Med Chem Res (2007) 15:463–470
DOI 10.1007/s00044-007-9000-4

MEDICINAL CHEMISTRY RESEARCH

Synthesis and antimycobacterial activity of novel 4-[5-(substituted phenyl)-1-phenyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methylphenol derivatives

Mohamed Ashraf Ali · Mohammad Shahar Yar

Received: 10 October 2006 / Accepted: 9 January 2007 / Published online: 30 June 2007

Abstract In the present investigation, 4-hydroxy-3-methylacetophenone, on condensation with appropriate aldehydes in methanolic potassium hydroxide solution, yielded the corresponding chalcones ($C_{I\text{-}XI}$). These corresponding chalcones were reacted with phenyl hydrazide in glacial acetic acid, which led to the formation of novel 4-[5-(substituted phenyl)-1-phenyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methylphenol derivatives. All newly synthesized compounds were evaluated for their antimycobacterial activities against isoniazid-resistant *Mycobacterium tuberculosis* using agar dilution. 4-[5-(4-Fluoro phenyl)-1-phenyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methylphenol showed good antimycobacterial activity, with a minimum inhibitory concentration of 0.62 μg/ml.

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 more than 2 million casualties annually worldwide. Mycobacteria are ubiquitous organisms that are becoming increasingly important intracellular pathogens that establish an infection in oxygen-rich macrophages of the lung (O'Brien and Nunn, 2001). The emergence of acquired immunodeficiency syndrome (AIDS), decline of socioeconomic standards, and a reduced emphasis on tuberculosis control programs contribute to the resurgence of the disease in industrialized countries (Barnes *et al*., 1991). Resistance of *Mycobacterium tuberculosis* strains to antimycobacterial

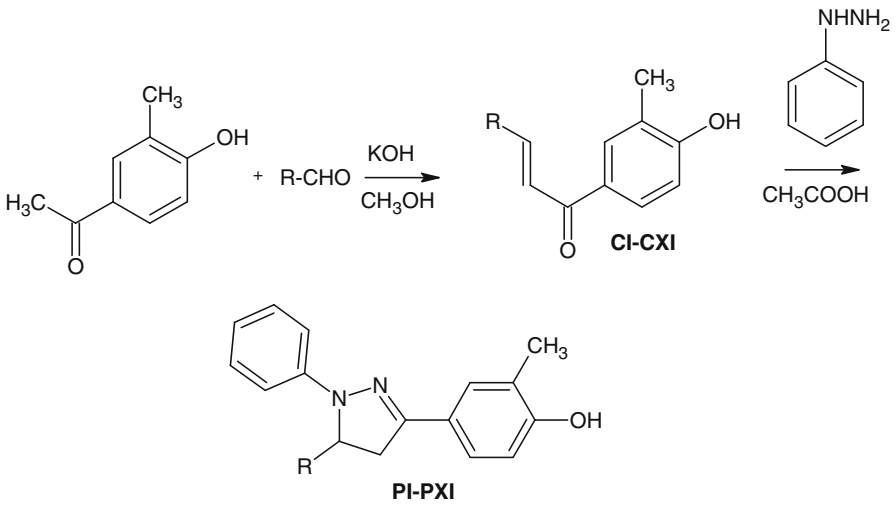
M. A. Ali · M. S. Yar (✉)
Faculty of Pharmacy, Jamia Hamdard University, Department of Pharmaceutical Chemistry, Hamdard Nagar, New Delhi 110062, India
e-mail: yarmsy@rediffmail.com

agents is an increasing problem worldwide (Schaberg *et al*., 1996; Fujiwara *et al*., 1997; Sbarbaro, 1997). However, powerful new anti-TB drugs with new mechanisms of action have not been developed in the last 40 years. In spite of severe toxicity on repeated dosing, isoniazid (INH) is still considered a first-line drug for chemotherapy of tuberculosis (Blair *et al*., 1985). A literature survey revealed pyrazoline derivatives are active against many mycobacteria (Nauduri and Reddy, 1998; Kucukguzel *et al*., 2000; Shenoy *et al*., 2001; Kucukguzel and Rolla, 2002). The current work describes the synthesis of novel pyrazoline moiety with encouraging antimycobacterial activity against INH-resistant *M. tuberculosis.*

Results and Discussion

Chemistry

The synthesis of chalcone and new 4-[5-(substituted phenyl)-1-phenyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methylphenol derivatives was carried via the steps shown in Scheme 1. In the initial step, chalcones were synthesized by condensing 4-hydroxy-3-methylacetophenone with appropriate aromatic aldehydes in dilute methanolic potassium hydroxide solution at room temperature. The 4-[5-(substituted phenyl)-1-phenyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methylphenol($P_{I\text{-}XI}$) compounds were synthesized via cyclocondensation with phenyl hydrazine in glacial acetic acid. The purity of the compounds was checked via thin-layer chromatography (TLC) using various mobile bases and elemental analyses. Both analytical and spectral data (^{1}H nuclear magnetic resonance [NMR], infrared [IR] spectroscopy) of all the synthesized compounds were in full agreement with the proposed structures.



Scheme 1 Protocol for the synthesis novel 4-[2-(substituted phenyl)-3-phenyl-2,3-dihydro-1*H*-5-pyrazolyl]-2-methylphenol derivatives

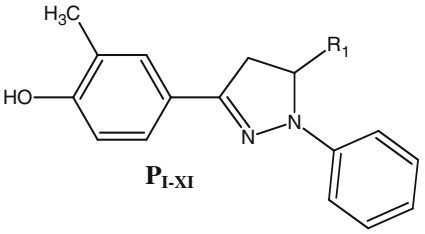
Antimycobacterial activity

The synthesized compounds ($P_{I\text{-}XI}$) were tested for their antimycobacterial activity *in vitro* against INH-resistant *Mycobacterium tuberculosis* (INHR-MTB) using the agar dilution method for the determination of minimum inhibitory concentration (MIC). The MIC was defined as the minimum concentration of compound required to inhibit 90% of bacterial growth, and MICs of the compounds are reported in Table 1 with the standard drug INH for reference.

Among the 11 newly synthesized compounds, 4 compounds were found to be most active, having an MIC of less than 1 μg/ml. Compound P_{VII}, bearing a 4-fluorophenyl radical at p-5 of the pyrazoline nucleus, was found to be the most active one against INHR-MTB at a concentration of 0.62 μg/ml. P_{XI}, P_{III}, and P_{IX} showed moderate inhibitory activity, with 0.81 μg/ml, 0.84 μg/ml, 0.92 μg/ml, respectively. The 4-flurophenyl group substitution (P_{VII}) derivatives displayed relatively higher inhibitory activity in general. However, electron-rich groups such as 4-cholrophenyl, 2-chlorophenyl, and 3-nitrophenyl substituted analog compounds produced moderate in inhibitory activity against INH-resistant *Mycobacterium*

Table 1 Physical constants and antimycobacterial activity of the synthesized compounds

$P_{I\text{-}XI}$



$P_{I\text{-}XI}$

Compound	R_1	Yield (%)	m.p. (°C)	Mol. formula	Mol. Wt.	(MIC) μg/ml
P_I	4-Methoxy-phenyl-	85	104	$C_{22}H_{19}ON_2$	358.43	1.45
P_{II}	4-Chloro-phenyl-	82	125	$C_{22}H_{19}ON_2Cl$	362.85	1.24
P_{III}	4-Dimethyl-amino phenyl-	76	102	$C_{24}H_{25}ON_3$	371.48	0.84
P_{IV}	Phenyl-	65	130	$C_{22}H_{20}ON_2$	328.41	1.60
P_V	3,4-Dimethoxy-phenyl-	75	111	$C_{24}H_{24}O_3N_2$	388.46	1.16
P_{VI}	3,4,5-Trimethoxy-phenyl-	65	118	$C_{25}H_{27}O_4N_2$	418.49	1.86
P_{VII}	4-Fluoro-phenyl-	80	151	$C_{22}H_{19}ON_2$ F	318.37	0.62
P_{VIII}	2-Chloro-phenyl-	86	166	$C_{22}H_{19}ON_2Cl$	362.85	1.00
P_{IX}	2,6-Dichloro-phenyl-	78	174	$C_{22}H_{18}ON_2Cl_2$	397.30	0.92
P_X	3-Nitro-phenyl-	72	173	$C_{22}H_{19}O_2N_3$	373.41	1.96
P_{XI}	2-Furfuryl-	80	136	$C_{20}H_{18}O_2N_2$	346.40	0.81
INH	–	–	–	–	–	0.63

[a] Recrystallization from ethanol

[b] INH-resistant Mycobacterium tuberculosis

tuberculosis. On the other hand, the analogue (OCH_3) group substituted 4-methoxy phenyl (P_I), 3,4-dimethoxy phenyl (P_V), and 3′,4,5-trimethoxy phenyl (P_{VI}) showed relatively moderate to low antitubercular activity.

All compounds were tested for cytotoxicity (IC_{50}) in VERO cells at concentrations of 62.5 μg/ml, or 10-fold. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT (3-(4,5-dimethylthiozole-2yl)-2,5-diphenyl tetrazolium bromide) assay into a formazan product using the Promega Cell Titer 96 nonradioactive cell proliferation method. Most of the active compounds were found to be nontoxic until 62.5 μg/ml.

Conclusion

Among the new synthesized 4-[5-(substituted phenyl)-1-phenyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methylphenol derivatives, 4-[5-(4-flurophenyl)-1-phenyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methylphenol (P_{VII}) has the highest antimycobacterial activity *in vitro.* It is conceivable that these derivatives showing antimycobacterial activity can be further modified to exhibit better potency than the standard drugs. Further studies to acquire more information about structure–activity relationships are in progress in our laboratory.

Experimental

All chemicals were supplied by E. Merck (Germany) and S.D. Fine Chemicals (India). Melting points were determined via the open tube capillary method and are uncorrected. Purity of the compounds was checked on TLC plates (silica gel G) in the solvent system toluene–ethyl formate–formic acid (5:4:1) and benzene–methanol (8:2). The spots were located under iodine vapors or UV light. IR spectrums were obtained on a Perkin Elmer 1720 FT-IR spectrometer (KBr Pellets). ^{1}H NMR spectra were recorded or a Bruker AC 300 MHz spectrometer using trimethyl silane (TMS) as internal standard in dimethyl sulfoxide (DMSO)–$CDCl_3$, Mass spectra were recorded on a Bruker Esquire LCMS using ESI, and elemental analyses were performed on a Carlo Erba 1106 elemental analyzer.

General procedure

Synthesis of 1-(4-hydroxy-3-methylphenyl)-3-(substituted phenyl)-2-propen-1-one ($C_{I\text{-}XI}$). 4-Hydroxy-3-methylacetophenone (0.01 mol) was dissolved in ethanol. Next, a solution of potassium hydroxide (30%, 5 ml) and suitable substituted aldehydes (0.01 mol) in 10 ml of pet. ether was added to the resulting solution with continuous stirring. The resulting solution was allowed to stand overnight. After 4 h of stirring, the solution was poured into ice-cold water and neutralized with hydrochloric acid. The solid product was filtered, dried, and purified from ethanol.

1-(4-Hydroxy-3-methyl-phenyl-3-(4-methoxyphenyl)-2-propen-1-one.

IR: (KBr) cm^{-1}: 3210(OH), 1682 (C=O), 3030 (CH); ^{1}H NMR (DMSO-d6) ppm: 2.2 (3H, s, CH_3), 3.9 (3H, s, OCH_3), 6.8–7.5 (1Hx2, dd J = 7.5 Hz, 8.5 Hz CH=CH), 7.7–8.2 (7H, s, aromatic), 9.2 (1H, s, OH).

1-(4-Hydroxy-3-methylphenyl-3-(4-cholorophenyl)-2-propen-1-one.

IR: (KBr) cm^{-1}: 3210 (OH), 782 (C-Cl), 1680 (C=O), 3042 (CH); ^1H NMR (DMSO-d6) ppm: 2.2 (3H, s, CH_3), 6.7–7.2 (1Hx2, dd J = 8.34 Hz, 6.79 Hz CH=CH), 7.7–8.0 (7H, m, aromatic), 9.2 (1H, s, OH).

1-(4-Hydroxy-3-methylphenyl-3-(4-dimethylaminophenyl)-2-propen-1-one. IR: (KBr) cm^{-1}: 3200 (OH), 1684 (C=O), 3040 (CH); ^1H NMR (DMSO-d6) ppm: 2.2 (3H, s, CH3), 3.9 (6H, s, N (CH_3x2), 6.8–7.5 (1Hx2, dd J = 7.61 Hz, 7.63 Hz CH=CH), 7.6–8.1 (7H, m, aromatic), 9.2 (1H, s, OH).

1-(4-Hydroxy-3-methylphenyl-3-phenyl-2-propen-1-one. IR: (KBr) cm^{-1}: 3210(OH), 1670 (C=O), 3040 (CH); ^1H NMR (DMSO-d6) ppm: 2.2 (3H, s, CH_3), 6.8–7.4 (1Hx2, dd *J* = 8.28 Hz, 6.70 Hz CH=CH), 7.7–8.2 (8H, m, aromatic), 9.2 (1H, s, OH).

1-(4-Hydroxy-3-methylphenyl-3-(3,4-dimethoxyphenyl)-2-propen-1-one. IR:(KBr) cm^{-1}: 3210 (OH), 1686 (C=O), 3030 (CH); ^1H NMR (DMSO-d6) ppm: 2.2 (3H, s, CH_3), 3.9 (6H, s, OCH_3 x2), 6.9–7.3 (1Hx2, dd *J* = 7.45 Hz, 7.29 Hz CH=CH), 7.6–8.2 (6H, m, aromatic), 9.2 (1H, s, OH).

1-(4-Hydroxy-3-methylphenyl-3-(3, 4, 5 trimethoxyphenyl)-2-propen-1-one. IR: (KBr) cm^{-1}: 3200 (OH), 1680 (C=O), 3040 (CH); ^1H NMR (DMSO-d_6 ppm): 9.2 (1H, s, OH), 2.2(3H, s, CH_3), 7.7–8.2 (5H, m, aromatic), 3.9 (9H, s, OCH_3x3), 6.9–7.5(1Hx2, dd J = 7.55 Hz, 7.27 Hz CH=CH).

1-(4-Hydroxy-3-methylphenyl-3-(4-fluorophenyl)-2-propen-1-one.

IR: (KBr) cm^{-1}: 3200 (OH), 1680 (C=O), 3040 (CH), 670(C-F); ^1H NMR (DMSO-d_6 ppm): 9.2 (1H, s, OH), 2.2 (3H, s, CH_3), 7.7–8.2 (7H, m, aromatic), 6.9–7.5 (1Hx2, dd J = 7.24 Hz, 7.29 Hz -CH=CH).

1-(4-Hydroxy-3-methylphenyl-3-(2-chlorophenyl)-2-propen-1-one.

IR: (KBr) cm^{-1}: 3200 (OH), 1680 (C=O), 3040 (CH), 770(C-Cl); ^1H NMR (DMSO-d_6 ppm): 9.2 (1H, s, OH), 2.2 (3H, s, CH_3), 7.6–8.0 (7H, m, aromatic), 6.9–7.5 (1Hx2, dd *J* = 8.35 Hz, 3.63 Hz -CH=CH).

1-(4-Hydroxy-3-methylphenyl-3-(2, 6 dichlorophenyl)-2-propen-1-one. IR: (KBr) cm^{-1}: 3200 (OH), 1680 (C=O), 3040 (CH), 770 (C-Cl); ^1H NMR (DMSO-d_6 ppm): 9.2 (1H, s, OH), 2.2 (3H, s, CH_3), 7.7–8.2 (6H, m, aromatic), 6.9–7.5 (1Hx2, dd *J* = 5.41 Hz, 15.68 Hz CH=CH).

1-(4-Hydroxy-3-methyl-phenyl-3-(3-nitrophenyl)-2-propen-1-one.

IR: (KBr) cm^{-1}: 3200 (OH), 1680 (C=O), 3040 (CH); ^1H NMR (DMSO-d_6 ppm): 9.2 (1H, s, OH), 2.2 (3H, s, CH_3), 7.7–8.2 (7H, m, aromatic), 6.9–7.5 1Hx2, dd *J* = 5.46 Hz, 16.3 Hz CH=CH).

1-(4-Hydroxy-3-methyl-phenyl-3-furfuryl-2-propen-1-one.

IR:(KBr) cm^{-1}: 3200 (OH), 1680 (C=O), 3040 (CH); ^1H NMR (DMSO-d_6 ppm): 9.2 (1H, s, OH), 2.2 (3H, s, CH_3), 7.7–8.2 (6H, m, aromatic), 7.43–7.48 (3H, m, furan), 6.9–7.5 (1Hx2, dd *J* = 3.0 Hz, 8.36 Hz, CH=CH).

General method

*Synthesis of novel 4-[5-(substituted phenyl)-1-phenyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methylphenol ($P_{I\text{-}XI}$).* To the solution of (0.002) mol of the appropriate ($P_{I\text{-}XI}$) derivatives in 15 ml of glacial acetic acid, 0.002 mol phenyl hydrazine was added

and the reaction mixture was refluxed for 12 h and cooled. Excess solvent was removed under reduced pressure, and the reaction mixture was poured on crushed ice (20 g) to cool. The product obtained was filtered, washed with water, and recrystallized from methanol.

*4-[5-(4-Methoxyphenyl)-1-phenyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methylphenol (P$_I$).* IR: (KBr) cm^{-1}: 3307 (OH), 1590 (C=N), 1320 (C-N). ^{1}H NMR(DMSO-d$_6$) ppm: 1.8 (3H, s, CH_3), 2.5(2H,s,CH_2), 3.8 (3H,s,OCH_3), 5.2(1H,t,CH), 6.9–7.9(12H, s, aromatic), 9.6 (1H, s, OH); EI-MS: *m/z*: 359 (M^+); calcd./analyz.: C (77.07) 77.15, H (6.19) 6.10, N (7.82) 7.80.

4-[5-(4-Chlorophenyl)-1-phenyl-4,5-dihydro-1H-3-pyrazolyl]-2-methylphenol (P$_{II}$). IR: (KBr) cm^{-1}: 3307 (OH), 1590 (C=N), 1320 (C-N); ^{1}H NMR (DMSO-d$_6$) ppm 1.8 (3H, s, CH_3), 2.5(2H, s, CH_2), 5.2 (1H, t, CH), 6.9–7.9 (12H, s, aromatic), 9.6 (1H, s, OH); EI-MS: *m/z*: 362 (M^+); calcd./analyz. C (72.82) 72.40, H (5.28) 5.42, N (7.72) 7.74.

*4-[5-(4-Dimethylaminophenyl)-1-phenyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methylphenol (P$_{III}$).* IR: (KBr) cm^{-1}: 3307 (OH), 1590 (C=N), 1320 (C-N); ^{1}H NMR (DMSO-d$_6$) ppm: 1.8 (3H, s, CH_3), 2.3 (6H, s, N-$(CH_3)_2$), 2.5 (2H, s, CH_2), 5.2 (1H, t, CH), 6.9–7.9 (12H, s, aromatic), 9.6 (1H, s, OH); EI-MS: *m/z*: 372 (M^{+1}); calcd./analyz.: C (77.60) 77.64, H (6.78) 6.76, N (11.31) 11.34.

4-(1,5-Diphenyl-4,5-dihydro-1H-3-pyrazolyl)-2-methylphenol (P_{IV}).

IR: (KBr) cm^{-1}: 3307 (OH), 1590 (C=N), 1320 (C-N). ^{1}H NMR (DMSO-d$_6$) ppm: 1.8 (3H, s, CH_3), 2.5 (2H, s, CH_2), 5.2 (1H, t, CH), 6.9–7.9 (13H, s, aromatic), 9.6 (1H, s, OH); EI-MS: *m/z*: 328 (M^+); calcd./analyz. C (80.46) 80.35, H (6.14) 6.12, N (8.53) 8.70.

*4-[5-(3,4-Dimethoxyphenyl)-1-phenyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methylphenol (P$_V$).* IR: (KBr) cm^{-1}: 3307(OH), 1590 (C=N), 1320 (C-N). ^{1}H NMR (DMSO-d$_6$) ppm: 2.1 (3H, s, CH_3), 2.5 (2H, s, CH_2), 3.8 (3H, s, OCH_3x2), 5.2 (1H, t, CH), 6.9–7.9 (11H, s, aromatic), 10.3 (1H, s, OH); EI-MS: *m/z*: 389(M^{+1}); calcd./analyz.: C (74.21) 74.18, H (6.23) 6.18, N (7.21) 7.28.

*4-[5-(3,4,5-Trimethoxyphenyl)-1-phenyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methylphenol(P$_{VI}$).* IR: (KBr) cm^{-1}: 3307 (OH), 1590 (C=N), 1320 (C-N). ^{1}H NMR (DMSO-d6) ppm: 2.1 (3H, s, CH_3), 2.5 (2H, s, CH_2), 3.8 (3H, s, OCH_3x3), 5.2 (1H, t, CH), 6.9–7.9 (10H, s, aromatic), 10.1 (1H, s, OH); EI-MS: *m/z*: 417 (M^{-1}); calcd./analyz.: C (71.75) 71.97, H (6.26) 6.19, N (6.69) 6.62.

*4-[5-(4-Fluorophenyl)-1-phenyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methylphenol (P$_{VII}$).* IR: (KBr) cm^{-1}: 3307 (OH), 1590 (C=N), 1320 (C-N); ^{1}H NMR (DMSO-d6) ppm: 1.8 (3H, s, CH_3), 2.5 (2H, s, CH_2), 5.2 (1H,t,CH), 6.6–7.6 (12H, s, aromatic), 9.6 (1H, s, OH); EI-MS: *m/z*: 319 (M^{+1}); calcd./analyz.: C (76.28) 76.24,H (5.53) 5.53, N (8.09) 8.03.

*4-[5-(2-Chlorophenyl)-1-phenyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methylphenol (P$_{VIII}$).* IR:(KBr) cm^{-1}: 3307 (OH), 1590 (C=N), 1320 (C-N); ^{1}H NMR (DMSO-d$_6$) ppm: 2.1 (3H, s, CH_3), 2.5 (2H, s, CH_2), 5.2 (1H, t, CH), 6.8–7.5 (12H, s, aromatic),10.3 (1H, s, OH); EI-MS: *m/z*: 361 (M^{-1}); calcd./analyz.: C (72.82) 72.60, H (5.28) 5.22, N (7.72) 7.12.

*4-[5-(2,6-Dichlorophenyl)-1-phenyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methylphenol (P$_{IX}$).* IR: (KBr) cm^{-1}: 3307 (OH), 1590 (C=N), 1320 (C-N); ^{1}H NMR (DMSO-

d6) ppm: 1.8 (3H, s, CH_3), 2.5 (2H, s, CH_2), 5.2 (1H, t, CH), 6.6–7.6 (11H, s, aromatic),10.3 (1H, s, OH); EI-MS: *m/z*: 397 (M^{+1}); calcd./analyz.: C (66.51) 66.50, H (4.57) 4.52, N (7.05) 7.0.

*4-[5-(3-Nitro phenyl)-1-phenyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methylphenol (*P_X*).* IR: (KBr) cm^{-1}: 3307 (OH), 1590 (C=N), 1320 (C-N); ^{1}H NMR (DMSO-d6) ppm: 1.8 (3H, s, CH_3), 2.5 (2H, s, CH_2), 5.2 (1H, t, CH), 6.6–8.4 (12H, s, aromatic), 9.6 (1H, s, OH); EI-MS: *m/z*: 372 (M^{-1}); calcd./analyz. C (70.76) 70.70, H (5.13) 5.10, N (11.25) 11.20.

*4-[5-(2-Furyl)-1-phenyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methylphenol (*P_{XI}*).* IR: (KBr) cm^{-1}: 3307 (OH), 1590 (C=N), 1320 (C-N); ^{1}H NMR (DMSO-d6) ppm: 1.8 (3H, s, CH_3), 2.5 (2H, s, CH_2), 5.2 (1H, t, CH), 6.6–7.9 (11H, s, aromatic),10.4 (1H, s, OH); EI-MS*: m/z*: 317 (M^{-1}); calcd./analyz.: C (75.45) 75.35, H (5.70) 5.72, N (8.80) 8.82.

Biology

The primary screening was conducted at a concentration of 6.25 μg/ml (or molar equivalent of the highest molecular weight compound in a series of congeners) against *Mycobacterium tuberculosis* H37Rv (ATCC27294) in BACTEC 12B medium using the BACTEC 460 radiometric system (Interleid, 1991; Colins and Franzblau, 1997). Compounds demonstrating at least 90% inhibition in the primary screen were reexamined at a lower concentration (MIC) in broth microdilution assay with almar 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 (IC_{50}) in VERO at concentrations equal to and greater than the MIC for *Mycobacterium tuberculosis* H37Rv after 72 h of exposure. Viability was assessed on the basis of cellular conversion of MTT in to a formazan product using the Promega cell Titer 96 nonradioactive cell proliferation assay (Heifets *et al*., 1989).

Acknowledgments M. Shahar Yar thanks the University Grant Commission-New Delhi, India for a research award. We thank the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), National Institute of Allergy and Infections Diseases Southern Research Institute/GW Long Hansen's Disease Center, Colorado State University Birmingham, AL, for *in vitro* antimycobacterial screening and Dr. Kiran Smith, National Cancer Institute, Bethesda, MD, for valuable suggestions.

References

Barnes P, Blotch AB, Davidson BT, Snyder Jr DE (1991) Tuberculosis in patients with immunodeficiency virus infection. N Engl J Med 324:1644–1650

Blair IA, Mansilla Tinoco R, Brodie MJ, Clare RA, Dollery CT, Timbrell JA, Beever IA (1985) Plasma hydrazine concentrations in man after isoniazid and hydralazine administration. Hum Toxicol 4:195

Collins L, Franzblau SG (1997) Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. Antimicrob Agents Chemother 41:1004–1009

Fujiwara PI, Cook SV, Rutherford CM, Crawford JT, Glickman SE, Kreiswirth BN, Sachdev PS, Osahan SS, Ebrahimzadeh A, Frieden TR (1997) A continuing survey of drug-resistant tuberculosis, New York City, April 1994. Arch Intern Med 157:531–536

Heifets LB, Flory MA, Lindholm-Levy PJ (1989) Does pyrazinoic acid as an active moiety of pyrazinamide have specific activity against *Mycobacterium tuberculosis*? Antimicrob Agents Chemother 33:1252–1254

Inderlied C (1991) Antimycobacterial agents: in vitro susceptibility testing, spectrums of activity, mechanisms of action and resistance, and assays for activity in biological fluids. In: Lorian V (ed.) Antibiotics in laboratory medicine, 3rd ed. The Williams & Wilkins Co., Baltimore p. 134–197

Güniz Küçükgüzel S, Rollas S (2002) Synthesis, characterization of novel coupling products and 4-arylhydrazono-2-pyrazoline-5-ones as potential antimycobacterial agents. Farmaco 57:583–587

Kucukguzel SG, Rollas S, Erdeniz H, Kiraz M, Ekinci AC, Vidin A (2000) Synthesis, characterization and pharmacological properties of some 4-arylhydrazono-2-pyrazoline-5-one derivatives obtained from heterocyclic amines. Eur J Med Chem 35:761–771

Nauduri D, Reddy GB (1998) Part-1: Antibactirials & antimycotics: Synthesis and structure activity relationship of 2-pyrazolines derivatives. Chem Pharm Bull 46(8):1254–1260

O'Brien RJ, Nunn PP (2001) The need for new drugs against tuberculosis. Am J Respir Crit Care Med 163:1055–1058

Sbarbaro JA (1997) Multidrug'-resistant tuberculosis. It is time to focus on the private sector of medicine. Chest 111:1149–1151

Schaberg T, Glocer G, Reichert B, Mauch H, Lode H (1996) Resistant lung tuberculosis in Berlin 1987–1993. Pneumologie 50:21–27

Shenoy GG, Bhat AR, Bhat GV, Kotian M (2001) Synthesis and antimicrobial activities of 1,3,5 trisubstituted 2-pyrazolines. Ind J Het Chem 10:197–200