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Synthesis and antituberculosis activity of new *N*-phenyl-*N′*-[4-(5-alkyl/arylamino-1,3,4-thiadiazole-2-yl)phenyl]thioureas

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Abstract

In this study, eight original *N*-phenyl-*N′*-[4-(5-alkyl/arylamino-1,3,4-thiadiazole-2-yl)phenyl]thiourea derivatives were synthesized and tested for antituberculosis activity. Antituberculosis activities of the synthesized compounds were screened in vitro using BACTEC 460 Radiometric System against *Mycobacterium tuberculosis* H37Rv at 6.25 μg/ml. The highest inhibition observed with the synthesized compounds is 67% for *N*-phenyl-*N′*-[4-(5-cyclohexylamino-1,3,4-thiadiazole-2-yl)phenyl]thiourea.

Keywords: 1,3,4-Thiadiazole; Thiourea; Antituberculosis activity

1. Introduction

Tuberculosis has become a particularly important social problem in the recent years. It is known that compounds that contain thiourea structure show antituberculosis activity [1–5]. In a previous work in our laboratory, *N*-allyl-*N′*-{4-[3-[(2,4-dichlorobenzyl)thio]-4-methyl-4*H*-1,2,4-triazole-5-yl]phenyl}thiourea was shown to possess a potent inhibitory activity against *Mycobacterium tuberculosis* H37Rv by a MIC value of 6.25 μg/ml [6]. Therefore, we synthesized eight compounds bearing the same thiourea pharmacophore with a different heterocyclic ring system.

2. Chemistry

The general formulae of the synthesized compounds are presented in Fig. 1. In the first part of research, compounds in the form of 2-(4-aminophenyl)-5-alkyl/arylamino-1,3,4-thiadiazoles were prepared from benzoyl chloride and ethyl 4-aminobenzoate according to the literature [7]. The product obtained was reacted with hydrazine hydrate to prepare 4-(benzoylamino)-benzoylhydrazine. 1-[4-(benzoylamino)benzoyl]-4-alkyl/arylthio-semicarbazides (IIIa–k) were then synthesized by the addition of methyl, ethyl, propyl, cyclohexyl, phenyl, benzyl, 4-fluorophenyl, 4-chlorophenyl, 2-methylphenyl, 4-methylphenyl, 4-methoxyphenyl and 4-nitrophenyl isothiocyanates to 4-(benzoylamino)-benzoylhydrazine [8]. From (IIIa–k), 2-(4-aminophenyl)-5-alkyl/arylamino-1,3,4-thiadiazoles (IVa–k) were synthesized by using a new method more economically in this study. In the second part, *N*-phenyl-*N′*-[4-(5-alkyl/arylamino-1,3,4-thiadiazole-2-yl)phenyl]-thioureas (Vc–e, Vg–i, Vj) were obtained from the addition of aromatic primary amine to phenyl isothiocyanate in dry acetone.

All the synthesized compounds were characterized by UV, IR, ^{1}H NMR (for Ve, Vf, Vg and Vj) and mass (for Vc, Ve, Vf and Vi) spectral methods besides elemental analysis. The UV spectra of Vc–e, Vg–i, Vj showed two absorption maxima as at 249.4–277.5 and 298.9–337.6 nm. The ^{1}H NMR (DMSO-d_6) spectra of Ve, Vf, Vg and Vj displayed NH resonance at 9.51–10.37 and 10.58–9.91 and 9.54 ppm, 10.00 and 10.06 ppm and 11.05 and 13.92 ppm. Mass spectra of compounds Vc, Ve and Vi gave molecular ion peaks at m/z: 370 (M.W.: 370); m/z: 403 (M.W.: 403.1); m/z: 417

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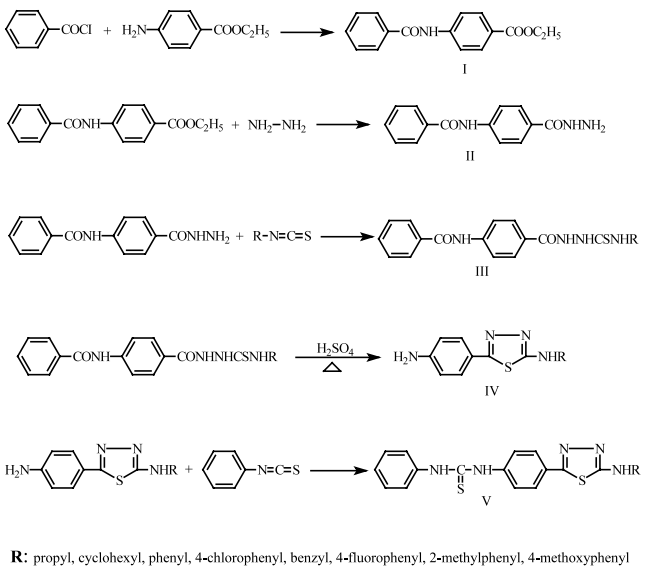


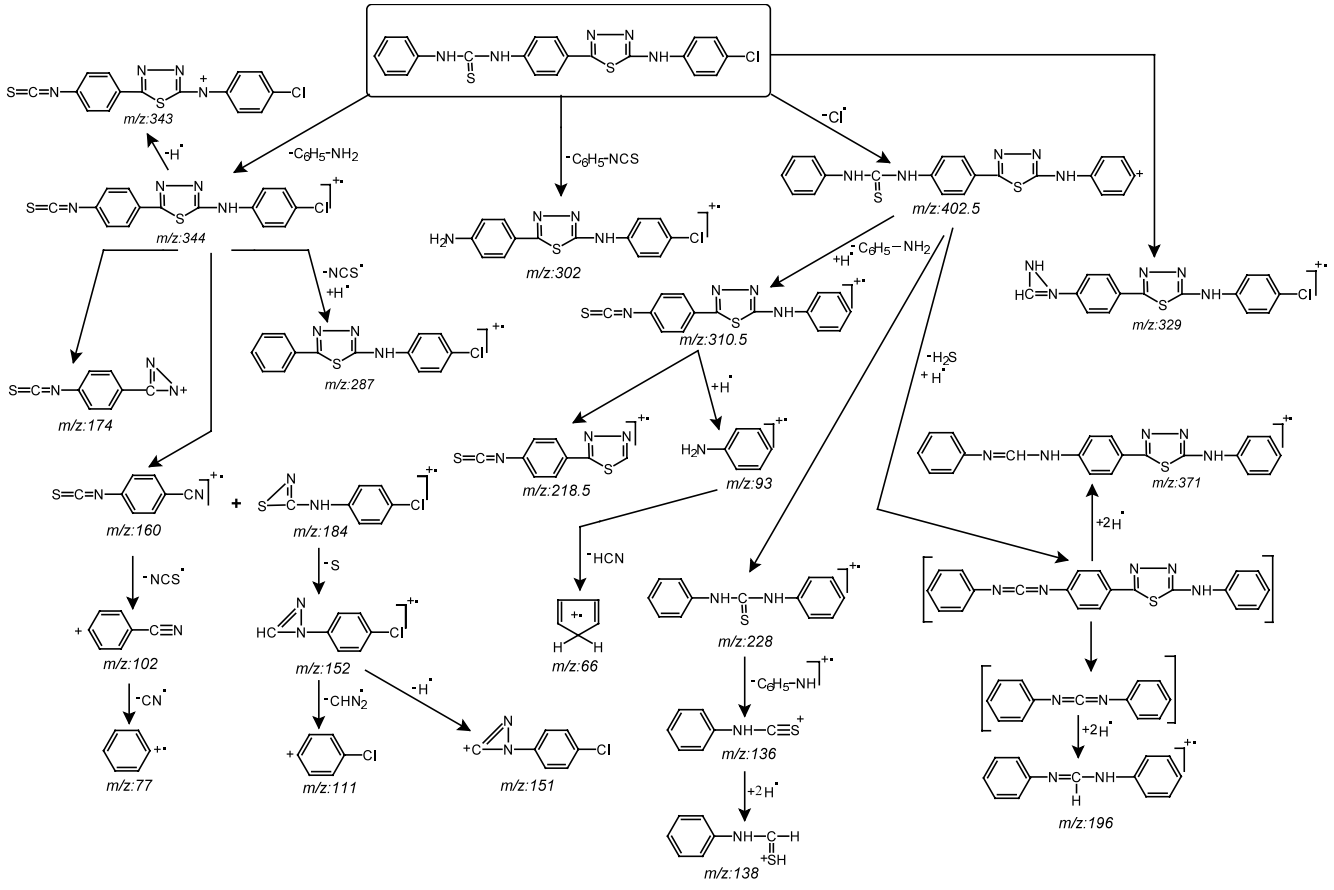
Fig. 1. The synthetic route of *N*-phenyl-*N*′-[4-(5-alkyl/arylamino-1,3,4-thiadiazole-2-yl)phenyl]thiourea derivatives.

(M.W.: 417.1). The molecular ion was not detected in the mass spectrum of compound Vf. All the synthesized compounds similar fragmentation patterns as observed previously for either thioureas such as losing the pertinent Ar–NH_2 or Ar–NCS from the molecular ion. The mass fragmentation pathway of compound Vf is shown in Scheme 1. Other characteristic data for compounds are given in Table 1.

3. Experimental

3.1. Chemistry

Acetone, benzocaine, hydrazine hydrate were purchased from Merck. All other chemicals were purchased from Fluka. Melting points were determined by using a Büchi-530 melting point apparatus (open capillaries) and uncorrected. Elemental analyses were performed on a Carlo Erba 1106. UV spectra were determined on a Shimadzu UV 2100 S spectrophotometer. IR spectra were run a Perkin–Elmer 1600 spectrophotometer as KBr pellets. ^{1}H NMR spectra were obtained on a Bruker DP X-400 spectrometer at MHz using TMS as the internal reference. Mass spectra were determined at 70 eV on a VG Zabspec Double Focussing Magnetic Sectot spectrometer.



Scheme 1.

Table 1
Physical and spectral data of Vc–e, Vg–i, Vj

Comp.	M.p. (°C)	Yields (%)	Molecular formula	Elemental analysis (Calc./Found)			UV (EtOH) λ_{max} (nm)	IR (KBr) (cm^{-1})
				C	H	N		
Vc	184	61.43	$C_{18}H_{19}N_5S_2$	58.51 57.76	5.14 4.84	18.95 18.22	330.6 250.0	3201, 1537, 1314, 1261, 991
Vd	189–190	50.94	$C_{21}H_{23}N_5S_2 \cdot \frac{1}{2}H_2O$	60.25 59.48	5.78 4.85	16.73 16.14	330.7 283.5	3201, 1543, 1320, 1267, 985
Ve	203–205	45.71	$C_{21}H_{17}N_5S_2 \cdot 1/2H_2O$	61.14 61.13	4.39 4.08	16.97 16.17	277.5	3272, 3201, 1543, 1314, 1267, 985
Vf	203–205	52.20	$C_{21}H_{16}ClN_5S_2$	57.59 57.86	3.68 3.80	15.99 15.44	298.9	3213, 1531, 1314, 1090, 973
Vg	175–177	53.89	$C_{22}H_{19}N_5S_2$	63.28 63.16	4.59 4.22	16.77 16.48	331.3 249.4	3319, 3213, 1531, 1308
Vh	217–218	48.86	$C_{21}H_{16}FN_5S_2 \cdot 3/2H_2O$	56.23 56.89	4.27 3.52	15.61 14.48	334.4 260.8	3213, 1537, 1318, 1290, 1186, 973
Vi	209–210	46.73	$C_{22}H_{19}N_5S_2 \cdot 2H_2O$	58.26 58.03	5.11 4.96	15.44 14.83	337.6 260.4	3619, 3285, 1587, 1319, 979
Vj	224–226	54.70	$C_{22}H_{19}N_5OS_2 \cdot 3H_2O$	54.19 53.84	5.17 5.04	14.36 13.70	308.3 225.5	3225, 1537, 1331, 1255, 973

3.1.1. General procedure for the preparation of N-phenyl-N′-[4-(5-alkyl/arylamno-1,3,4-thiadiazole-2-yl)-phenyl]thioureas [Vc–e, Vg–i, Vj]

A solution of the 0.001 mol phenyl isothiocyanate in 1.2 ml anhydrous C_3H_6O was added dropwise to the solution of 0.001 mol amine in 1.2 ml anhydrous C_3H_6O [9]. The solution was refluxed for 4–5 h. The mixture was evaporated and the precipitated product was purified by washing with petroleum ether and boiled EtOH.

3.2. Biological activity

Antituberculosis activities of the synthesized compounds were screened in vitro using BACTEC 460 Radiometric System against *M. tuberculosis* H37Rv at 6.25 μg/ml. Primary antituberculosis activity screening results of these compounds are shown in Table 2.

3.2.1. BACTEC radiometric method of susceptibility testing

Inocula for susceptibility testing were either from a positive BACTEC isolation vial with a growth index (GI) of 500 or more, or a suspension of organisms isolated earlier on a conventional medium. The culture was well mixed with a syringe and 0.1 ml of a positive BACTEC culture was added to each of the vials containing the test drugs. The drug vials contained rifampicin (0.25 μg/ml). A control vial was inoculated with 1:100 dilution of the culture. A suspension equivalent to a McFarland No. 1 standard was prepared in the same manner as a BACTEC positive vial, when growth from a solid medium was used. Each vial was tested immediately on a BACTEC instrument to provide CO_2 in the headspace. The vials were incubated at 37 °C and tested daily with a BACTEC instrument. When the GI in the control reads at least 30, the increase in GI (ΔGI) from the previous day in the control was compared with that in the drug vial [10,11]. The following formula was used to interpret results:

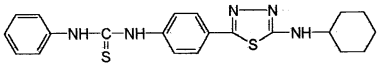
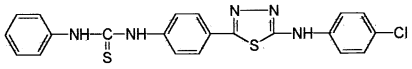
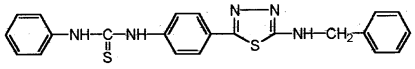
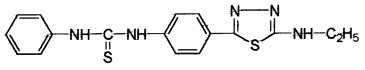
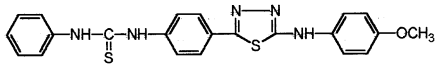
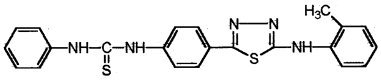
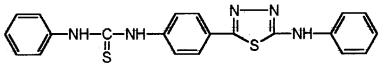
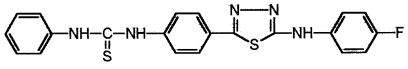
$$\Delta GI \text{ control} > \Delta GI \text{ drug} = \text{susceptible}$$

$$\Delta GI \text{ control} < \Delta GI \text{ drug} = \text{resistant}$$

4. Results and discussion

Antituberculosis activities of the synthesized compounds were screened in vitro using BACTEC 460 Radiometric System against *M. tuberculosis* H37Rv at 6.25 μg/ml. Rifampicin was used as the standard in these tests. The compound Vd has cyclohexyl group showed the highest inhibition with 67%. While the compound Vf has chlorine on the fourth position of aromatic ring showed 32% inhibition against *M. tuberculosis* H37Rv, the compound Vg has fluorine on the fourth position of aromatic ring did not exhibited inhibition. Other compounds showed varying degrees of inhibition in the primary screen. We concluded from our investigation that Vd may be considered promising for the development of new antituberculosis agents.

Table 2
Antimycobacterial activity of Vc–e, Vg–i and Vj

Compound	Chemical formula	MIC (μg/ml)	% Inhibition
Vd		> 6.25	67
Vf		> 6.25	32
Vh		> 6.25	16
Vb		> 6.25	6
Vj		> 6.25	5
V1		> 6.25	3
Ve		> 6.25	1
Vg		> 6.25	0
Rifampicin		0.25	98

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