Journal of Enzyme Inhibition and Medicinal Chemistry, 2010; 25(6): 893–899

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RESEARCH ARTICLE

Synthesis and antimycobacterial evaluation of various 6-substituted pyrazolo[3,4-*d*]pyrimidine derivatives

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Abstract

Various pyrazolo[3,4-*d*]pyrimidines carrying a variety of substituents in the 6-position have been synthesised and their ability to inhibit growth of *Mycobacterium tuberculosis in vitro* has been determined. Compounds **5a**, **5b**, **6c**, **7a**, **7b**, **8d**, **8e** and **8f** demonstrated a minimum inhibitory concentration (MIC) of <6.25 µg/mL and were found to be active against *Mycobacterium tuberculosis* strain $H_{37}RV$. Compound **8d** was found to be the most active compound *in vitro* with a MIC of <6.25 µg/mL and inhibitory concentration IC_{90} of 1.53 µg/mL.

Keywords: *Pyrazolopyrimidines; antitubercular activity; antimycobacterial activity*

Introduction

Tuberculosis (TB) is by far the most frequently encountered mycobacterial disease in the world. Among infectious diseases, TB is the number one killer with over two million casualties annually worldwide. The World Health Organization (WHO) considers tuberculosis to be the most dangerous chronic communicable disease in the world [1]. The emergence of AIDS, decline of socioeconomic standards and a reduced emphasis on tuberculosis control programmes have contributed to the resurgence of the disease in industrialised countries [2]. Resistance of *Mycobacterium tuberculosis* strains to anti-mycobacterial agents is an increasing problem worldwide [3–5]. However, powerful new anti-TB drugs with new mechanisms of action have not been developed in the last forty years.

The currently recommended treatment regimens for active pulmonary TB are both lengthy and cumbersome. The treatment duration is a minimum of six months, with four drugs (isoniazid, rifampin, pyrazinamide, and ethambutol) typically given daily for the first two months and then two drugs (isoniazid and rifampin) administered for four additional months [6]. Partly due to this lengthy and complex treatment regimen, in 1993 the World Health Organization (WHO) introduced a global strategy for TB control known as "directly observed therapy, short-course" (DOTS) [7]. One of the crucial components of this strategy is the direct observation by trained personnel of patients taking their medications, to ensure compliance and to help prevent the emergence of drug resistance. Although the direct observation and monitoring of patient adherence to the regimen is important to treatment success, it also increases the cost of treatment and makes TB therapy more burdensome.

One additional difficulty associated with the current 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 metabolise certain of the protease inhibitors and non-nucleoside reverse-transcriptase inhibitors commonly used to treat HIV/AIDS. Therefore, it is difficult to co-administer effective treatment for TB and AIDS.

The treatment of multi-drug resistant (MDR)-TB is characterised by relatively less effective, poorly tolerated, and expensive drugs that may need to be administered for years. Equally inadequate is the treatment available for latent TB infection. It has been estimated that 2 billion individuals are infected with M. tuberculosis, so there is an enormous human reservoir of the infecting organism. The currently recommended treatment for latent TB infection is isoniazid given for six to nine months. The long duration of this therapy and the potential

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(Received 04 August 2009; revised 25 September 2009; accepted 08 December 2009)

ISSN 1475-6366 print/ISSN 1475-6374 online © 2010 Informa UK, Ltd.
DOI: 10.3109/14756360903540276

toxicities of isoniazid means there is a major compliance problem associated with the treatment regimen. Although new drugs are needed to shorten the duration of treatment of latent TB infection, the safety profile for these drugs must be excellent, because most patients with latent infection are destined never to experience activation of their TB.

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 programme to synthesise and screen diverse heterocyclic entities like pyrimidines, phenothiazines and pyrazolo[3,4-*d*]pyrimidines as potential anti-tubercular agents. Based on our previous results [8–10], we set upon a programme of making anti-tubercular agents, using the central pyrazolo[3,4-*d*]pyrimidine as the template and adding substituents as necessary to impart activity, on the various positions of pyrazolo[3,4-*d*]pyrimidine ring. As a part of the programme we have synthesised various 6-substituted pyrazolo[3,4-*d*]pyrimidine derivatives and subjected them to antimycobacterial screening against Mycobacterium tuberculosis H37Rv (ATCC 27294) in BACTEC 12B medium using the microplate alamarBlue® assay (MABA).

Materials and methods

All chemicals were supplied by Merck (Germany) and SD Fine Chemicals (India). Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by thin layer chromotography (TLC; Merck, Germany) 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 synthesised compounds was carried out on Carlo Erba 1108 analyser and are found within the range of theoretical value.

Chemistry

*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 [11].

*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 to 10 hours. The reaction mixture was kept at room temperature for 2 to 3 hours. The product was filtered, dried and recrystallised 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, 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), 8.5 (s, 1H, -NH); ^{13}C NMR (δ): 163.3, 148.5, 147.3, 145.4, 139.5, 128.9, 128, 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), 3.7 (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, 148.7, 147.4, 143.4, 139.5, 133.5, 129.7, 129, 127.5, 126.8, 122.5, 119, 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, 139.8, 137.3, 133.5, 129.9, 128.3, 127.8, 126.5, 121, 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, 148.7, 141, 130.2, 128.8, 128, 127.7, 126, 121.1, 119.4, 117.3, 41.2, 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 **(4a–f)** (0.01 mol), dimethyl sulphate (0.01 mol) and K_2CO_3 (0.01 mol)

in DMF (20 mL) was stirred for 4 hours. The reaction mixture was poured in to ice cold water, filtered, dried and recrystallised from ethanol to give **5a–f**.

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

Yield: 76%; mp 164°C; 1H NMR (DMSO-*d6*) 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); 13C NMR (δ): 159.9, 158.2, 149.2, 139.6, 135.7, 130.2, 128.1, 127.7, 125.3, 120, 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-1H-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, 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-1H-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-1H-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, 121.9, 120, 117.8, 40.8, 13, 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-1H-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; 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-1H-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, 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-(methylsulphonyl)-1-phenyl-1H-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 **(5a–f)** (0.01 mol) in glacial acetic acid (10 mL), hydrogen peroxide (10 mL) 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 recrystallised from ethanol to give **6a–f**.

4-(4-methoxyphenyl)-3-methyl-6-(methylsulphonyl)-1-phenyl-1H-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, 163.2, 160.6, 150.9, 145.4, 139.7, 129.6, 127.4, 126.1, 125, 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-(methylsulphonyl)-1-phenyl-1H-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, 129.9, 128.6, 128, 126, 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-(methylsulphonyl)-1-phenyl-1H-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, 140, 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-(methylsulphonyl)-1-phenyl-1H-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), 8.67-7.2 (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, 128.8, 126.9, 122.5, 121, 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-(methylsulphonyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (6e)

Yield: 72%; mp 208°C; ^{1}H NMR (DMSO-d_6) δ ppm: 3.1 (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, 128.8, 125.9, 121, 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-(methylsulphonyl)-1-phenyl-1H-pyrazolo [3,4-d]pyrimidine (6f)
Yield: 78%; mp 182°C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.9 (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, 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-1H-pyrazolo[3,4-d] pyrimidin-6-yl)hydrazines (7a–f)
A mixture of appropriate 4-(aryl)-3-methyl-6-(methylthio)-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine **(5a–f)** (0.01 mol) and hydrazine hydrate (0.01 mol) was heated under reflux condition for ten hours. After completion of the reaction, the reaction mixture was poured into ice cold water, filtered, dried and recrystallised from ethanol to give **7a–f**.

1-(4-(4-methoxyphenyl)-3-methyl-1-phenyl-4,5-dihydro-1H-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, 160.1, 145.7, 138.4, 133, 129.5, 127.3, 125, 118.7, 116.2, 112.4, 56.5, 40.7, 13; Anal. calcd for $C_{19}H_{20}N_6O$: C, 56.5; 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-1H-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-1H-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-1H-pyrazolo[3,4-d]pyrimidin-6-yl)hydrazine (7d)
Yield: 72%; mp 205°C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.5 (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, 118.9, 117.9, 38.1, 12.2; Anal. calcd for $C_{18}H_{17}N_7O_2$: C, 59.5; 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-1H-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, 59.5; 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-1H-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, 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-1H-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 **(4a–f)** (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 reaction mixture was poured into ice cold water, filtered, dried and recrystallised from ethanol to give **8a–f**.

6-(2,4-dinitrophenylthio)-4-(4-methoxyphenyl)-3-methyl-1-phenyl-4,5-dihydro-1H-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, 159.4, 150.9, 148.7, 145.1, 141, 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, 3.9; 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-1H-pyrazolo[3,4-d]pyrimidine (8b)
Yield: 72%; mp 240°C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.3 (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-1H-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), 5.2 (s, 1H, -CH), 9.07-7.26 (m, 12H, Ar-H), 10.44 (s, 1H, -NH); ^{13}C NMR (δ): 167, 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, 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-1H-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, 134.3, 132.1, 130.7, 128.6, 127.7, 127, 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; H, 3.34; N, 18.24%; MS: *m/z* 531.

6-(2,4-dinitrophenylthio)-4-(2-nitrophenyl)-3-methyl-1-phenyl-4,5-dihydro-1H-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, 146.1, 145.2, 139.5, 136.9, 133.8, 133, 130.9, 128.8, 127.9, 127.2, 125.6, 122.1, 121, 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-1H-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, 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.

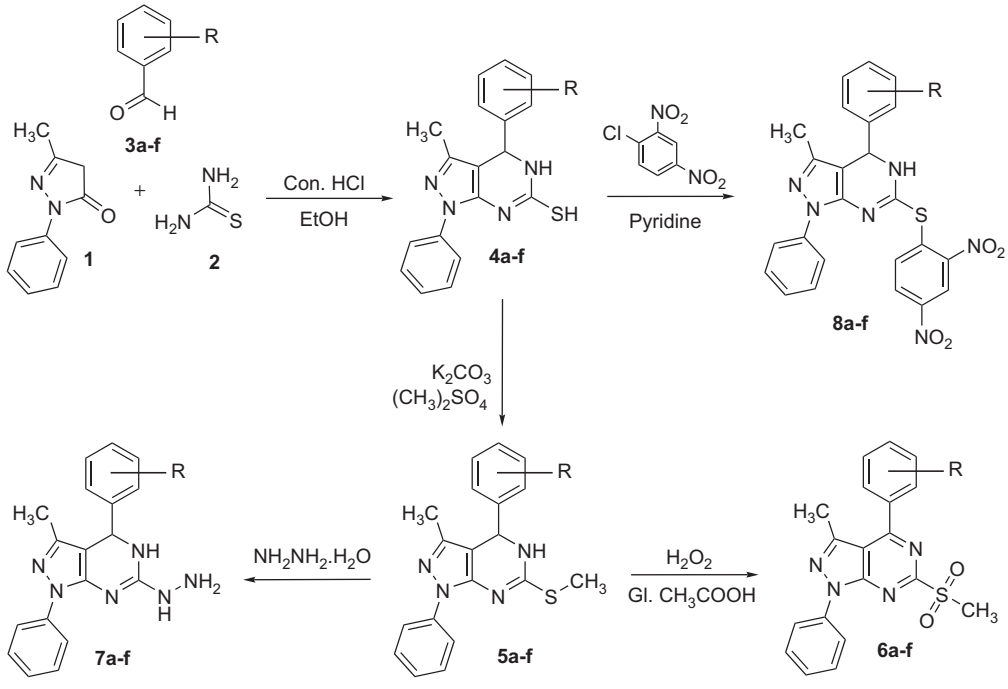
Biology

Primary screening of compounds **5a–f** to **8a–f** was conducted at 6.25 µg/mL against *M. tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the microplate Alamar blue assay [12]. Compounds exhibiting >90% inhibition in the initial screen were retested at and below 6.25 µg/mL using two-fold dilution to determine the actual minimum inhibitory concentration (MIC) using MABA. The assay afforded percentage inhibition, IC_{90} and IC_{50} values at the corresponding tested concentrations. IC stands for inhibitory concentration and is the concentration where a drug inhibits the TB strain by 90% or 50%. Compounds are considered active in the screen if $IC_{90} \leq 10$ µg/mL.

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 summarised in Scheme 1. Synthesis of 4,5-dihydro-4-(aryl)-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-thiols (**4a–f**) was achieved in excellent (80–92%) yields as per our previously published method [13]. Compounds **4a–f** were used as a core nucleus to generate various 6-substituted pyrazolo[3,4-*d*]pyrimidines. A sharp peak representing methine proton of pyrimidine was observed in the range of 5.09-5.28 δ ppm, which confirmed the formation of



3a-f to **8a-f** R = 4-OCH_3, 4-Cl, 4-NO_2, 3-NO_2, 2-NO_2, 2-OH

Scheme 1. Synthesis of various 6-substituted pyrazolo[3,4-d]pyrimidines.

pyrazolo[3,4-*d*]pyrimidine nucleus. Also, in ^{13}C NMR spectra, methine proton was observed between 39.5-41.9 δ ppm. Compounds **4a–f** on S-methylation with dimethyl sulphate in the presence of K_2CO_3 afforded 4,5-dihydro-4-(aryl)-3-methyl-6-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*] pyrimidines (**5a–f**), which was confirmed by presence of S-methyl protons as a singlet in the range of 2.01-2.16 δ ppm in ^{1}H NMR spectra of **5a–f**. S-methyl protons were observed at 12.3-14.3 δ ppm In ^{13}C NMR spectra. These were oxidised to 4-(aryl)-3-methyl-6-(methylsulphonyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**6a–f**) with the help of H_2O_2. Absence of characteristic methine proton peak in ^{1}H NMR spectra due to the oxidation of pyrazolo[3,4-*d*] pyrimidine nucleus proved the formation of **6a–f**. Also, confirmatory singlet due to methyl sulphonyl protons in the range of 2.9–3.12 δ ppm was observed. In ^{13}C NMR spectra the characteristic peak of methine proton was also absent.

Refluxing **5a–f** with hydrazine hydrate yielded 1-(4,5-dihydro-4-(aryl)-3-methyl-1-phenyl-1*H*-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-1*H*-pyrazolo[3,4-*d*]pyrimidines **8a–f** was accomplished by reacting **4a–f** with 1-chloro-2,4-dinitrobenzene using pyridine as a solvent. Disappearance of characteristic S-methyl proton peak in ^{1}H NMR and ^{13}C NMR confirmed the formation of hydrazinyl derivatives **8a–f**. The structures of the synthesised compounds were assigned on the basis of ^{1}H NMR, ^{13}C NMR, mass spectral data and purity was proven by elemental analysis.

Antimycobacterial activity

Preliminary antimycobacterial activity screening results show that compounds **5a**, **5b**, **6c**, **7a**, **7b**, **8d**, **8e** and **8f** exhibited excellent anti-tubercular activity with MIC of <6.25 μg/mL and IC_{90} of 7.12, 3.57, 3.1, 3.11, 2.98, 3.75, 1.53 and 5.99 μg/mL respectively. The results are depicted in Table 1.

Structure–activity relationship highlighted that altering the sixth position of core pyrazolo[3,4-*d*]pyrimidine nucleus (**4a–f**) alters the antimycobacterial activity considerably. Pyrazolo[3,4-*d*]pyrimidines carrying various substituents on the C-4 aryl ring with different electronic properties (Cl, NO_2, OH and OMe) exhibit high inhibitory activity against MTB, indicating that the electronic properties of the substituents have only minor influence on the antimycobacterial activity.

With respect to the structure-MTB activity relationship, at C-6 position we have studied various substitutions namely –SCH_3 (**5a–f**), –SO_2CH_3 (**6a–f**), -$NHNH_2$ (**7a–f**), -$S(C_6H_3)(NO_2)_2$ (**8a–f**). A comparison of the substitution pattern at C-6 demonstrated that the order of activity with respect to the substituents was -$S(C_6H_3)(NO_2)2$ > -$NHNH_2$ > –SO_2CH3 > –SCH_3. Compound 6-(2,4-dinitrophenylthio)-4-(3-nitrophenyl)-3-methyl 1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine (**8d**) was found to be the most active compound *in vitro* with MIC of < 6.25 μg/mL and IC_{90} of 1.53 μg/mL against MTB possibly due to the presence of bulky substituents at the sixth position. In future extensive structure–activity relations could be derived with various other modifications.

Table 1. *In vitro* antitubercular screening data for **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	3.1	2.32
6d	3-Nitrophenyl	<6.25	92	24.8	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	1.4
8e	2-Nitrophenyl	>6.25	54	–	–
8f	2-Hydroxyphenyl	<6.25	99	5.99	5.39

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 classes of antimycobacterial drugs or prodrugs with potential use in tuberculosis treatment. Further studies in this area are in progress in our laboratory.

Acknowledgements

The authors thank the Professor and Head of the Department of Chemistry, Saurashtra University, Rajkot for providing research facilities. The authors thank the University Grant Commission, New Delhi for providing Major Research Project (Sanction No. 32-254/2006).

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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