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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antimycobacterial activities of some new *N*-acylhydrazone and thiosemicarbazide derivatives of 6-methyl-4,5-dihydropyridazin-3(2*H*)-one

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Abstract In this study, twenty-five new 6-methyl-4,5-dihydropyridazin-3(2*H*)-one derivatives having *N*-acylhydrazone and thiosemicarbazide moieties were synthesized. The target compounds were tested for their antimycobacterial activity in vitro against *Mycobacterium tuberculosis* $H_{37}R_v$ using the agar dilution method. Among the synthesized compounds, *N*′-(2,4-dichlorobenzylidene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4*H*)-yl)acetohydrazide **4g** was found to be the most active compound with minimum inhibitory concentration of 0.78 μM and was more potent than ethambutol and ciprofloxacin.

Keywords Antimycobacterial activity · 6-Methyl-4,5-dihydropyridazin-3(2H)-one · *N*-Acylhydrazone · Thiosemicarbazide

Introduction

Tuberculosis is an infection caused by *Mycobacterium tuberculosis* and is the leading cause of infectious disease mortality in the world (Dye, 2006). No new drugs have been developed specifically against mycobacteria since the 1960s (Janin, 2007). The increase in *M. tuberculosis* strains resistant to first-line antimycobacterial drugs (such as rifampin, isoniazid (INH), and ethambutol) and toxicities of these compounds have further complicated the problem, which clearly indicates the need for more effective, faster acting chemotherapeutics with the lower toxicity for the efficient management of tuberculosis.

Within the scope of antitubercular drug design, a number of *N*-acylhydrazones (NAH) having different heterocyclic systems, such as thiadiazole, triazole, quinoline, pyrazine, and imidazo[4,5-d]pyridine have been reported as active compounds (Abdel-Aziz and Abdel-Rahman, 2010; Bukowski and Janowiec, 1996; Mamolo, *et al.*, 2001; Nayyar *et al.*, 2007; Ulusoy *et al.*, 2001). In the light of these studies, it can be assumed that NAH moiety is a suitable pharmacophore group for the treatment of tuberculosis. Thiosemicarbazides are less studied compared with NAHs, however, there is an interesting report which claims that some thiosemicarbazide derivatives of isoniazid show higher activity than isoniazid (Cardia *et al.*, 2006). On the other hand, some compounds bearing pyridazine ring have been reported to possess antimycobacterial activity (Islam *et al.*, 2008; Mantu *et al.* 2010).

On the basis of these findings, this study aimed to investigate the synthesis and antimycobacterial activities of some new 6-methyl-4,5-dihydropyridazin-3(2*H*)-one derivatives having NAH and thiosemicarbazide moieties at position 2 of the ring.

Materials and methods

Chemistry

Melting points were determined using a Thomas-Hoover Capillary Melting Point Apparatus (Philadelphia, PA, USA) and are uncorrected. ATR-FTIR spectra were

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obtained using the MIRacle ATR accessory (Pike technologies) in conjunction with a Spectrum BX FTIR spectrometer (Perkin Elmer) and were reported in cm^{-1}. The ^1H NMR spectra (DMSO-d_6) were measured on a Varian Mercury 400 FT NMR spectrophotometer using TMS as an internal reference. Chemical shifts are reported in δ values (ppm). The ESI-MS spectra were recorded on a micromass ZQ-4000 single quadruple mass spectrometer. Elemental analyses (C, H, N) were performed on Leco CHNS 932 analyzer. The results were within 0.4% of the theoretical values.

*6-Methyl-4,5-dihydropyridazin-3(2H)-one (**1**)*

This compound was prepared according to the method described in the literature (Reichelt and Reissig, 1984).

*Ethyl 2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetate (**2**)*

The mixture of 1(0.05 mol) and anhydrous potassium carbonate (0.075 mol) in acetone (100 ml) was refluxed for 4 h. To the mixture, a solution of 0.05 mol ethyl bromoacetate in acetone (25 ml) was added dropwise. After refluxing for 2 h, the reaction mixture was poured into ice-cold water (250 ml) and extracted with ethyl acetate. The oily residue obtained was used in the next step without further purification.

*2-(3-Methyl-6-oxo-5,6-dihydropyridazin-1-(4H)-yl)acetohydrazide (**3**)*

Compound **2** (0.01 mol) was refluxed with hydrazine (0.20 mol) in 50 ml of absolute ethanol for 1 h. The solvent was evaporated under reduced pressure and the residue was crystallized from ethanol to give **3**. mp 194°C. IR; 3306, 3210 (N–H), 1667 (C=O, ester), 1644 (C=O, pyridazinone). ^1H-NMR (DMSO-d_6, 400 MHz); 1.95 (3H; s; –CH_3), 2.35–2.40 (2H; m; –CH_2–), 2.49–2.53 (2H; m; –CH_2–), 4.15 (2H; s; –CH_2–CONH–), 4.21 (2H; b; –NHNH_2), 9.02 (1H; b; –NHNH_2). ESI-MS (*m/z*); 207 $[M+Na]^+$, 185 [M+H] (100%).

*General procedure for the preparation of N-acylhydrazones (**4a–m** and **5a–h**)*

To a stirred mixture of 0.01 mol hydrazide (**3**) in 20 ml of ethanol, 0.01 mol of appropriate aldehyde or acetophenone was added and stirring was continued for 2 h in the presence of HCl (2 drops). The mixture was poured into cold water and neutralized with 10% aqueous sodium bicarbonate solution. The obtained solid was filtered, washed with water, and crystallized from acetonitrile.

*N′-Benzylidene-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**4a**)* Yield 51%, mp 197°C. IR; 1698 (C=O, pyridazinone), 1630 (C=O, hydrazide). ^1H-NMR (DMSO-d_6, 400 MHz); δ 1.97 (3H; s; –CH_3), 2.40–2.43 (2H; m; –CH_2–), 2.49–2.51 (2H; m; –CH_2–), 4.32 and 4.71 (2H; s; –CH_2–CONH–), 7.41–7.69 (5H; m; –C_6H_5), 7.98 and 8.21 (1H; s; –N=CH–), 11.46 and 11.54 (1H; s; –CO–NH–N=). ESI-MS (*m/z*); 295 $[M+Na]^+$, 273 $[M+H]^+$ (100%).

*N ′-(4-Chlorobenzylidene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**4b**)* Yield 55%, mp 190°C. IR; 1683 (C=O, pyridazinone), 1656 (C=O, hydrazide). ^1H-NMR (DMSO-d_6, 400 MHz); δ 1.94 (3H; s; –CH_3), 2.35–2.40 (2H; m; –CH_2–), 2.46–2.50 (2H; m; –CH_2–), 4.29 and 4.68 (2H; s; –CH_2–CONH–), 7.45–7.50 (2H; m; –C_6H_4–Cl, H_3, H_5), 7.67–7.70 (2H; m; –C_6H_4–Cl, H_2, H_6), 7.94 and 8.18 (1H; s; –N=CH–), 11.51 and 11.57 (1H; s; –CO–NH–N=). ESI-MS (*m/z*); 331 $[M+Na+2]^+$, 329 $[M+Na]^+$, 307 $[M+H]^+$ (100%).

*N′-(4-Bromobenzylidene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**4c**)* Yield 80%, mp 211°C. IR; 1688 (C=O, pyridazinone), 1656 (C=O, hydrazide). ^1H-NMR (DMSO-d_6, 400 MHz); δ 1.97 (3H; s; –CH_3), 2.40–2.42 (2H; m; –CH_2–), 2.49–2.51 (2H; m; –CH_2–), 4.32 and 4.70 (2H; s; –CH_2–CONH–), 7.61–7.66 (4H; m; –C_6H_4–Br), 7.95 and 8.18 (1H; s; –N=CH–), 11.54 and 11.60 (1H; s; –CO–NH–N=). ESI–MS (*m/z*); 375 $[M+Na+2]^+$, 373 $[M+Na]^+$, 351 $[M+H]^+$ (100%).

*N′-(4-Methylbenzylidene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**4d**)* Yield 55%, mp 193°C. IR; 1666 (C=O, pyridazinone, hydrazide). ^1H-NMR (DMSO-d_6, 400 MHz); δ 1.97 (3H; s; –CH_3), 2.33 (3H; s; ar-CH_3), 2.40–2.42 (2H; m; –CH_2–), 2.48–2.51 (2H; m; –CH_2–), 4.31 and 4.69 (2H; s; –CH_2–CONH–), 7.23–7.26 (2H; m; ar, H_3, H_5), 7.56–7.59 (2H; m; ar, H_2, H_6), 7.94 and 8.16 (1H; s; –N=CH–), 11.39 and 11.46 (1H; s; –CO–NH–N=). ESI-MS (*m/z*); 309 $[M+Na]^+$, 287 $[M+H]^+$ (100%).

*N′-(4-Methoxybenzylidene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**4e**)* Yield 54%, mp 185°C. IR; 1673 (C=O, pyridazinone), 1651 (C=O, hydrazide). ^1H-NMR (DMSO-d_6, 400 MHz); δ 1.97 (3H; s; –CH_3), 2.40–2.42 (2H; m; –CH_2–), 2.48–2.51 (2H; m; –CH_2–), 3.79 (3H; s; ar-OCH_3), 4.30 and 4.68 (2H; s; –CH_2–CONH–), 6.98–7.02 (2H; m; ar, H_3, H_5), 7.61–7.64 (2H; m; ar, H_2, H_6), 7.92 and 8.15 (1H; s; –N=CH–), 11.31 and 11.39 (1H; s; –CO–NH–N=). ESI-MS (*m/z*); 325 $[M+Na]^+$, 203 $[M+H]^+$ (100%).

*N′-(4-Nitrobenzylidene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**4f**)* Yield 94%, mp 216°C. IR; 1687 (C=O, pyridazinone), 1640 (C=O, hydrazide). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.97 (3H; s; –CH_3), 2.41–2.43 (2H; m; –C$\underline{H}_2$–), 2.50–2.51 (2H; m; –C$\underline{H}_2$–), 4.36 and 4.76 (2H; s; –C$\underline{H}_2$–CONH–), 7.95–8.32 (5H; m; ar, H_2, H_3, H_5, H_6, –N=C$\underline{H}$–), 11.80 and 11.84 (1H; s; –CO–N$\underline{H}$–N=). ESI-MS (*m/z*); 340 $[M+Na]^+$, 318 $[M+H]^+$ (100%).

*N′-(2,4-Dichlorobenzylidene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**4g**)* Yield 62%, mp 203°C. IR; 1694 (C=O, pyridazinone), 1640 (C=O, hydrazide). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.97 (3H; s; –CH_3), 2.40–2.42 (2H; m; –C$\underline{H}_2$–), 2.49–2.52 (2H; m; –C$\underline{H}_2$–), 4.33 and 4.73 (2H; s; –C$\underline{H}_2$–CONH–), 7.48 (1H; d; ar, H_5, *J*: 8.8 Hz), 7.72 (1H; s; ar, H_3), 7.99 (1H; d; ar, H_6, *J*: 8.8 Hz), 8.31 and 8.56 (1H; s; –N=C$\underline{H}$–), 11.77 (1H; s; –CO–N$\underline{H}$–N=). ESI-MS (*m/z*); 365 $[M+Na+2]^+$, 363 $[M+Na]^+$, 341 $[M+H]^+$ (100%).

*N′-(4-(Trifluoromethyl)benzylidene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**4h**)* Yield 59%, mp 165°C. IR; 1688 (C=O, pyridazinone), 1639 (C=O, hydrazide). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.97 (3H; s; –CH_3), 2.38–2.42 (2H; m; –C$\underline{H}_2$–), 2.48–2.50 (2H; m; –C$\underline{H}_2$–), 4.37 and 4.74 (2H; s; –C$\underline{H}_2$–CONH–), 7.30–7.53 (2H; m; ar, H_3, H_5), 7.79–7.84 (2H; m; ar, H_2, H_6), 8.05 and 8.28 (1H; s; –N=C$\underline{H}$–), 11.73 (1H; s; –CO–N$\underline{H}$–N=). ESI-MS (*m/z*); 363 $[M+Na]^+$, 341 $[M+H]^+$ (100%).

*N′-(4-(Dimethylamino)benzylidene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**4i**)* Yield 67%, mp 200°C. IR; 1698 (C=O, pyridazinone), 1646 (C=O, hydrazide). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.94 (3H; s; –CH_3), 2.37–2.39 (2H; m; –C$\underline{H}_2$–), 2.46–2.49 (2H; m; –C$\underline{H}_2$–), 2.94 (6H; s; N(C$\underline{H}_3$)$_2$), 4.25 and 4.63 (2H; s; –C$\underline{H}_2$–CONH–), 6.69–6.73 (2H; m; ar, H_3, H_5), 7.44–7.48 (2H; m; ar, H_2, H_6), 7.81 and 8.02 (1H; s; –N=C$\underline{H}$–), 11.10 and 11.20 (1H; s; –CO–N$\underline{H}$–N=). ESI-MS (*m/z*); 338 $[M+Na]^+$, 316 $[M+H]^+$ (100%).

*N′-(4-Fluorobenzylidene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**4j**)* Yield 58%, mp 180°C. IR; 1701 (C=O, pyridazinone), 1650 (C=O, hydrazide). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.96 (3H; s; –CH_3), 2.38–2.43 (2H; m; –C$\underline{H}_2$–), 2.51–2.52 (2H; m; –C$\underline{H}_2$–), 4.35 and 4.70 (2H; s; –C$\underline{H}_2$–CONH–), 7.25–7.29 (2H; m; ar, H_3, H_5), 7.73–7.78 (2H; m; ar, H_2, H_6), 7.99 and 8.25 (1H; s; –N=C$\underline{H}$–), 11.18 and 11.57 (1H; s; –CO–N$\underline{H}$–N=). ESI–MS (*m/z*); 313 $[M+Na]^+$, 291 $[M+H]^+$ (100%).

*N′-(Furan-2-ylmethylene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**4k**)* Yield 43%, mp 199°C. IR; 1692 (C=O, pyridazinone), 1661 (C=O, hydrazide). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.97 (3H; s; –CH_3), 2.39–2.42 (2H; m; –C$\underline{H}_2$–), 2.48–2.51 (2H; m; –C$\underline{H}_2$–), 4.30 and 4.62 (2H; s; –C$\underline{H}_2$–CONH–), 6.61 (1H; d; furan, H_4), 6.89 (1H; t; furan, H_3), 7.82 (1H; d; furan, H_5), 7.86 and 8.09 (1H; s; –N=C$\underline{H}$–), 11.39 and 11.48 (1H; s; –CO–N$\underline{H}$–N=). ESI-MS (*m/z*); 285 $[M+Na]^+$, 263 $[M+H]^+$ (100%).

*N′-(Thiophen-2-ylmethylene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**4l**)* Yield 73%, mp 209°C. IR; 1694 (C=O, pyridazinone), 1626 (C=O, hydrazide). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.97 (3H; s; –CH_3), 2.39-2.42 (2H; m; –C$\underline{H}_2$–), 2.48–2.51 (2H; m; –C$\underline{H}_2$–), 4.30 and 4.61 (2H; s; –C$\underline{H}_2$–CONH–), 7.10–7.14 (1H; m; thiophene, H_4), 7.42–7.46 (1H; m; thiophene, H_3), 7.63–7.66 (1H; m; thiophene, H_5), 8.15 and 8.43 (1H; s; –N=C$\underline{H}$–), 11.41 and 11.52 (1H; s; –CO–N$\underline{H}$–N=). ESI-MS (*m/z*); 301 $[M+Na]^+$, 279 $[M+H]^+$ (100%).

*N′-(4-Ethoxybenzylidene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**4m**)* Yield 64%, mp 166°C. IR; 1679 (C=O, pyridazinone), 1643 (C=O, hydrazide). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.35 (3H; t; –OCH$_2$–C$\underline{H}_3$), 1.97 (3H; s; –CH_3), 2.39–2.41 (2H; m; –C$\underline{H}_2$–), 2.48–2.51 (2H; m; –C$\underline{H}_2$–), 4.08 (3H; q; –OC$\underline{H}_2$–CH_3), 4.30 and 4.68 (2H; s; –C$\underline{H}_2$–CONH–), 7.59–7.62 (2H; m; ar, H_3, H_5), 7.78–7.81 (2H; m; ar, H_2, H_6), 7.91 and 8.14 (1H; s; –N=C$\underline{H}$–), 11.31 and 11.39 (1H; s; –CO–N$\underline{H}$–N=). ESI-MS (*m/z*); 339 $[M+Na]^+$, 317 $[M+H]^+$ (100%).

*N′-(1-Phenylethylidene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**5a**)* Yield 50%, mp 171°C. IR; 1685 (C=O, pyridazinone), 1661 (C=O, hydrazide). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.97 (3H; s; –CH_3), 2.25 and 2.27 (3H; s; –N=C–C$\underline{H}_3$), 2.38–2.42 (2H; m; –C$\underline{H}_2$–), 2.49–2.51 (2H; m; –C$\underline{H}_2$–), 4.44 and 4.74 (2H; s; –C$\underline{H}_2$–CONH–), 7.39–7.80 (5H; m; ar), 10.48 and 10.76 (1H; s; –CO–N$\underline{H}$–N=). ESI-MS (*m/z*); 309 $[M+Na]^+$, 287 $[M+H]^+$ (100%).

*N′-(1-(4-Chlorophenyl)ethylidene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**5b**)* Yield 66%, mp 212°C. IR; 1660 (C=O, pyridazinone), 1643 (C=O, hydrazide). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.97 (3H; s; –CH_3), 2.24 and 2.26 (3H; s; –N=C–C$\underline{H}_3$), 2.38–2.42 (2H; m; –C$\underline{H}_2$–), 2.49–2.51 (2H; m; –C$\underline{H}_2$–), 4.44 and 4.74 (2H; s; –C$\underline{H}_2$–CONH–), 7.45–7.59 (2H; m; ar, H_3, H_5), 7.80–7.82 (2H; m; ar, H_2, H_6), 10.53 and 10.82 (1H; s; –

CO–NH–N=). ESI-MS (*m/z*); 345 $[M+Na+2]^+$, 343 $[M+Na]^+$, 321 $[M+H]^+$ (100%).

*N′-(1-(4-Bromophenyl)ethylidene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**5c**)* Yield 63%, mp 225°C. IR; 1660 (C=O, pyridazinone, hydrazide). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.97 (3H; s; $-CH_3$), 2.23 and 2.26 (3H; s; –N=C–CH_3), 2.38–2.42 (2H; m; $-CH_2-$), 2.49–2.51 (2H; m; $-CH_2-$), 4.44 and 4.73 (2H; s; $-CH_2-$CONH–), 7.58–7.63 (2H; m; ar, H_3, H_5), 7.73–7.75 (2H; m; ar, H_2, H_6), 10.53 and 10.83 (1H; s; –CO–NH–N=). ESI-MS (*m/z*); 389 $[M+Na+2]^+$, 387 $[M+Na]^+$, 365 $[M+H]^+$ (100%).

*N′-(1-(4-Methylphenyl)ethylidene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**5d**)* Yield 67%, mp 211°C. IR; 1683 (C=O, pyridazinone), 1658 (C=O, hydrazide). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.97 (3H; s; $-CH_3$), 2.22 and 2.24 (3H; s; –N=C–CH_3), 2.32 (3H; s; ar-CH_3), 2.38–2.42 (2H; m; $-CH_2-$), 2.49–2.51 (2H; m; $-CH_2-$), 4.42 and 4.72 (2H; s; $-CH_2-$CONH–), 7.20–7.22 (2H; d; ar, H_3, H_5, *J*:8.4 Hz), 7.67–7.69 (2H; d; ar, H_2, H_6, *J*:8.4 Hz), 10.42 and 10.70 (1H; s; –CO–NH–N=). ESI-MS (*m/z*); 323 $[M+Na]^+$, 301 $[M+H]^+$ (100%).

*N′-(1-(4-Methoxyphenyl)ethylidene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**5e**)* Yield 56%, mp 185°C. IR; 1675 (C=O, pyridazinone), 1659 (C=O, hydrazide). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.97 (3H; s; $-CH_3$), 2.21 and 2.23 (3H; s; –N=C–CH_3), 2.38–2.42 (2H; m; $-CH_2-$), 2.49–2.50 (2H; m; $-CH_2-$), 3.79 (3H; s; ar-OCH_3), 4.41 and 4.72 (2H; s; $-CH_2-$CONH–), 6.94–6.98 (2H; m; ar, H_3, H_5), 7.72–7.75 (2H; m; ar, H_2, H_6), 10.38 and 10.65 (1H; s; –CO–NH–N=). ESI-MS (*m/z*); 339 $[M+Na]^+$, 317 $[M+H]^+$ (100%).

*N′-(1-(4-Nitrophenyl)ethylidene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**5f**)* Yield 88%, mp >270°C. IR; 1672 (C=O, pyridazinone, hydrazide). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.98 (3H; s; $-CH_3$), 2.31 and 2.42 (3H; s; –N=C–CH_3), 2.40–2.44 (2H; m; $-CH_2-$), 2.50–2.52 (2H; m; $-CH_2-$), 4.47 and 4.78 (2H; s; $-CH_2-$CONH–), 8.03–8.07 (2H; m; ar, H_2, H_6,), 8.23–8.29 (2H; m; ar, H_3, H_5), 10.72 and 10.98 (1H; s; –CO–NH–N=). ESI-MS (*m/z*); 354 $[M+Na]^+$, 332 $[M+H]^+$ (100%).

*N′-(1-(2,4-Dichlorophenyl)ethylidene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**5g**)* Yield 72%, mp 192°C. IR; 1682 (C=O, pyridazinone), 1660 (C=O, hydrazide). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.98 (3H; s; $-CH_3$), 2.33 and 2.39 (3H; s; –N=C–CH_3), 2.41–2.43 (2H; m; $-CH_2-$), 2.50–2.53 (2H; m; $-CH_2-$), 4.44 and 4.78 (2H; s; $-CH_2-$CONH–), 7.40 (1H; d; ar, H_5, *J*: 8.8 Hz), 7.71 (1H; s; ar, H_3), 7.96 (1H; d; ar, H_6, *J*: 8.8 Hz), 10.73 and 10.96 (1H; s; –CO–NH–N=). ESI-MS (*m/z*); 379 $[M+Na+2]^+$, 377 $[M+Na]^+$, 355 $[M+H]^+$ (100%).

*N′-(1-(4-Phenylphenyl)ethylidene)-2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetohydrazide (**5h**)* Yield 70%, mp 221°C. IR; 1683 (C=O, pyridazinone), 1661 (C=O, hydrazide). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.98 (3H; s; $-CH_3$), 2.28 and 2.31 (3H; s; –N=C–CH_3), 2.40–2.43 (2H; m; $-CH_2-$), 2.50–2.54 (2H; m; $-CH_2-$), 4.45 and 4.77 (2H; s; $-CH_2-$CONH–), 7.37–7.89 (9H; m; ar), 10.51 and 10.81 (1H; s; –CO–NH–N=). ESI-MS (*m/z*); 385 $[M+Na]^+$, 363 $[M+H]^+$ (100%).

*General procedure for the preparation of thiosemicarbazides (**6a–c**)*

An equimolar amount of appropriate isothiocyanate was added to a solution of hydrazide **6a–c** in 50 ml of ethanol. The reaction mixture was refluxed for 4 h and cooled to room temperature. The precipitated solid was filtered, washed with water, and purified by crystallization from ethyl acetate.

*4-Methyl-1-[2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetyl]thiosemicarbazide (**6a**)* Yield 52%, mp 198°C. IR; 1724 (C=O, pyridazinone), 1634 (C=O, hydrazide), 1201 (C=S). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.96 (3H; s; $-CH_3$), 2.39–2.41 (2H; m; $-CH_2-$), 2.47–2.51 (2H; m; $-CH_2-$), 2.86 (3H; d; NH–CH_3), 4.31 (2H; s; $-CH_2-$CONH–), 7.81 (1H; br; N$H-CH_3$), 9.30 (1H; br; –NH–NH–CS), 9.86 (1H; br; –NH–NH–CS). ESI-MS (*m/z*); 280 $[M+Na]^+$, 258 $[M+H]^+$ (100%).

*4-Ethyl-1-[2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetyl]thiosemicarbazide (**6b**)* Yield 65%, mp 190°C. IR; 1727 (C=O, pyridazinone), 1634 (C=O, hydrazide), 1192 (C=S). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.06 (3H; t; –NH–CH_2–CH_3), 1.96 (3H; s; $-CH_3$), 2.37–2.41 (2H; m; $-CH_2-$), 2.48–2.51 (2H; m; $-CH_2-$), 3.43–3.46 (2H; m; –NH–CH_2–CH_3), 4.29 (2H; s; $-CH_2-$CONH–), 7.73 (1H; br; N$H-CH_2-CH_3$), 9.26 (1H; br; –NH–NH–CS), 9.87 (1H; br; –NH–NH–CS). ESI-MS (*m/z*); 294 $[M+Na]^+$, 272 $[M+H]^+$ (100%).

*4-Phenyl-1-[2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4H)-yl)acetyl]thiosemicarbazide (**6c**)* Yield 74%, mp 158°C. IR; 1703 (C=O, pyridazinone), 1645 (C=O, hydrazide), 1200 (C=S). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.96 (3H; s; $-CH_3$), 2.41–2.42 (2H; m; $-CH_2-$), 2.49–2.50 (2H; m;

–CH$_2$–), 4.36 (2H; s; –CH$_2$–CONH–), 7.15–7.47 (5H; m; NH–C$_6$H$_5$), 9.41 (1H; br; NH–C$_6$H$_5$), 9.71 (1H; br; –NH–NH–CS), 10.14 (1H; br; –NH–NH–CS). ESI–MS (*m/z*); 342 $[M+Na]^+$, 320 $[M+H]^+$ (100%).

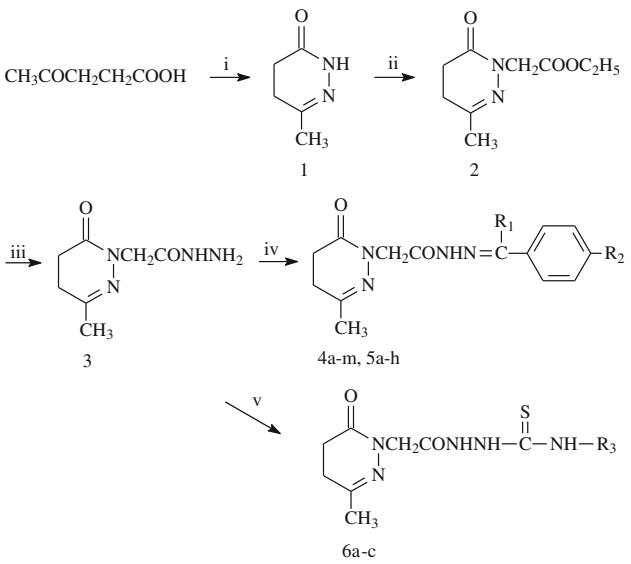
Evaluation of antimycobacterial activity

The target compounds were screened for their in vitro antimycobacterial activity against *M. tuberculosis*, in Middlebrook 7H11 agar medium supplemented with OADC by agar dilution method similar to that recommended by the National Committee for Clinical Laboratory Standards for the determination of MIC in triplicate (1995). The minimum inhibitory concentration (MIC) was defined as the minimum concentration of the compound required to provide complete inhibition of bacterial growth.

Results and discussion

Chemistry

The target compounds (**3**, **4a–6c**) were synthesized via the route outlined in Scheme 1. The starting compound, 6-methyl-4,5-dihydropyridazin-3(2H)-one **1** was prepared by the reaction of levulinic acid with hydrazine hydrate according to the method reported earlier (Reichelt and Reissig, 1984). Although **2** and **3** are already known (Scifinder), the synthetic methods and spectral data of them are also given in this study. Ethyl 2-(3-methyl-6-oxo-5,6-dihydropyridazin-1(4*H*)-yl)acetate **2** was prepared by the



Scheme 1 Synthesis of the compounds. Reagents and conditions: (*i*) $NH_2NH_2.H_2O$, reflux; (*ii*) $BrCH_2COOC_2H_5$, K_2CO_3, reflux; (*iii*) $NH_2NH_2.H_2O$, reflux; (*iv*) appropriate aldehydes and acetophenones, rt; (*v*) R_3NCS, reflux

treatment of **1** with ethyl bromoacetate in the presence of potassium carbonate. The reaction of **2** with excess of hydrazine hydrate gave 2-(3-Methyl-6-oxo-5,6-dihydropyridazin-1-(4*H*)-yl)acetohydrazide **3**. Compound **3** was treated with the appropriate benzaldehydes and acetophenones in the presence of HCl to synthesize **4a–m** and **5a–h**, respectively. Finally, thiosemicarbazide derivatives **6a–c** were prepared by refluxing **3** with the appropriate isothiocyanates.

The structures of the target compounds **3** and **4a–6c** were characterized using spectral methods (IR, ^{1}H-NMR, and ESI-MS). The bands at 3306 and 3210 cm^{-1} in the IR spectra and the signals at 4.21 and 9.02 ppm in the ^{1}H-NMR spectra of **3** were the evidences of the presence of hydrazide structure. *N*-Acylhydrazones may exist as geometrical isomers (*E/Z*) with respect to the C=N double bonds and as rotamers (*cis/trans*) about amide N–C(O) bond (Palla *et al.*, 1986; Syakaev *et al.*, 2006). As mentioned in our previous article (Unsal-Tan *et al.*, 2010), in the ^{1}H-NMR spectra of **4a–m** and **5a–h,** the double signals were observed due to the presence of *cis* and *trans* isomers. In the ^{1}H-NMR spectra of **4a–m,** the signals belonging to methylidene and imine protons were observed at around 4.30 (*trans*), 4.70 (*cis*) and 8.20 (*trans*), 7.90 (*cis*) ppm, respectively. Different from **4a–m**, in the ^{1}H-NMR spectra of **5a–h**, the methyl protons were observed as double signals at around 2.20 (*trans*) and 2.30 (*cis*) ppm in place of imine proton. The signals of the amide protons in **4a–m** and **5a–h** were also seen at around 11–12 ppm as double singlets with some exceptions. The strong absorption band at around 1200 cm^{-1} due to C=S stretching in the IR and three broad signals at around 7.70, 9.30, and 9.90 ppm in the ^{1}H-NMR spectra of **6a–c** exhibited the presence of thiosemicarbazide structure. Furthermore, the structures of all the target compounds were confirmed by the peaks belonging to $[M+Na]^+$ and $[M+H]^+$ seen in the ESI mass spectra.

Antimycobacterial activity

The synthesized compounds **3** and **4a–6c** were evaluated for in vitro antimycobacterial activity against *M. tuberculosis* by agar dilution method using Middlebrook 7H11 agar medium supplemented with OADC. The results for the Minimum Inhibitory Concentrations (MICs) are presented in Table 1.

When the results were examined, benzaldehyde-derived NAH compounds showed higher antimycobacterial activity than their acetophenone-derived counterparts with some exceptions. The semicarbazide derivatives **6a–c** were found as the least active compounds in the series.

In general, the benzaldehyde-derived NAH compounds showed remarkable antimycobacterial activity except **4k**,

Table 1 Physical data and antimycobacterial activity of synthesized compounds

Compound	R_1	R_2	R_3	Yield (%)	M.p. (°C)	MIC (μM)
3	–	–		–	194	25
4a	H	H		51	197	12.5
4b	H	Cl		55	190	3.13
4c	H	Br		80	211	1.56
4d	H	CH_3		55	193	12.5
4e	H	OCH_3		54	185	25
4f	H	NO_2		94	216	6.25
4g	H	2,4-Cl		62	203	0.78
4h	H	CF_3		59	165	1.56
4i	H	$N(Me)_2$		67	200	12.5
4j	H	F		58	180	1.56
4k	H	Furfural		43	199	>25
4l	H	Thiophene		73	209	25
4m	H	OC_2H_5		64	166	>25
5a	CH_3	H		50	171	25
5b	CH_3	Cl		66	212	12.5
5c	CH_3	Br		63	225	12.5
5d	CH_3	CH_3		67	211	25
5e	CH_3	OCH_3		56	185	25
5f	CH_3	NO_2		88	>270	12.5
5g	CH_3	2,4-Cl		72	192	12.5
5h	CH_3	Phenyl		70	221	25
6a	–	–	Methyl	52	198	>25
6b	–	–	Ethyl	65	190	>25
6c	–	–	Phenyl	74	158	>25
INH						0.05
Rifampin						0.1
Ethambutol						1.56
Ciprofloxacin						1.56

MIC minimum inhibitory concentration

4l having heteroaromatic moiety and **4e**, **4m** carrying alkoxy groups on the phenyl ring. Among them, **4g** was the most active compound with MIC of 0.78 μM against *M. tuberculosis.* This is a quite good activity compared with ethambutol and ciprofloxacin. Besides, **4c**, **4 h,** and **4j** (MIC = 1.56 μM) were found as active as ethambutol and ciprofloxacin.

It was also observed that the presence of halogen atoms (Cl, Br, and F) on phenyl ring produced noticeable improvements in antimycobacterial activity (MIC = 0.78–3.13 μM) based on the number and type on the phenyl ring. The 2,4-dichloro-substituted compound (**4g)** was the most active, with MIC of 0.78 μM. The substitution of Br and F (**4c** and **4j** with MIC of 1.56 μM) on the phenyl ring slightly enhanced the activity compared to Cl (**4b** with MIC of 3.13 μM). On the other hand, strong electron withdrawing groups such as NO_2 and CF_3 (**4f** and **4 h** with MIC of 6.25 and 1.56 μM, respectively) on the phenyl ring increased the antimycobacterial activity while electron donating groups (CH_3, OCH_3 $N(CH_3)_2$, and OC_2H_5) diminished the activity (**4d**, **4e**, **4i,** and **4m** with MIC ≥12.5 μM).

It is noteworthy that the acetophenone-derived NAH compounds **5a–h** were devoid of antimycobacterial activity (≥12.5 μM). This observation may be due to the introduction of a bulky group (methyl) in the C=N double bond of NAH moiety. Among the **5a–h**, compounds substituted with Cl, Br, NO_2 (**5b**, **5c**, **5f,** and **5g** with MIC of 12.5 μM) are more active than rest of the series.

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