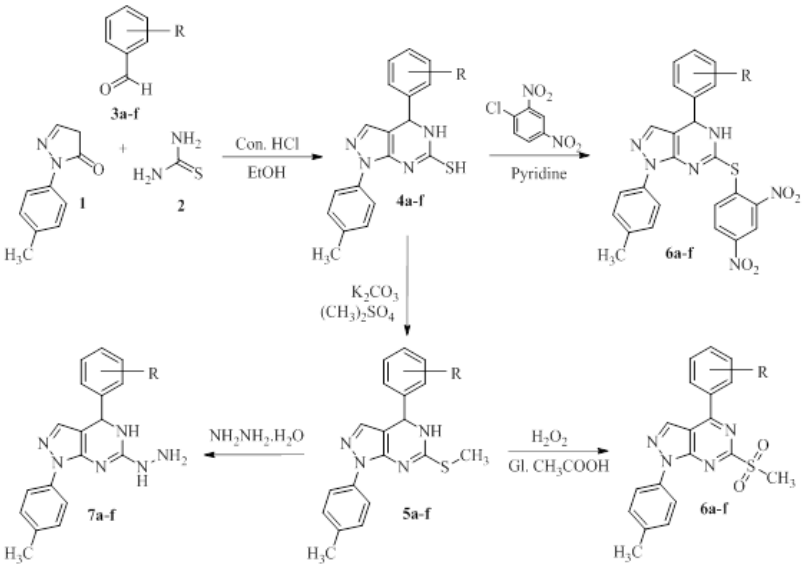
AIDS. Rifampin induces some of the cytochrome P-450 enzymes that metabolize certain of the protease inhibitors and nonnucleoside reverse-transcriptase inhibitors commonly used to treat HIV/AIDS. Therefore, it is difficult to co-administer effective treatment for TB and AIDS.

Therefore, the need for newer, more effective drugs that can achieve multiple goals in improving TB control is pressing. Recognizing these serious facts, we initiated a program to synthesize and screen diverse heterocyclic entities like pyrimidines, phenothiazines and pyrazolo[3,4-d]pyrimidines as potential anti-tubercular agents. Inspired by our previous results[4], [5], [6], we set upon a program of making anti-tubercular agents, using the central pyrazolo[3,4-d]pyrimidine as the template and adding substituents as we deemed necessary to impart activity, on the various positions of pyrazolo[3,4-d]pyrimidine ring. As a part of the program we have synthesized various 6-substituted pyrazolo[3,4-d]pyrimidine derivatives and subjected them to antimycobacterial screening against Mycobacterium tuberculosis $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA).

II. RESULTS AND DISCUSSION

Chemistry

The synthetic routes for the preparation of 6-substituted pyrazolo[3,4-d]pyrimidine derivatives (4a-f to 8a–f) are summarized in scheme 1. Synthesis of 4,5-dihydro-4-(aryl)-3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine-6-thiols (4a-f) was achieved in excellent (80-92%) yields as per our previously published method.[6] Compounds 4a-f were used as a core nucleus to generate various 6-substituted pyrazolo[3,4-d]pyrimidines. Compounds 4a-f on S-methylation with DMS in the presence of K_2CO_3 afforded 4,5-dihydro-4-(aryl)-3-methyl-6-(methylthio)-1-phenyl-1H-pyrazolo [3,4-d]pyrimidines (5a-f), which was oxidized to 4-(aryl)-3-methyl-6-(methylsulfonyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (6a-f) with the help of H_2O_2. While, refluxing 4a-f with hydrazine hydrate yielded 1-(4,5-dihydro-4-(aryl)-3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-6-yl)hydrazines (7a-f). The synthesis of 6-(2,4-dinitrophenylthio)-4,5-dihydro-4-(aryl)-3-methyl-1-phenyl-1H-pyrazolo [3,4-d]pyrimidines 8a-f was accomplished by reacting 4a-f with 1-chloro-2,4-dinitrobenzene using pyridine as a solvent. The structures of the synthesized compounds were assigned on the basis of ^{1}H NMR spectra, ^{13}C NMR, mass spectra and purity was proven by elemental analysis. In ^{1}H NMR spectra of 4a-f, 5a-f, 7a-f and 8a-f, a sharp peak representing methine proton of pyrimidine is observed in the range of 5.08-6.81 δppm, which confirms the formation of pyrazolo[3,4-d]pyrimidine nucleus. While, ^{1}H NMR spectra of 6a-f showed absence of this characteristic methine proton peak due to the oxidation of pyrazolo[3,4-d]pyrimidine nucleus along with the methylthio group.



Antimycobacterial activity

Compounds 5a-f to 8a-f were initially screened against Mycobacterium tuberculosis strain $H_{37}Rv$ at 6.25 µg/mL in the Dose Response assay by the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF) in BACTEC 12B medium using the microplate Alamar Blue assay.[7] This assay is the TAACF's primary screen. Compounds exhibiting >90% inhibition in the initial screen were retested at and below 6.25 µg/mL using 2-fold dilution to determine the MIC. The assay returned IC_{90}, IC_{50} and all of the % Inhibition values at the tested concentrations. IC stands for 'inhibitory concentration' - this is the concentration where a drug inhibits the TB strain by 90% or 50%. The significance of this value depends on several factors such as compound structure, novelty, toxicity, and potential mechanism of action. Compounds are considered active in the dose response screen if $IC_{90} \leq 10$ µg/mL.

Preliminary screening results show that, compounds 5a, 5b, 6c, 7a, 7b, 8d, 8e and 8f exhibited excellent anti-tubercular activity with IC_{90} of 7.12, 3.57, 3.10, 3.11, 2.98, 3.75, 1.53 and 5.99 respectively, and percentage inhibition of 95-100. The results are depicted in Table 1.

Table 1. In vitro antitubercular screening data of 5a-f to 8a-f

Compound.	R	MIC (µg/mL)	% Inhibition	IC_{90} (µg/mL)	IC_{50} (µg/mL)
5a	4-Methoxyphenyl	<6.25	95	7.12	3.23
5b	4-Chlorophenyl	<6.25	96	3.57	2.55
5c	4-Nitrophenyl	>6.25	56	-	-
5d	3-Nitrophenyl	>6.25	53	-	-
5e	2-Nitrophenyl	>6.25	49	-	-
5f	2-Hydroxyphenyl	>6.25	65	-	-
6a	4-Methoxyphenyl	>6.25	64	-	-
6b	4-Chlorophenyl	>6.25	59	-	-
6c	4-Nitrophenyl	<6.25	100	3.10	2.32
6d	3-Nitrophenyl	<6.25	92	24.80	22.65
6e	2-Nitrophenyl	>6.25	63	-	-
6f	2-Hydroxyphenyl	>6.25	46	-	-
7a	4-Methoxyphenyl	<6.25	98	3.11	2.32
7b	4-Chlorophenyl	>6.25	54	-	-
7c	4-Nitrophenyl	>6.25	55	-	-
7d	3-Nitrophenyl	>6.25	52	-	-
7e	2-Nitrophenyl	<6.25	96	2.98	2.71
7f	2-Hydroxyphenyl	>6.25	65	-	-
8a	4-Methoxyphenyl	>6.25	59	-	-
8b	4-Chlorophenyl	<6.25	99	3.75	2.55
8c	4-Nitrophenyl	>6.25	63	-	-
8d	3-Nitrophenyl	<6.25	99	1.53	1.40
8e	2-Nitrophenyl	>6.25	54	-	-
8f	2-Hydroxyphenyl	<6.25	99	5.99	5.39

Structure activity relationship bought to the fore that altering the 6th position of core pyrazolo[3,4-*d*]pyrimidine nucleus (4a-f) along with substitutions at the phenyl ring affects the antimycobacterial activity considerably. Compounds 5a and 5b having methylthio functional group at 6th position along with methoxy and chloro substituents at 4th position of phenyl ring showed better activity compared to other compounds of the series. While, in the case of 6a-f, compound 6c having methylsulfonyl functional group at 6th position along

with nitro group at 4th position of phenyl ring showed better activity then any other compound of the series. Compounds 7a and 7e bearing a mehtoxy and nitro group at 4th and 2nd position of phenyl ring, respectively, in addition to hydrazinyl group at 6th position, showed far better inhibition then any other substituents at any other position. Three compounds belonging to series 8a-f, namely 8a, 8d and 8f demonstrated excellent inhibition possibly due to the presence of bulky substituents at 6th position and chloro, nitro and hydroxy functional group at 4th, 3rd and 2nd position of the phenyl ring, respectively.

III. CONCLUSIONS

In the present paper, we report the synthesis, spectral studies and antimycobacterial activity of various 6-substituted pyrazolo[3,4-*d*]pyrimidine derivatives. The high bioactivity of these compounds makes them suitable hits for additional in vitro and in vivo evaluations, in order to develop new class of antimycobacterial drugs or prodrugs with potential use in the tuberculosis treatment. Further studies in this area are in progress in our laboratory.

IV. EXPERIMENTAL

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 $CDCl_3$ 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 °C, in $CDCl_3$. Elemental analysis of the newly synthesized compounds was carried out on Carlo Erba 1108 analyzer and are found within the range of theoretical value.

Synthesis of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (1)

Synthesis of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (1) was achieved by reported method.[8]

General procedure for the synthesis of 4-(aryl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-thiols (4a-f)

An equimolar mixture of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (0.01 mol), an appropriate aldehyde (0.01 mol), and thiourea (0.01 mol) was heated under reflux condition in ethanol (30 ml) for 8-10 h. The reaction mixture was kept at room temperature for 2-3 hours. The product was filtered, dried and recrystallized from ethanol to give 4a-f.

4-(4-methoxyphenyl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-thiol (4a)

Yield: 75%; mp 121 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.52 (s, 3H, -CH_3), 3.69 (s, 3H, -OCH_3), 5.11 (s, 1H, -CH), 7.41-6.68 (m, 10H, Ar-H), 8.47 (s, 1H, -NH); ^{13}C NMR (δ): 162.9, 157.9, 148.5, 138.3, 132.9 129.8, 127.9, 126.3, 121.1, 119.5, 115.2, 56.1, 41.9, 11.3; Anal. Calcd for $C_{19}H_{18}N_4OS$: C, 65.12; H, 5.18; N, 15.99; Found: C, 64.74; H, 5.22; N, 15.67; MS: m/z 350.

4-(4-chlorophenyl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-thiol (4b)

Yield: 67%; mp 105 °C; ^{1}H NMR (DMSO- d_6) δ ppm: 2.47 (s, 3H, -CH_3), 3.65 (s, 3H, -OCH_3), 5.17 (s, 1H, -CH), 7.56-6.95 (m, 10H, Ar-H), 8.51 (s, 1H, -NH); ^{13}C NMR (δ): 162.0, 147.8, 141.1, 137.9, 131.3, 129.8, 128.3, 127.9, 126.7, 121.4, 118.4, 41.3, 10.8; Anal. Calcd for $C_{18}H_{15}ClN_4S$: C, 60.92; H, 4.26; N, 15.79; Found: C, 60.17; H, 4.41; N, 15.31; MS: m/z 354.

4-(4-nitrophenyl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-thiol (4c)

Yield: 76%; mp 158 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.51 (s, 3H, -CH_3), 3.72 (s, 3H, -OCH_3), 5.21 (s, 1H, -CH), 8.17-7.24 (m, 10H, Ar-H), 8.50 (s, 1H, -NH); ^{13}C NMR (δ): 163.3, 148.5, 147.3, 145.4, 139.5, 128.9, 128.0, 127.1, 125.7, 120.8, 118.7, 39.5, 11.7; Anal. Calcd for $C_{18}H_{15}N_5O_2S$: C, 59.16; H, 4.14; N, 19.17; Found: C, 58.37; H, 4.22; N, 18.67; MS: m/z 365.

4-(3-nitrophenyl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-thiol (4d)

Yield: 74%; mp 141 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.57 (s, 3H, -CH_3), 3.70 (s, 3H, -OCH_3), 5.28 (s, 1H, -CH), 8.12-7.28 (m, 10H, Ar-H), 8.48 (s, 1H, -NH); ^{13}C NMR (δ): 162.0, 148.7, 147.4, 143.4, 139.5, 133.5, 129.7, 129.0, 127.5, 126.8, 122.5, 119.0, 40.3, 11.6; Anal. Calcd for $C_{18}H_{15}N_5O_2S$: C, 59.16; H, 4.14; N, 19.17; Found: C, 58.42; H, 4.27; N, 18.61; MS: m/z 365.

4-(2-nitrophenyl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-thiol (4e)

Yield: 66%; mp 103 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.49 (s, 3H, -CH_3), 3.71 (s, 3H, -OCH_3), 5.18 (s, 1H, -CH), 8.23-7.30 (m, 10H, Ar-H), 8.41 (s, 1H, -NH); ^{13}C NMR (δ): 161.4, 148.1, 146.0, 139.8, 137.3, 133.5, 129.9, 128.3, 127.8, 126.5, 121.0, 119.8, 118.7, 41.2, 12.1; Anal. Calcd for $C_{18}H_{15}N_5O_2S$: C, 59.16; H, 4.14; N, 19.17; Found: C, 58.33; H, 4.12; N, 18.92; MS: m/z 365.

4-(2-hydroxyphenyl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-thiol (4f)

Yield: 79%; mp 138 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.42 (s, 3H, -CH_3), 3.66 (s, 3H, -OCH_3), 5.09 (s, 1H, -CH), 7.33-6.56 (m, 10H, Ar-H), 8.54 (s, 1H, -NH), 12.09 (s, 1H, -OH); ^{13}C NMR (δ): 164.3, 154.0, 148.7, 141.0, 130.2, 128.8, 128.0, 127.7, 126.0, 121.1, 119.4, 117.3, 31.2, 10.6; Anal. Calcd for $C_{18}H_{16}N_4OS$: C, 64.26; H, 4.79; N, 16.65; Found: C, 63.57; H, 4.31; N, 15.98; MS: m/z 336.

General procedure for the synthesis of 4-(aryl)-3-methyl-6-(methylthio)-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidines (5a-f)

A mixture of appropriate 4-(aryl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-thiol (0.01 mol), dimethyl sulphate (0.01 mol) and K_2CO_3 (0.01 mol) in DMF (20 ml) was stirred for 4 h. The reaction mixture was poured in to ice cold water, filtered, dried and recrystallized from ethanol to give 5a-f.

4-(4-methoxyphenyl)-3-methyl-6-(methylthio)-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine (5a)

Yield: 76%; mp 164 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.15 (s, 3H, -SCH_3), 2.58 (s, 3H, -CH_3), 3.71 (s, 3H, -OCH_3), 5.12 (s, 1H, -CH), 7.37-6.71 (m, 10H, Ar-H), 8.41 (s, 1H, -NH); ^{13}C NMR (δ): 159.9, 158.2, 149.2, 139.6, 135.7, 130.2, 128.1, 127.7, 125.3, 120.0, 118.8, 58.1, 41.7, 14.1, 11.6; Anal. Calcd for $C_{20}H_{20}N_4OS$: C, 65.91; H, 5.53; N, 15.37; Found: C, 65.34; H, 5.71; N, 15.31; MS: m/z 364.

4-(4-chlorophenyl)-3-methyl-6-(methylthio)-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine (5b)

Yield: 70%; mp 175 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.16 (s, 3H, -SCH_3), 2.53 (s, 3H, -CH_3), 5.14 (s, 1H, -CH), 7.32-6.39 (m, 10H, Ar-H), 8.32 (s, 1H, -NH); ^{13}C NMR (δ): 158.4, 148.3, 142.6, 139.4, 132.5, 130.9, 128.7, 128.0, 127.5, 124.6, 120.4, 41.3, 13.3, 11.4; Anal. Calcd for $C_{19}H_{17}ClN_4S$: C, 61.86; H, 4.65; N, 15.19; Found: 61.96; H, 4.37; N, 14.62; MS: m/z 368.

4-(4-nitrophenyl)-3-methyl-6-(methylthio)-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine (5c)

Yield: 68%; mp 190 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.12 (s, 3H, -SCH_3), 2.61 (s, 3H, -CH_3), 5.17 (s, 1H, -CH), 8.21-7.13 (m, 10H, Ar-H), 8.26 (s, 1H, -NH), 12.12 (s, 1H, -OH); ^{13}C NMR (δ): 157.6, 147.1, 145.6, 143.4, 138.8, 129.5, 128.1, 127.8, 125.9, 120.7, 119.9, 118.7, 41.5, 12.8, 11.3; Anal. Calcd for $C_{19}H_{17}N_5O_2S$: C, 60.14; H, 4.52; N, 18.46; Found: C, 59.67; H, 4.32; N, 18.87; MS: m/z 379.

4-(3-nitrophenyl)-3-methyl-6-(methylthio)-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine (5d)

Yield: 75%; mp 172 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.14 (s, 3H, -SCH_3), 2.62 (s, 3H, -CH_3), 5.19 (s, 1H, -CH), 8.39-7.06 (m, 10H, Ar-H), 8.29 (s, 1H, -NH); ^{13}C NMR (δ): 156.2, 148.3, 147.4, 141.6, 139.9, 135.3, 131.7, 129.2, 126.0, 121.9, 120.0, 117.8, 40.8, 13.0, 11.9; Anal. Calcd for $C_{19}H_{17}N_5O_2S$: C, 60.14; H, 4.52; N, 18.46; Found: C, 59.43; H, 4.58; N, 18.31; MS: m/z 379.

4-(2-nitrophenyl)-3-methyl-6-(methylthio)-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine (5e)

Yield: 66%; mp 168 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.09 (s, 3H, -SCH_3), 2.66 (s, 3H, -CH_3), 5.22 (s, 1H, -CH), 8.32-7.23 (m, 10H, Ar-H), 8.44 (s, 1H, -NH); ^{13}C NMR (δ): 158.4, 147.9, 146.8, 142.1, 137.9, 132.9, 129.9, 128.3, 127.1, 126.5, 123.8, 120.7, 118.6, 34.9, 14.3, 11.0; Anal. Calcd for $C_{19}H_{17}N_5O_2S$: C, 60.14; H, 4.52; N, 18.46; Found: C, 59.27; H, 21; N, 17.93; MS: m/z 379.

4-(2-hydroxyphenyl)-3-methyl-6-(methylthio)-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine (5f)

Yield: 71%; mp 208 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.01 (s, 3H, -SCH_3), 2.55 (s, 3H, -CH_3), 5.08 (s, 1H, -CH), 7.39-6.52 (m, 10H, Ar-H), 8.08 (s, 1H, -NH), 11.98 (s, 1H, -OH); ^{13}C NMR (δ): 160.0, 153.4, 145.8, 140.5, 131.1, 129.7, 128.3, 127.8, 125.3, 121.8, 120.4, 117.5, 114.9, 32.7, 12.3, 11.8; Anal. Calcd for

$C_{19}H_{18}N_4OS$: C, 65.12; H, 5.18; N, 15.99; Found: C, 64.67; H, 4.78; N, 15.01; MS: m/z 350.

General procedure for the synthesis of 4-(aryl)-3-methyl-6-(methylsulfonyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidines (6a-f)

To a solution of appropriate 4-(aryl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo [3,4-*d*]pyrimidine-6-thiol (0.01 mol) in glacial acetic acid (10 ml), 10 ml of hydrogen peroxide solution was added. The reaction mixture was stirred at room temperature for 48 h. After completion of the reaction, the content was poured in to ice cold water, filtered, dried and recrystallized from ethanol to give 6a-f.

4-(4-methoxyphenyl)-3-methyl-6-(methylsulfonyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (6a)

Yield: 79%; mp 178 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.92 (s, 3H, -SO_2CH_3), 2.58 (s, 3H, -CH_3), 3.72 (s, 3H, -OCH_3), 7.37-6.72 (m, 10H, Ar-H); ^{13}C NMR (δ): 165.0, 163.2, 160.6, 150.9, 145.4, 139.7, 129.6, 127.4, 126.1, 125.0, 120.8, 113.8, 55.4, 46.1, 14.2; Anal. Calcd for $C_{20}H_{18}N_4O_3S$: C, 60.90; H, 4.60; N, 14.20; Found: C, 60.54; H, 4.31; N, 14.02; MS: m/z 394.

4-(4-chlorophenyl)-3-methyl-6-(methylsulfonyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (6b)

Yield: 68%; mp 172 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.98 (s, 3H, -SO_2CH_3), 2.65 (s, 3H, -CH_3), 7.45-6.91 (m, 10H, Ar-H); ^{13}C NMR (δ): 166.4, 164.7, 149.6, 144.1, 140.3, 134.1, 132.0, 129.9, 128.6, 128.0, 126.0, 122.3, 44.9, 15.8; Anal. Calcd for $C_{19}H_{15}ClN_4O_2S$: C, 57.21; H, 3.79; N, 14.05; Found: C, 56.85; H, 3.86; N, 13.73; MS: m/z 398.

4-(4-nitrophenyl)-3-methyl-6-(methylsulfonyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (6c)

Yield: 70%; mp 230 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 3.12 (s, 3H, -SO_2CH_3), 2.71 (s, 3H, -CH_3), 8.39-7.11 (m, 10H, Ar-H); ^{13}C NMR (δ): 168.1, 165.1, 150.7, 147.9, 143.0, 140.0, 139.1, 131.8, 129.2, 125.9, 121.6, 119.7, 44.1, 16.3; Anal. Calcd for $C_{19}H_{15}N_5O_4S$: C, 55.74; H, 3.69; N, 17.11; Found: C, 55.12; H, 3.39; N, 16.83; MS: m/z 409.

4-(3-nitrophenyl)-3-methyl-6-(methylsulfonyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (6d)

Yield: 68%; mp 218 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 3.02 (s, 3H, -SO_2CH_3), 2.67 (s, 3H, -CH_3), 8.67-7.20 (m, 10H, Ar-H); ^{13}C NMR (δ): 167.5, 163.7, 151.1, 148.7, 143.8, 140.3, 134.5, 133.2, 130.0, 128.8, 126.9, 122.5, 121.0, 119.5, 44.3, 15.8; Anal. Calcd for $C_{19}H_{15}N_5O_4S$: C, 55.74; H, 3.69; N, 17.11; Found: C, 54.98; H, 3.47; N, 16.69; MS: m/z 409.

4-(2-nitrophenyl)-3-methyl-6-(methylsulfonyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (6e)

Yield: 72%; mp 208 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 3.10 (s, 3H, -SO_2CH_3), 2.71 (s, 3H, -CH_3), 8.49-7.17 (m, 10H, Ar-H); ^{13}C NMR (δ): 168.7, 164.5, 150.1, 147.1, 142.8, 138.3, 135.9, 133.2, 131.1, 129.0, 128.8, 125.9, 121.0, 117.8, 43.4, 15.1; Anal. Calcd for $C_{19}H_{15}N_5O_4S$: C, 55.74; H, 3.69; N, 17.11; Found: C, 55.21; H, 3.17; N, 16.84; MS: m/z 409.

4-(2-hydroxyphenyl)-3-methyl-6-(methylsulfonyl)-1-phenyl-1*H*-pyrazolo [3,4-*d*]pyrimidine (6f)

Yield: 78%; mp 182 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.90 (s, 3H, -SO_2CH_3), 2.54 (s, 3H, -CH_3), 7.47-6.61 (m, 10H, Ar-H), 12.25 (s, 1H, -OH); ^{13}C NMR (δ): 169.0, 165.1, 155.8, 151.6, 146.1, 138.5, 131.9, 128.2, 127.7, 125.6, 122.4, 120.0, 117.3, 111.9, 43.7, 13.8; Anal. Calcd for $C_{19}H_{16}N_4O_3S$: C, 59.99; H, 4.24; N, 14.73; Found: C, 55.67; H, 4.21; N, 14.66; MS: m/z 380.

General procedure for the synthesis of 1-(4-(aryl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl)hydrazines (7a-f)

A mixture of appropriate 4-(aryl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-thiol (0.01 mol) and hydrazine hydrate (0.01 mol) was heated under reflux condition for 10 h. After completion of the reaction, the content was poured in to ice cold water, filtered, dried and recrystallized from ethanol to give 7a-f.

1-(4-(4-methoxyphenyl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl)hydrazine (7a)

Yield: 74%; mp 178 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.33 (s, 3H, -CH_3), 3.90 (s, 3H, -OCH_3), 6.78 (s, 1H, -CH) 8.10-6.81 (m, 10H, Ar-H), 8.11 (s, 1H, -NH_2); ^{13}C NMR (δ): 163.0, 160.1, 145.7, 138.4, 133.0, 129.5, 127.3, 125.0, 118.7, 116.2, 112.4, 56.5, 40.7, 13.0; Anal.

Calcd for $C_{19}H_{20}N_6O$: C, 56.50; H, 5.79; N, 24.12; Found: C, 55.87; H, 5.27; N, 24.06; MS: m/z 348.

1-(4-(4-chlorophenyl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl)hydrazine (7b)

Yield: 79%; mp 162 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.47 (s, 3H, -CH_3), 6.69 (s, 1H, -CH) 7.79-6.83 (m, 10H, Ar-H), 8.06 (s, 1H, -NH_2); ^{13}C NMR (δ): 164.7, 146.3, 139.4, 138.1, 132.9, 130.6, 128.7, 127.5, 125.4, 121.2, 118.4, 38.4, 12.3; Anal. Calcd for $C_{18}H_{17}ClN_6$: C, 61.28; H, 4.86; N, 23.82; Found: C, 60.45; H, 4.53; N, 23.18; MS: m/z 352.

1-(4-(4-nitrophenyl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl)hydrazine (7c)

Yield: 72%; mp 212 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.53 (s, 3H, -CH_3), 6.53 (s, 1H, -CH) 8.37-7.02 (m, 10H, Ar-H), 8.35 (s, 1H, -NH_2); ^{13}C NMR (δ): 165.0, 149.1, 147.5, 145.2, 138.7, 130.3, 128.6, 127.8, 124.9, 122.3, 119.7, 117.8, 41.2, 11.7; Anal. Calcd for $C_{18}H_{17}N_7O_2$: C, 59.50; H, 4.72; N, 26.98; Found: C, 58.34; H, 4.87; N, 26.79; MS: m/z 363.

1-(4-(3-nitrophenyl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl)hydrazine (7d)

Yield: 72%; mp 205 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.50 (s, 3H, -CH_3), 6.57 (s, 1H, -CH) 8.34-7.13 (m, 10H, Ar-H), 8.49 (s, 1H, -NH_2); ^{13}C NMR (δ): 165.4, 148.7, 147.8, 142.5, 137.9, 131.8, 129.9, 128.2, 125.2, 121.9, 120.0, 118.9, 117.9, 38.1, 12.2; Anal. Calcd for $C_{18}H_{17}N_7O_2$: C, 59.50; H, 4.72; N, 26.98; Found: C, 58.17; H, 4.83; N, 26.69; MS: m/z 363.

1-(4-(2-nitrophenyl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl)hydrazine (7e)

Yield: 70%; mp 198 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.61 (s, 3H, -CH_3), 6.66 (s, 1H, -CH) 8.42-6.99 (m, 10H, Ar-H), 8.45 (s, 1H, -NH_2); ^{13}C NMR (δ): 164.7, 147.8, 146.1, 141.7, 137.1, 133.9, 130.3, 128.8, 127.2, 126.5, 125.7, 121.7, 119.7, 32.1, 11.8; Anal. Calcd for $C_{18}H_{17}N_7O_2$: C, 59.50; H, 4.72; N, 26.98; Found: C, 58.47; H, 4.78; N, 26.78; MS: m/z 363.

1-(4-(2-hydroxyphenyl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl)hydrazine (7f)

Yield: 73%; mp 152 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.39 (s, 3H, -CH_3), 6.81 (s, 1H, -CH) 7.56-6.41 (m, 10H, Ar-H), 8.14 (s, 1H, -NH_2), 11.86 (s, 1H, -OH); ^{13}C NMR (δ): 162.8, 153.4, 147.0, 140.9, 129.7, 128.6, 128, 127.5, 126.2, 121.3, 119.9, 115.5, 30.1, 12.8; Anal. Calcd for $C_{18}H_{18}N_6O$: C, 64.66; H, 5.43; N, 25.13; Found: C, 64.37; H, 5.87; N, 25.79; MS: m/z 334.

General procedure for the synthesis of 6-(2,4-dinitrophenylthio)-4-(aryl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidines (8a-f)

A mixture of appropriate 4-(aryl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-thiol (0.01 mol) and 1-chlro-2,4-dinitrobenzene was heated under reflux condition for 10-12 h using pyridine (20 ml) as a solvent. After completion of the reaction, the content was poured in to ice cold water, filtered, dried and recrystallized from ethanol to give 8a-f.

6-(2,4-dinitrophenylthio)-4-(4-methoxyphenyl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine (8a)

Yield: 78%; mp 210 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.27 (s, 3H, -CH_3), 3.85 (s, 3H, -OCH_3), 5.28 (s, 1H, -CH) 8.91-6.76 (m, 12H, Ar-H), 10.27 (s, 1H, -NH); ^{13}C NMR (δ): 166.0, 159.4, 150.9, 148.7, 145.1, 141.0, 135.7, 133.3, 131.8, 128.6, 127.8, 126.9, 125.7, 120.7, 119.4, 117.1, 114.6, 55.1, 43.3, 10.9; Anal. Calcd for $C_{25}H_{20}N_6O_5S$: C, 58.13; H, 3.90; N, 16.27; Found: C, 57.49; H, 3.56; N, 16.05; MS: m/z 516.

6-(2,4-dinitrophenylthio)-4-(4-chlorophenyl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine (8b)

Yield: 72%; mp 240 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.30 (s, 3H, -CH_3), 5.34 (s, 1H, -CH) 8.99-7.01 (m, 12H, Ar-H), 10.34 (s, 1H, -NH); ^{13}C NMR (δ): 166.3, 151.2, 148.3, 146.1, 141.7, 138.8, 133.6, 131.5, 130.7, 129.2, 128.1, 127.8, 127.2, 126.3, 125.7, 120.3, 118.5, 55.1, 42.8, 11.1; Anal. Calcd for $C_{24}H_{17}ClN_6O_4S$: C, 55.33; H, 3.29; N, 16.13; Found: C, 54.78; H, 3.01; N, 15.89; MS: m/z 521.

6-(2,4-dinitrophenylthio)-4-(4-nitrophenyl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine (8c)

Yield: 67%; mp 265 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.38 (s, 3H, -CH_3), 5.20 (s, 1H, -CH) 9.07-7.26 (m, 12H,

Ar-H), 10.44 (s, 1H, -NH); ^{13}C NMR (δ): 167.0, 151.8, 147.4, 146.6, 146.1, 145.7, 143.7, 138.5, 132.9, 130.8, 128.3, 127.2, 126.7, 126.0, 120.7, 119.2, 118.6, 40.4, 10.1; Anal. Calcd for $C_{24}H_{17}N_7O_6S$: C, 54.23; H, 3.22; N, 18.45; Found: C, 53.69; H, 3.08; N, 18.15; MS: m/z 531.

6-(2,4-dinitrophenylthio)-4-(3-nitrophenyl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine (8d)

Yield: 73%; mp 264 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.34 (s, 3H, -CH_3), 5.25 (s, 1H, -CH) 9.12-7.31 (m, 12H, Ar-H), 10.39 (s, 1H, -NH); ^{13}C NMR (δ): 166.7, 151.1, 148.3, 146.5, 145.8, 144.7, 138.0, 134.3, 132.1, 130.7, 128.6, 127.7, 127.0, 126.9, 122.1, 119.4, 118.3, 117.6, 42.8, 10.4; Anal. Calcd for $C_{24}H_{17}N_7O_6S$: C, 54.23; H, 3.22; N, 18.45; Found: C, 54.00; H, 3.34; N, 18.24; MS: m/z 531.

6-(2,4-dinitrophenylthio)-4-(2-nitrophenyl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine (8e)

Yield: 75%; mp 258 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.35 (s, 3H, -CH_3), 5.19 (s, 1H, -CH) 8.97-7.32 (m, 12H, Ar-H), 10.37 (s, 1H, -NH); ^{13}C NMR (δ): 167.2, 151.4, 147.0, 146.1, 145.2, 139.5, 136.9, 133.8, 133.0, 130.9, 128.8, 127.9, 127.2, 125.6, 122.1, 121.0, 119.5, 118.1, 33.6, 10.6; Anal. Calcd for $C_{24}H_{17}N_7O_6S$: C, 54.23; H, 3.22; N, 18.45; Found: C, 53.87; H, 3.21; N, 17.96; MS: m/z 531.

6-(2,4-dinitrophenylthio)-4-(2-hydroxyphenyl)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine (8f)

Yield: 80%; mp 208 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.43 (s, 3H, -CH_3), 5.22 (s, 1H, -CH) 8.86-6.68 (m, 12H, Ar-H), 10.27 (s, 1H, -NH), 12.47 (s, 1H, -OH); ^{13}C NMR (δ): 166.6, 155.1, 150.8, 146.5, 145.7, 140.8, 132.9, 130.4, 129.0, 128.5, 127.7, 127.1, 124.9, 120.6, 119.7, 117.7, 114.8, 32.3, 10.9; Anal. Calcd for $C_{24}H_{18}N_6O_5S$: C, 57.36; H, 3.61; N, 16.72; Found: C, 56.32; H, 3.27; N, 16.65; MS: m/z 502.

V. REFERENCES

[1]. Corbett EL, Watt CJ, Walker N. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. Arch Intern Med 2003;163:1009-21.

[2]. Blumberg HM, Burman WJ, Chaisson RE, Daley CL, Etkind SC, Friedman LN, Fujiwara P, Grzemska M, Hopewell PC, Iseman MD, Jasmer RM, Koppaka V, Menzies RI, O'Brien RJ, Reves RR, Reichman LB, Simone PM, Starke JR, Vernon AA. American Thoracic Society/Centers for Disease Control and Prevention/ Infectious Diseases Society of America: treatment of tuberculosis. Am J Respir Crit CareMed 2003;167:603-62.

[3]. Yew WW. Directly Observed Therapy, Short-Course: The Best Way to Prevent Multidrug-Resistant Tuberculosis. Chemotherapy 1999;45:26-33.

[4]. Trivedi AR, Siddiqui AB, Shah VH. Design synthesis characterization and antitubercular activity of some newer 2-hetrocycle phenothiazines. ARKIVOC 2008;2:210-17.

[5]. Trivedi AR, Dodiya DK, Ravat NR, Shah VH. Synthesis and biological evaluation of some new pyrimidines via a novel chalcone series. ARKIVOC 2008;11:131-37.

[6]. Trivedi A, Dodiya D, Surani J, Mathukia H, Ravat N, Shah V. Facile one-pot synthesis and antimycobacterial evaluation of pyrazolo[3,4-d]pyrimidines. Archiv der pharmazie 2008;341:435-39.

[7]. Collins L, Franzblau SG. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against Mycobacterium tuberculosis and Mycobacterium avium. Antimicrob Agents Chemother 2007;41:1004-1009.

[8]. Tiwari S, Khupse N, Kumar A. Intramolecular Diels-Alder reaction in ionic liquids: effect of ion-specific solvent friction. Journal of organic chemistry 2008;73:9075-83.