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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization, and in vitro anti-*Mycobacterium tuberculosis* activity of terpene Schiff bases

Mashooq A. Bhat · Mohamed A. Al-Omar

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Abstract A novel series of Schiff bases (**3**–**13**) were synthesized by the reaction of isoniazid (INH), nalidixic acid hydrazide, and fenamic acid hydrazides with monoterpenes (citral, camphor, and carvone) to obtain anti-mycobacterial agents in good yield and purity. The structures of the compounds were confirmed on the basis of their elemental analysis and spectral data. The structural modification of INH, nalidixic acid hydrazide, and fenamic acid hydrazides provided lipophilic adaptation of the respective hydrazides. The anti-mycobacterial activity of the synthesized compounds was investigated against four *Mycobacterium strains*: *M. intercellulari* (ATCC 35743), *M. xenopi* (ATCC 14470), *M. cheleneo* (ATCC 35751), and *M. smegmatis* (ATCC 35797). Compound **5**, N′-[(1E)-2-methyl-5-(prop-1-en-2-yl) cyclohex-2-en-1-ylidene] pyridine-4-carbohydrazide with minimum inhibitory concentration (MIC 12 ± 0.03 μg/mL) was found to be more potent than INH under the in vitro investigation conditions. It was found that there is no evident relation between the anti-tubercular activity of the tested compounds and their lipophilicity. However, lipophilicity has an influence on the activity, but it does not solely determine the anti-tubercular activity of these compounds. All compounds presented lipophilicity higher than that of respective parent hydrazide.

Keywords Schiff bases · Terpenes · Isoniazid · Nalidixic acid hydrazide · Fenamic acid hydrazide · Anti-tubercular activity

M. A. Bhat (✉) · M. A. Al-Omar
Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box. 2457, Riyadh 11451, Kingdom of Saudi Arabia
e-mail: mashooqbhat@rediffmail.com

Introduction

Tuberculosis (TB) is a chronic disease caused by several species of *Mycobacteriae*. The incidence of TB is increasing worldwide, partly due to poverty and inequity and partly due to the HIV/AIDS pandemic. The problem of clinical treatment has become more acute especially in immuno-compromised AIDS patients where the rise in TB incidence and consequent deaths over the past two decades has escalated by more than 12 % (Patole *et al*., 2006). The major issue is the increase of multidrug-resistant tuberculosis. There is emerging demand for the development of new anti-tubercular agents effective against pathogens resistant to current treatment regimens, which is limited to five drugs including rifampicin, isoniazid (INH), ethambutol, streptomycin, and pyrazinamide. There are several strategies for the development of new anti-mycobacterial drugs. These include design of analogs of existing agents, broad screening, and target-directed drug design. In spite of major advances that have been made in the discovery process, no new drugs have been introduced in clinic since the discovery of rifampicin (Burman and Jones, 2001). The need for newer compounds remains urgent due to increasing resistance of *Mycobacterial* strains to certain type of currently used anti-mycobacterials. Therefore, there is an urgent need for anti-TB drugs with improved properties such as enhanced activity against MDR strains, reduced toxicity, shortened duration of therapy, rapid mycobactericidal mechanism of action, the ability to penetrate host cells, and exert anti-mycobacterial effects in the intracellular environment. INH is still maintaining its importance as a first line drug for treatment of TB. Although there are many reports on synthesis and anti-TB screening of a large number of compounds containing isoniazid moiety (Judge *et al.*, 2012), reports suggest that INH, a prodrug which is converted into its active form by mycobacterial

catalase-peroxidase, acts on mycobacterial cell wall by inhibiting the fatty acid synthetase II system to produce long chain fatty acid precursors for mycolic acid synthesis (Fu and Shinnick, 2007). Nalidixic acid hydrazide derivatives have been reported to possess anti-tubercular activities (Aboul-Fadl *et al*., 2010). Mefenamic and flufenamic acid are congeners of diclofenac acid, which is reported to have anti-tubercular activity (Dutta *et al.*, 2004). Incorporation of hydrophobic moieties into the framework of INH, nalidixic acid hydrazide, and fenamic acid hydrazides can enhance penetration of the drug into tissues of mammalian host and waxy cell wall of bacterium. This strategy has been used for augmenting fundamental drug activity (Mohamad *et al*., 2004). There are reports that structural-modified drugs have increased efficacy because of suppression of transformation (Hearn and Cynamon, 2003). Our rationale has been to prepare Schiff bases of INH, nalidixic acid hydrazide, and fenamic acid hydrazides with monoterpenes (citral, camphor and carvone) with enhanced lipophilicity and to examine their activity against four *Mycobacterium* strains: *M. intercellulari* (ATCC 35743), *M. xenopi* (ATCC 14470), *M. cheleneo* (ATCC 35751), and *M. smegmatis* (ATCC 35797).

Chemistry

Experimental

All the solvents were of LR grade and were obtained from Merck. The elemental analysis (C, H, N) of all compounds were performed on the CHN Elementar (Analysen systeme GmbH, Germany) and Vario EL III (Elementar Americas Corporation) and were within a limit of ±0.4 %, of the theoretical values. The homogeneity of the compounds was checked by TLC performed on Silica gel G-coated plates (Merck). Iodine chamber was used for visualization of TLC spots. The FT-IR spectra were recorded in SHIMADZU spectrophotometer by dissolving samples in carbon tetrachloride (CCl_4). Melting points were determined on a Gallenkamp melting point apparatus, and are uncorrected. NMR Spectra were scanned in DMSO-d_6 on a Bruker NMR spectrophotometer operating at 500 MHz for ^{1}H and 125.76 MHz for ^{13}C at the Research Center, College of Pharmacy, King Saud University, Saudi Arabia. Chemical shifts are expressed in δ-values (ppm) relative to TMS as an internal standard and D_2O was added to confirm the exchangeable protons. Mass spectra were measured on Agilent Triple Quadrupole 6410 QQQ LC/MS with ESI (Electrospray ionization) source.

Pyridine-4-carbohydrazide (isoniazid) was purchased from Aldrich Chemicals. Synthesis of 1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carbohydrazide (nalidixic acid hydrazide) and fenamic acid hydrazides were carried out by reported methods (Grover and Kini, 2006; Aboul-Fadl *et al*., 2011), respectively.

*N′-[(1E, 2E)-3,7-dimethyloct-2-en-1-ylidene] pyridine-4-carbohydrazide (**3**) (Hearn et al., 2009)*

The pyridine-4-carbohydrazide, (isoniazid) Schiff base was prepared by reaction of 3,7-dimethylocta-2,6-dienal (citral), (0.15 g, 1 mmol) with isoniazid (0.14 g, 1 mmol) in ethanol/H_2O (10 mL), initially dissolving the isoniazid in H_2O and adding the respective solution of citral in ethanol. After stirring for 1–3 h, at room temperature, the resulting mixture was concentrated under reduced pressure. The residue purified by washing with cold ethyl alcohol and ethyl ether, afforded the pure compound. Colorless blocks of the compound suitable for X-ray determination were recrystallized from ethanol by slow evaporation of solvent at room temperature. Compounds **4** and **5** were prepared by the same method by reaction of isoniazid with camphor and carvone, respectively.

Yield: (50 %). m. p.: 110–112 °C. FT-IR (ν, cm^{-1}): 3200 (NH str.), 3000 (C–H str.), 1636 (C=O str.), 1549 (C=N str.). ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 11.7 (1H, s, –NH, D_2O exch.), 7.8–8.7 (4H, m, Ar–H), 6.0 (1H, s, –CH), 5.0 (1H, s, –CH), 3.38 (1H, s, –CH), 2.17 (4H, s, 2 × CH_2), 1.88 (3H, s, CH_3), 1.63 (6H, s, 2 × CH_3). ^{13}C NMR (125.76 MHz, DMSO-d_6, δ ppm): 167.2, 161.0, 150.2, 149.4, 149.1, 148.3, 144.6, 141.7, 140.4, 131.8, 123.4, 122.8, 121.5, 121.3, 121.1, 120.9, 26.3, 25.5, 23.9, 17.5, 16.9, 16.7. MS (ESI) $m/z = 272.3$ $[M\text{-}1]^+$. Anal.: Calcd. for $C_{16}H_{21}N_3O$ (273.37): C (70.82), H (7.80), N (15.49). Found: C (70.62), H (7.83), N (15.43). The single crystal X-ray of the compound **3** has been reported (Bhat *et al*., 2012a).

*N′-[(1Z)-5-methyl-2-(propan-2-yl) cyclohexylidene]pyridine-4-carbohydrazide (**4**)*

Yield: (50 %). m. p.: 205–207 °C. FT-IR (ν cm^{-1}): 3190 (NH str.), 3000 (C–H str.), 1668 (C=O str.), 1559 (C=N str.). ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.65 (s, 1H, –NH, D_2O exch.), 7.6–8.7 (m, 4H, Ar–H), 0.7–0.9 (s, 9H, CH_3), 1.5 (s, 1H, –CH), 1.7 (s, 6H, –CH_2); ^{13}C NMR (125.76 MHz, DMSO-d_6, δ ppm): 175.4, 167.1, 164.4, 150.0, 149.2, 142.0, 141.2, 123.2, 121.6, 52.7, 47.6, 43.2, 34.9, 34.2, 32.2, 26.7, 19.2, 11.3; MS (ESI) $m/z = 274.5$ $[M + 1]^+$. Anal.: Calcd. for $C_{16}H_{21}N_3O$ (273.37): C (70.82), H (7.80), N (15.49). Found: C (71.00), H (7.77), N (15.55).

*N′-[(1E)-2-methyl-5-(prop-1-en-2-yl) cyclohex-2-en-1-ylidene]pyridine-4-carbohydrazide (**5**)*

Yield: (70 %). m. p.: 140–142 °C. FT-IR (ν cm^{-1}): 3250 (NH str.), 3000 (C–H str.), 1655 (C=O str.), 1540 (C=N str.). ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.89 (1H, s, –NH, D_2O exch.), 7.6–8.7 (4H, m, Ar–H), 6.2 (1H, s, CH), 4.6 (2H, s, CH_2), 2.3 (1H, s, CH_2), 2.2 (4H, s, CH_2), 1.7 (6H, s, 2 × CH_3). ^{13}C NMR (125.76 MHz, DMSO-d_6, δ ppm): 168.5, 163.7, 162.1, 158.4, 151.1, 150.1, 149.1, 147.4, 142.2, 141.2, 134.9, 133.3, 132.1, 123.0, 121.7, 120.9, 110.1, 30.0, 29.7, 29.3, 29.1, 20.6, 17.8. MS (ESI) $m/z = 269.2$ $[M]^+$. Anal.: Calcd. for $C_{16}H_{19}N_3O$ (269.34): C (71.35), H (7.11), N (15.60). Found: C (71.37), H (7.14), N (15.66).

*(17E)-1-ethyl-1,4-dihydro-7-methyl-N′-((Z)-3,7-dimethylocta-2,6-dienylidene)-4-oxo-1,8-naphthyridine-3-carbohydrazide (**6**)*

The 1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carbohydrazide, (nalidixic acid hydrazide) Schiff base was prepared by reaction of citral, 3,7-dimethylocta-2,6-dienal (0.15 g, 1 mmol) with nalidixic acid hydrazide (0.24 g, 1 mmol) in ethanol (10 mL). After stirring for 1–3 h, at room temperature, the resulting mixture was concentrated under reduced pressure. The residue purified by washing with cold ethyl alcohol and ethyl ether, afforded the pure compound. Compounds **7** and **8** were prepared by the same method by reaction of 1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carbohydrazide with camphor and carvone, respectively.

Yield: (60 %). m. p.: 148–150 °C. FT-IR (ν cm^{-1}): 3300 (NH str.), 3050 (C–H str.), 1677 (C=O str.), 1527 (C=N str.). ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 12.73 (1H, s, –NH, D_2O exch.), 8.25–9.06 (3H, m, Ar–H), 7.51 (1H, s, –CH), 6.0 (1H, s, –CH), 5.1 (1H, s, –CH), 4.6 (2H, s, –CH_2), 2.6 (3H, s, CH_3), 2.2 (4H, s, 2 × CH_2), 1.9 (3H, s, CH_3), 1.6 (6H, s, 2 × CH_3), 1.2 (3H, s, CH_3); ^{13}C NMR (125.76 MHz, DMSO-d_6, δ ppm): 175.5, 163.3, 160.2, 148.4, 148.0, 146.9, 135.8, 131.3, 123.5, 121.6, 119.3, 111.3, 46.1, 32.0, 26.3, 25.6, 25.4, 24.8, 24.0, 17.5, 17.0, 15.0; MS (ESI) $m/z = 380.2$ $[M]^+$. Anal.: Calcd. $C_{22}H_{28}N_4O_2$ (380.48): C (69.45), H (7.42), N (14.73). Found: C (69.25), H (7.45), N (14.78).

*(8E)-1-ethyl-1,4-dihydro-7-methyl-N′-(1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)-4-oxo-1,8-naphthyridine-3-carbohydrazide (**7**)*

Yield: (60 %). m. p.: 238–240 °C. FT-IR (ν cm^{-1}): 3300 (NH str.), 3050 (C–H str.), 1677 (C=O str.), 1527 (C=N str.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 12.4 (s, 1H, –NH, D_2O exch.), 7.5–9.0 (m, 3H, Ar–H), 4.6 (s, 2H, –CH_2), 2.6 (s, 3H, CH_3), 2.0 (s, 6H, CH_2). 1.4 (s, 1H, –CH), 1.3 (s, 3H, CH_3), 0.7–1.0 (s, 9H, CH_3); ^{13}C NMR (125.76 MHz, DMSO-d_6, δ ppm): 175.8, 168.7, 163.4, 159.7, 148.1, 135.9, 121.6, 119.3, 111.8, 55.9, 52.1, 47.7, 46.2, 43.3, 34.4, 32.3, 26.7, 24.8, 19.2, 18.5, 15.0, 11.4; MS (ESI) $m/z = 382.2$ $[M]^+$. Anal.: Calcd. for $C_{22}H_{28}N_4O_2$ (382.4) C (69.45), H (7.42), N (14.73). Found: C (69.65), H (7.40), N (14.79).

*(17Z)-1-ethyl-1,4-dihydro-7-methyl-N′-(2-methyl-5-(prop-1-en-2-yl)cyclohex-2-enylidene)-4-oxo-1,8-naphthyridine-3-carbohydrazide (**8**)*

Yield: (70 %). m.p.: 230–232 °C. Yield: (70 %). m.p.: 230–232 °C. FT-IR (ν cm^{-1}): 3300 (NH str.), 3000 (C–H str.), 1682 (C=O str.), 1533 (C=N str.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 12.93 (1H, s, –NH, D_2O exch.), 7.5–9.0 (3H, m, Ar–H), 6.2 (1H, s, CH), 4.8 (2H, s, CH_2), 4.6 (2H, s, CH_2), 2.5 (3H, s, CH_3), 2.3 (4H, s, 2 × CH_2), 2.2 (1H, s, CH), 1.8 (3H, s, CH_3), 1.7 (3H, s, CH_3), 1.4 (3H, s, CH_3). MS (ESI) $m/z = 378.2$ $[M]^+$. Anal.: Calcd. for $C_{22}H_{26}N_4O_2$ (378.4): C (69.82), H (6.92), N (14.80). Found: C (69.60), H (0.95), N (14.76).

*2-(2,3-dimethylphenylamino)-N′-((Z)-3,7-dimethylocta-2,6-dienylidene)benzohydrazide (**9**)*

The 2-[(2,3-dimethylphenyl)amino]benzohydrazide, (mefanimic acid hydrazide) Schiff base was prepared by reaction of citral, 3,7-dimethylocta-2,6-dienal (0.15 g, 1 mmol) with mefenamic acid hydrazide (0.25 g, 1 mmol) in ethanol (10 mL). After stirring for 1–3 h, at room temperature, the resulting mixture was concentrated under reduced pressure. The residue purified by washing with cold ethyl alcohol and ethyl ether, afforded the pure compound. Compounds **10** and **11** were prepared by the same method by reaction of 2-[(2,3-dimethylphenyl) amino]benzohydrazide with camphor and carvone, respectively. Colorless blocks of the compound **11** suitable for X-ray structure determination were recrystallized from ethanol by slow evaporation of the solvent at room temperature.

Yield: (65 %). m. p.: 135–137 °C. FT-IR (ν cm^{-1}): 3200 (NH str.), 3000 (C–H str.), 1647 (C=O str.), 1516 (C=N str.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 11.51 (1H, s, –NH, D_2O exch.), 9.28 (1H, s, –NH, D_2O exch.), 6.7–8.3 (7H, m, Ar–H), 7.6 (1H, s, –CH), 5.9 (1H, s, –CH), 5.09 (1H, s, –CH), 2.36 (6H, s, 2 × CH_3), 2.1 (4H, s, 2 × CH_2), 1.2 (9H, s, 3 × CH_3. ^{13}C NMR (125.76 MHz, DMSO-d_6, δ ppm): 165.0, 148.4, 148.1, 147.3, 147.0, 146.4, 139.0, 137.6, 132.3, 131.7, 131.3, 129.4, 128.6, 125.8, 125.2, 123.4, 123.3, 122.6, 121.7, 119.8, 116.8,

115.9, 114.0, 32.32, 26.3, 25.6, 25.4, 23.9, 20.2, 17.5, 16.9, 13.4. MS (ESI) $m/z = 389.2$ $[M]^+$. Anal.: Calcd. for $C_{25}H_{31}N_3O$ (389.53): C (77.08), H (8.02), N (10.79). Found: C (77.38), H (8.06), N (10.83).

*(E)-2-(2,3-dimethylphenylamino)-N′-(2-isopropyl-5-methylcyclohexylidene)benzohydrazide (**10**)*

Yield: (70 %). m.p.: 200–202 °C. Yield: (70 %). m.p.: 200–202 °C. FT-IR (ν cm^{-1}): 3100 (NH str.), 3000 (C–H str.), 1631 (C=O str.), 1581 (C=N str.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.51 (1H, s, –NH, D_2O exch.), 10.25 (1H, s, –NH, D_2O exch.), 6.86–9.0 (7H, m, Ar–H), 2.27 (6H, s, 2 × CH_3), 2.0 (6H, s, 3 × CH_2), 1.9 (2H, s, –CH), 1.09 (9H, s, 3 × CH_3); ^{13}C NMR (125.76 MHz, DMSO-d_6, δ ppm): 155.5, 145.2, 139.2, 137.5, 131.8, 128.9, 125.7, 124.8, 119.1, 117.1, 114.2, 47.5, 43.2, 34.4, 32.2, 26.6, 25.0, 20.1, 19.1, 18.4, 17.9, 13.3, 11.3; MS (ESI) $m/z = 391.2$ $[M]^+$. Anal.: Calcd. for $C_{25}H_{31}N_3O$ (391.5): C (77.08), H (8.02), N (10.79). Found: C (77.38), H (8.05), N (10.75).

*(E)-2-(2,3-dimethylphenylamino)-N′-(2-methyl-5-(prop-1-en-2-yl)cyclohex-2-enylidene)benzohydrazide (**11**)*

Yield: (65 %). m. p.: 170–172 °C. FT-IR (ν cm^{-1}): 3200 (NH str.), 3000 (C–H str.), 1636 (C=O str.), 1579 (C=N str.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.75 (1H, s, –NH, CONH, D_2O exch.), 8.8 (1H, s, –NH, D_2O exch.), 6.8–7.6 (7H, m, Ar–H), 6.2 (1H, s, CH), 4.8 (2H, s, –CH_2), 2.5 (1H, s, CH), 2.3 (4H, s, 2 × CH_3), 2.27 (6H, s, 2 × CH_3), 1.7 (6H, s, CH_3); ^{13}C NMR (125.76 MHz, DMSO-d_6, δ ppm): 157.0, 147.5, 145.7, 139.3, 137.6, 134.2, 132.3, 129.5, 125.8, 119.4, 118.0, 114.5, 110.1, 29.9, 20.4, 20.2, 17.8, 13.4. MS (ESI) $m/z = 387.0$ $[M]^+$. Anal.: Calcd. for $C_{25}H_{29}N_3O$ (387.5): C (77.48), H (7.54), N (10.84). Found: C (77.18), H (7.51), N (10.88). The single crystal x-ray of compound **11** has been reported (Bhat *et al*., 2012b).

*(E)-2-(3-(trifluoromethyl) phenylamino)-N′-(2-isopropyl-5-methylcyclohexylidene) benzohydrazide (**12**)*

The 2-{[2-methyl-3-(trifluoromethyl)phenyl]amino}benzohydrazide, (flufenamic acid hydrazide) Schiff base was prepared by reaction of camphor, 1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (0.15 g, 1 mmol) with flufenamic acid hydrazide (0.30 g, 1 mmol) in ethanol (10 mL). After stirring for 1–3 h, at room temperature, the resulting mixture was concentrated under reduced pressure. The residue purified by washing with cold ethyl alcohol and ethyl ether, afforded the pure compound. Compound **13** was prepared by the same method by reaction of 2-{[2-methyl-(trifluoromethyl) phenyl]amino}benzohydrazide with carvone.

Yield: (70 %). m. p.: 160–162 °C. FT-IR (ν cm^{-1}): 3300 (NH str.), 3000 (C–H str.), 1634 (C=O str.), 1582 (C=N str.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.2 (s, 1H, –CONH, D_2O exch.), 8.9 9s, 1H, –NH, D_2O exch.), 7.0–7.6 (m, 8H, Ar–H), 0.5–1.7 (s, 6H, –CH_2), 1.2 (s, 1H, –CH), 0.9 (s, 9H, CH_3); ^{13}C NMR (125.76 MHz, DMSO-d_6, δ ppm): 172.4, 163.8, 144.1, 141.1, 131.6, 130.0, 129.7, 124.2, 121.3, 120.1, 119.2, 116.2, 112.9, 52.4, 47.5, 43.2, 34.2, 32.2, 26.7, 19.1, 11.3; MS (ESI) $m/z = 431.0$ $[M]^+$. Anal.: Calcd. for $C_{24}H_{26}F_3N_3O$ (431.4): C (67.12), H (6.10), N (9.78). Found: C (67.32), H (6.12), N (9.80).

*(20E)-2-(3-(trifluoromethyl) phenylamino)-N′-(2-methyl-5-(prop-1-en-2-yl) cyclohex-2-enylidene)benzohydrazide (**13**)*

Yield: (70 %). m. p.: 170–172 °C. FT-IR (ν cm^{-1}): 3200 (NH str.), 3000 (C–H str.), 1673 (C=O str.), 1517 (C=N str.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.8 (1H, s, –NH, CONH, D_2O exch.) 8.9 (1H, s, –NH, D_2O exch.), 7.0–7.7 (8H, m, Ar–H), 6.1 (1H, s, –CH), 4.7 (2H, s, –CH_2), 2.8 (1H, s, –CH), 2.29 (4H, s, 2 × CH_2), 1.68 (6H, s, 2 × CH_3). ^{13}C NMR (125.76 MHz, DMSO-d_6, δ ppm): 170.1, 164.3, 156.0, 147.4, 144.0, 141.4, 134.1, 132.3, 131.6, 129.9, 127.4, 125.2, 124.3, 123.0, 121.3, 120.4, 119.0, 116.3, 114.8, 113.1, 111.2, 109.9, 29.6, 20.3, 17.7 MS (ESI) $m/z = 427.0$ $[M]^+$. Anal.: Calcd. for $C_{24}H_{24}F_3N_3O$ (427.4): C (67.43), H (5.66), N (9.83). Found: C (67.63), H (5.63), N (9.79).

Anti-mycobacterial activity

Anti-mycobacterial activity was performed at the Research Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The tested *Mycobacterium tuberculosis* strains are *M. intercellulari* (ATCC 35743), *M. xenopi* (ATCC 14470), *M. cheleneoi* (ATCC 35751), and *M. smegmatis* (ATCC 35797) using Rist and Grosset proportion method, agar dilution method (Canetti *et al*., 1963). The synthesized compounds **(3–13)** and INH was dissolved in dimethylsulfoxide (DMSO) at a concentration of 1 mg/mL. The appropriate aliquot of each solution was diluted with 10 % molten agar to give concentrations of 100 μg/mL. The agar and the compound solution were mixed thoroughly and the mixture was poured into Petri-dishes on a level surface to result in an agar depth of 3–4 mm and allowed to harden. The inocula were prepared by growing overnight culture in Muller-Hinton broth. The cultures were diluted 1:100. Tested organisms were streaked in a radial pattern, and plates were incubated at 35 °C for 48 h to check the growth of the tested strains at this single concentration. Active compounds were further

diluted and tested by the same way to determine the minimum inhibitory concentration (MIC) of these compounds. Experiment using the tested strains in a medium free of investigated compounds was also carried out.

Results and discussion

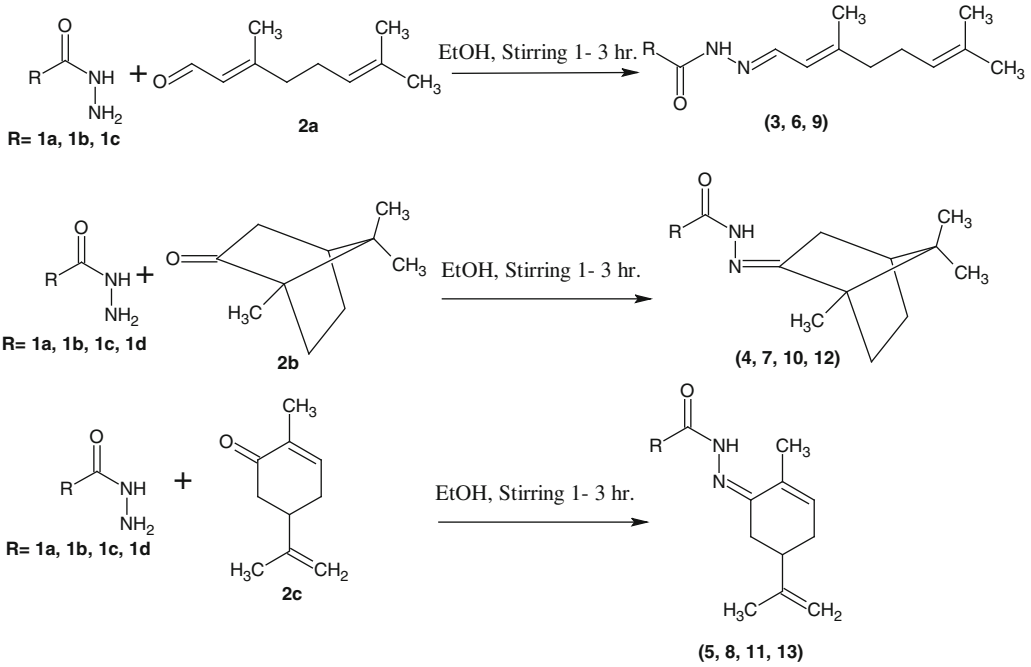
The synthesized compounds (**3**–**13**) were prepared by condensation reaction between the Isoniazid, hydrazides of drugs; nalidixic acid, mefenamic acid and flufenamic acid (**1a**–**d**) with respective monoterpenes; citral, camphor, and carvone (**2a**–**c**) in ethanol by stirring for 1–3 h at room temperature. The resulting mixture was concentrated under reduced pressure. The residue was purified by washing with cold ethyl alcohol and ethyl ether to get the pure compound. The purity of synthesized compounds was checked by thin layer chromatography (TLC) and elemental analyses, and the structures were identified by spectral data. The compounds were obtained in good yields ranging from 50 to 70 % with high purity (Scheme 1). Compounds **3** and **11** were further recrystallized by slow evaporation of solvent (ethanol) for single crystal X-ray spectroscopy. The IR spectra of compounds, exhibited in each case, a band in the region of 1,686–1,631 cm^{-1} due to carbonyl absorption, 1,582–1,516 cm^{-1} due to C=N stretching whereas the absorption band of NH function appeared in the region of 3,300–3,100 cm^{-1}. The ^{1}H NMR and ^{13}C NMR spectra, the signals of the respective prepared derivatives were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The spectra of the compounds showed the characteristic D_2O exchangeable NH protons at δ 10.2–12.93 ppm in addition to the aromatic protons of the phenyl moieties. The mass spectra of compounds showed the mass peaks of $[M^+]$ and $[M-1]^+$. The elemental analysis results were within ±0.4 % of the theoretical values. The lipophilicities of the synthesized compounds were calculated using Chem Office 6.0 software. ACD/Chem Sketch (freeware) and Chem Draw Ultra 8.0 version program were used to draw the chemical structures.

Anti-mycobacterial activity

The synthesized compounds (**3**–**13**) were evaluated for their anti-mycobacterial activity in vitro against four *Mycobacterium* strains: *M. intercellulari* (ATCC35743), *M. xenopi* (ATCC 14470), *M. cheleneo* (ATCC 35751) and *M. smegmatis* (ATCC 35797) by agar dilution method according to the protocol described in the experimental section similar to that recommended by the National Committee for Clinical Laboratory Standards (NCCLS) for the determination of MIC [Wayne, 2000]. Isoniazid (INH) was used as a reference drug and control experiments were done using a growth media free from drugs or the tested compounds. Results of the in vitro anti-tubercular activity of the tested compounds along with the standard drug for comparison are given in (Table 1). Compound **5** (*N*′-[(1E)-2-methyl-5-(prop-1-en-2-yl) cyclohex-2-en-1-ylidene]pyridine-4-carbohydrazide) presented significant growth inhibition against all strains mycobacterium with minimum inhibitory concentration (MIC 12 ± 0.03 μg/mL). Compounds **4** (*N*′-[(1Z)-5-methyl-2-(propan-2-yl)cyclohexylidene]pyridine-4-carbohydrazide) and compound **6** (17E)-1-ethyl-1,4-dihydro-7-methyl-*N*′-((Z)-3,7-dimethylocta-2,6-dienylidene)-4-oxo-1,8-naphthyridine-3-carbohydrazide also showed growth inhibition against all strains of *Mycobacterium* at concentration of 50 μg/mL. The data of the anti-*Mycobacterium* screening of compounds **3**, **6**, **8**, **9**, **10**, **11**, **12**, and **13** revealed no activity on the tested strains up to concentration of 100 μg/mL. The active compound **5** was found to be more potent than first line anti-tubercular drug INH under the investigation conditions. The lipophilicity is a well-known physicochemical factor affecting biological activities, characterizing the distribution process of compound in the human organism, and being a key factor of both pharmacokinetic and pharmacodynamic properties of drug molecules (plasma protein binding, blood–brain barrier (BBB) penetration, and penetration through cell membranes (Lesyk *et al*., 2006). Correlation between lipophilicity and anti-TB was reported, as lipophilicity of drug molecules may render them more capable of penetrating various biomembranes consequently improving their permeation properties toward microbial cell membranes (Sivakumar *et al*., 2007). Lipophilicity of the synthesized compounds expressed in the term of their C log *P* values, is shown in (Table 1). It was found that there is no evident relation between the anti-TB activity of the tested compounds and their lipophilicity. Clearly, the lipophilicity has an influence on the activity, but it does not solely determine the anti-*Mycobacterium* activity of these compounds. All compounds presented lipophilicity higher than that of respective parent hydrazide.

Conclusion

The Schiff bases of INH, Nalidixic acid hydrazide, and Fenimic acid hydrazides were readily prepared for evaluation against four *Mycobacterium strains*; *M. intercellulari* (ATCC 35743), *M. xenopi* (ATCC 14470), *M. cheleneo* (ATCC 35751), and *M. smegmatis* (ATCC 35797) in good yield and purity. The structural modification of INH, nalidixic acid hydrazide, and fenamic acid hydrazides provided lipophilic adaptation of respective hydrazides. Compound **5** *N*′-[(1E)-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-ylidene]pyridine-4-carbohydrazide (MIC 12 ± 0.03 μg/mL)



R	2a	2b	2c
1a	3	4	5
1b	6	7	8
1c	9	10	11
1d	-	12	13

Scheme 1 Synthesis of Schiff bases of hydrazide drugs (**3**–**13**)

Table 1 Lipophilicity (C log *P*) and in vitro anti-mycobacterial activities of compounds (**3**–**13**)

Compound no.	C log P^a	MIC (μg/ML)[b]			
		M. intercellulari	*M. xenopi*	*M. cheleneoi*	*M. smegmatis*
3	3.88	>100	>100	>100	>100
4	2.20	50	50	50	50
5	2.80	12 ± 0.03[c]	12	12	12
6	4.9	>100	>100	>100	>100
7	3.22	50	50	50	50
8	3.83	>100	>100	>100	>100
9	7.93	>100	>100	>100	>100
10	6.25	>100	>100	>100	>100
11	6.86	>100	>100	>100	>100
12	6.54	>100	>100	>100	>100
13	7.15	>100	>100	>100	>100
Isoniazid	0.67	12.5	12.5	12.5	12.5

[a] C log *P* was calculated using software Chem Office 6.0, nalidixic acid hydrazide (C log *P* 0.27); mefenamic acid hydrazide (C log *P* 3.04); flufenamic acid hydrazide (C log *P* 3.33)

[b] Minimal inhibition concentration (MIC) is expressed in μg/mL

[c] Value are expressed as mean ±SEM and analyzed by ANOVA with $P < 0.05$

was found to be more potent than first line anti-tubercular drug INH under the investigation conditions.

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