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SYNTHESIS AND ANTI-MYCOBACTERIAL ACTIVITY OF NEW 4-THIAZOLIDINONE AND 1,3,4-OXADIAZOLE DERIVATIVES OF ISONIAZID

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Abstract: A new series of 4-thiazolidinone **(3a–e)** and 1,3,4-oxadiazole **(4a–e)** derivatives of isoniazid were synthesized and evaluated for their *in vitro* anti-mycobacterial activity. The structures of the compounds were confirmed on the basis of spectral data and elemental analysis. Some compounds showed interesting activity against four *Mycobacterium* strains: *M. intercellulari* (ATCC 35743), *M. xenopi* (ATCC 14470), *M. cheleneo* (ATCC 35751) and *M. smegmatis* (ATCC 35797). Compounds **3e**, N-(4-oxo-2-undecylthiazolidin-3-yl) isonicotinamide and **4e** N-acetyl-4-(5-undecyl-1,3,4-oxadiazol-2-yl) pyridine with minimum inhibitory concentration (MIC), 6.0 μg/mL were found to be more potent than isoniazid under the *in vitro* investigational conditions. Compound **3e** and **4e** bear a high lipophilic chain bonded to the 5-position of the thiazolidinone and 1,3,4-oxadiazole moiety, respectively. This fact indicates that there exists a contribution of lipophilicity, which would facilitate the transport of these molecules through membranes.

Keywords: 4-thiazolidinone, 1,3,4-oxadiazole, isoniazid, anti-mycobacterium agents

Tuberculosis is a chronic disease caused by several species of *Mycobacteriae*. The major issue is the increase of multidrug resistant tuberculosis. The appearance of multidrug resistant strains of *Mycobacterium tuberculosis*, which exhibit *in vitro* resistance to at least two major anti-tubercular drugs (usually isoniazid and rifampicin) and cause intractable tuberculosis, has greatly contributed to the increased incidence of tuberculosis. Another serious problem is the extensively drug-resistant tuberculosis, which are strains resistant to first and second line anti-tubercular drugs. The emergence of drug-resistant tuberculosis is an important fact that made the resurgence of tuberculosis especially alarming. Due to the high impact of multidrug resistant and extensively drug-resistant tuberculosis treatment, there is an urgent need for new drugs to treat this disease efficiently. Hence, there is emerging demand for the development of new anti-tubercular agents effective against pathogens resistant to current treatment regimens, which are limited to five drugs including rifampicin, isoniazid, ethambutol, streptomycin and pyrazinamide. 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 (1). Isoniazid (INH) is still maintaining its importance as a first line drug for treatment of tuberculosis. There are many reports on synthesis and anti-tuberculosis screening of a large number of compounds containing the isoniazid moiety (2–8). Several recent experiments indicate that incorporation of hydrophobic moieties into the framework of INH can enhance the penetration of drug into the tissues of mammalian host and into waxy cell wall of bacterium. This strategy for drug design has been proposed as a vehicle for controlled study of the growth cycle of the pathogen, as these compounds have demonstrated good activities (9–12). The need for newer compounds of this kind remains urgent due to increasing resistance of *Mycobacterial* strains to certain type of currently used anti-mycobacterials. Reports suggest that INH, a pro-drug, is converted into its active form by mycobacterial catalase peroxidase and acts on mycobacterial cell wall by inhibiting the fatty acid synthetase-II system to produce long chain fatty acid precursors for mycolic acid syn-

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thesis (13). Modification of INH at N^2 atom blocks the resulting molecule against the action of *N*-arylaminoacetyl transferases (NATs) and structurally blocking INH towards the action of NAT at N^2 may thus combat the rise of resistance. 4-Thiazolidinone derivatives have many interesting activity profiles; have been reported to possess antifungal (14), anti-TB (15), anti HIV (16), antihistaminic (17) and anticonvulsant properties (18). 4-Thiazolidinones have been reported to exhibit their anti-mycobacterial effect *via* a different route, by inhibiting bacterial enzyme Mur B, which is a precursor acting during biosynthesis of peptidoglycan (19). Newly designed molecules might be regarded as double action molecules and components involved may act synergistically. It has also been reported that conversion of INH to oxadiazoles produces the corresponding 5-substituted-3*H*-1,3,4-oxadiazol-2-thione and 3*H*-1,3,4-oxadiazol-2-one derivatives, which are characterized by high activity against *M. tuberculosis* strain H37Rv (20). Literature survey revealed that 4-(5-pentadecyl-1,3,4-oxadiazole-2-yl)pyridine was ten times more active than INH and 4-(5-heptadecyl-1,3,4-oxadiazol-2-yl)pyridine also showed the same activity, because these compounds bear a high lipophilic chain bonded to the 5-position of oxadiazole moiety (21). Also oxadiazoles conform to an important class of heterocyclic compounds with a wide range of biological activities such as: anticonvulsant (22), tyrosinase inhibitors (23), antimicrobial (24), cathepsin K inhibitors (25) and anti-neoplastic properties (26). There is an urgent need for anti-TB drugs with improved properties such as enhanced activity against multidrug resistant strains, reduced toxicity, shortened duration of therapy, rapid mycobactericidal mechanism of action and the ability to penetrate host cells and exert anti-mycobacterial effects in the intracellular environment.

Chemical modification of hydrazine unit of INH with a functional group that blocks acetylation, while maintain strong anti TB action has the potential to improve clinical outcomes and reduce the emergence in patients of acquired INH resistance.

To investigate the effect of length of lipophilic chain at 5-position and change of 1,3,4-oxadiazole scaffold with thiazolidinone moiety on the anti-mycobacterial activity, in the present study we incorporated the 4-thiazolidinone and 1,3,4-oxadazole scaffolds with different lengths of side chain at 5-position in the INH moiety and evaluated their *in vitro* anti-mycobacterial activity.

EXPERIMENTAL

All the solvents were obtained from Merck. The elemental analyses (C, H, N and S) of all compounds were performed on the CHNS Elementar (Analysen Systeme GmbH, Germany) and Vario EL III (Elementar Americas Corporation) and were within a limit of ± 0.4% and ± 0.3%, respectively, 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 thermometer was uncorrected. NMR spectra were scanned in DMSO-d_6 on a Bruker NMR spectrophotometer operating at 500 MHz for ^1H 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 apparatus with ESI (electrospray ionization) source.

N'-[(1*E*)-propylidene]pyridine-4-carbohydrazide (2a)

The isonicotinoyl hydrazide Schiff base (**2a**) was prepared by reaction between propanal (1.0 equiv.) with INH (1.0 equiv.) in ethanol/H_2O (10 mL), initially dissolving the INH in H_2O and adding the respective solution to a solution of the propanal in ethanol. After 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 diethyl ether and afforded pure derivatives (**2a**). The compound was assigned (*E*) configuration (27). The other Schiff bases (**2b–e**) were synthesized similarly.

Yield: 75%; m.p.: 170–172°C. FT-IR (ν, cm^{-1}): 3430 (NH, str.), 2970 (C-H, str.), 1660 (C=O, str.), 1606 (C=C, str.), 1555 (C=N, str.). ^1H NMR (500 MHz, DMSO-d_6, δ, ppm): 0.8 (3H, t, CH_3), 1.29 (2H, m, CH_2), 7.70 (1H, s, CH), 7.75 (2H, d, J = 4 Hz, CH pyridyl), 8.75 (2H, d, J = 4 Hz, CH pyridyl), 11.6 (1H, s, -NH, D_2O exch.). ^{13}C NMR (125.76 MHz, DMSO-d_6, δ, ppm): 149.8 (C-1), 149.8 (C-3), 122.8 (C-4), 140.9 (C-5), 122.8 (C-6), 163 (C-7), 158.3 (C-11), 22.2 (C-12), 10.4 (C-13). MS (ESI) m/z = 177.0 $[M]^+$. Analysis: calcd. for $C_9H_{11}N_3O$ (177.2): C 61.00, H 6.26, N 23.71%; found: C 61.20, H 6.24, N 23.61%.

N'-[(1*E*)-octylidene]pyridine-4-carbohydrazide (2b)

Yield: 80%; m.p.: 90–92°C. FT-IR (ν, cm^{-1}): 3430 (NH, str.), 2970 (C-H, str.), 1660 (C=O, str.), 1606 (C=C, str.), 1555 (C=N, str.). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 0.8 (3H, t, CH_3), 1.4 (12H, m, CH_2), 7.70 (1H, s, CH), 7.8 (2H, d, J = 4 Hz, CH pyridyl), 8.70 (2H, d, J = 4 Hz, CH pyridyl), 11.6 (1H, s, -NH, D_2O exch.). ^{13}C NMR (125.76 MHz, DMSO-d_6, δ, ppm): 149.8 (C-1), 149.8 (C-3), 122.8 (C-4), 140.9 (C-5), 122.8 (C-6), 163 (C-7), 158.3 (C-11), 26.1 (C-12), 26.1 (C-13), 29.5 (C-14), 29.1 (C-15), 31.9 (C-16), 22.8 (C-17), 14.1 (C-18). MS (ESI) m/z = 246.9 $[M]^+$. Analysis: calcd. for $C_{14}H_{21}N_3O$ (247.3): C 67.98, H 8.56, N 16.99%; found: C 67.75, H 8.54, N 16.93%.

N'-[(1*E*)-decylidene]pyridine-4-carbohydrazide (2c)

Yield: 65%; m.p.: 78–80°C. FT-IR (ν, cm^{-1}): 3430 (NH, str.), 2972 (C-H, str.), 1661 (C=O, str.), 1556 (C=C, str.), 1496 (C=N, str.). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 0.8 (3H, t, CH_3), 1.3 (16H, m, CH_2), 7.70 (1H, s, CH), 7.85 (2H, d, J = 4 Hz, CH pyridyl), 8.7 (2H, d, J = 4 Hz, CH pyridyl), 11.6 (1H, s, -NH, D_2O exch.). ^{13}C NMR (125.76 MHz, DMSO-d_6, δ, ppm): 149.8 (C-1), 149.8 (C-3), 122.8 (C-4), 140.9 (C-5), 122.8 (C-6), 163 (C-7), 158.3 (C-11), 26.1 (C-12), 26.1 (C-13), 29.5 (C-14), 29.4 (C-15), 29.7 (C-16), 29.4 (C-17), 31.9 (C-18), 22.8 (C-19), 14.1 (C-20). MS (ESI) m/z = 275.3 $[M]^+$. Analysis: calcd. for $C_{16}H_{25}N_3O$ (275.3): C 69.78, H 9.15, N 15.26%; found: C 69.88, H 9.37, N 15.20%.

N'-[(1*E*)-undecylidene]pyridine-4-carbohydrazide (2d)

Yield: 70%; m.p.: 80–82°C. FT-IR (ν, cm^{-1}): 3261 (NH, str.), 2923 (C-H, str.), 1655 (C=O, str.), 1535 (C=C, str.), 1464 (C=N, str.). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 0.8 (3H, t, CH_3), 1.29 (18H, m, CH_2) 7.50 (1H, s, CH), 7.8 (2H, d, J = 4 Hz, CH pyridyl), 8.6 (2H, d, J = 4 Hz, CH pyridyl), 11.6 (1H, s, -NH, D_2O exch.). ^{13}C NMR (125.76 MHz, DMSO-d_6, δ, ppm): 149.8 (C-1), 149.8 (C-3), 122.8 (C-4), 140.9 (C-5), 122.8 (C-6), 163 (C-7), 158.3 (C-11), 26.1 (C-12), 26.1 (C-13), 29.5 (C-14), 29.4 (C-15), 29.7 (C-16), 29.7 (C-17), 29.7 (C-18), 31.9 (C-19), 22.8 (C-20), 14.1 (C-21). MS (ESI) m/z = 290.0 $[M + 1]^+$. Analysis: calcd. for $C_{17}H_{27}N_3O$ (289): C 70.5, H 9.4, N 14.5%; found: C 70.75, H 9.38, N 14.40%.

N'-[(1*E*)-dodecylidene]pyridine-4-carbohydrazide (2e)

Yield: 60%; m.p.: 85–87°C. FT-IR (ν, cm^{-1}): 3257 (NH, str.), 2923 (C-H, str.), 1655 (C=O, str.), 1546 (C=N, str.). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 0.8 (3H, t, CH_3), 1.4 (20H, m, CH_2), 7.75 (1H, s, CH), 7.8 (2H, d, J = 4 Hz, CH pyridyl), 8.7 (2H, d, J = 4 Hz, CH pyridyl), 11.6 (1H, s, -NH, D_2O exch.). ^{13}C NMR (125.76 MHz, DMSO-d_6, δ, ppm): 149.8 (C-1), 149.8 (C-3), 122.8 (C-4), 140.9 (C-5), 122.8 (C-6), 163 (C-7), 158.3 (C-11), 26.1 (C-12), 26.1 (C-13), 29.5 (C-14), 29.4 (C-15), 29.7 (C-16), 29.7 (C-17), 29.7 (C-18), 29.4 (C-19), 31.9 (C-20), 22.8 (C-21), 14.1 (C-22). MS (ESI) m/z = 304.0 $[M + 1]^+$. Analysis: calcd. for $C_{18}H_{29}N_3O$ (303.4), C 71.25, H 9.63, N 13.85%; found: C 71.03, H 9.60, N 13.90.

N-(2-ethyl-4-oxothiazolidin-3-yl)isonicotinamide (3a)

A mixture of Schiff base of INH **2a** (0.1 mol), mercaptoacetic acid (0.15 mol) and silica chloride (0.025 mol) was heated at 50°C under solvent-free condition for 1 h. The progress of the reaction was monitored by TLC using hexane-ethyl acetate (7 : 3, v/v). After the completion of reaction, the reaction mixture was extracted with ethyl acetate and organic layer was washed with 5% sodium bicarbonate solution and brine. Organic layer was separated and dried over anhydrous sodium sulfate. From the organic extract the solvent was removed under reduced pressure and the residual crude solid was crystallized from ethanol. The other thiazolidinone derivatives (**3b–e**) were synthesized similarly.

Yield: 60%; semisolid. FT-IR (ν, cm^{-1}): 3300 (NH, str.), 3000 (C-H, str.), 1700 (C=O, str., thiazolidinone), 1680 (C=O, str.). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 1.0 (3H, t, CH_3), 1.8 (2H, m, CH_2), 3.7 (2H, s, -CH_2, thiazolidinone), 5.0 (1H, t, CH, thiazolidinone), 7.7 (2H, d, J = 4 Hz, CH pyridyl), 8.7 (2H, d, J = 4 Hz, CH pyridyl), 11.1 (1H, s, -NH, D_2O exch.). ^{13}C NMR (125.76 MHz, DMSO-d_6, δ ppm): 168.8 (C-1), 164.0 (C-3), 138.0 (C-4), 150.0 (C-5), 122.3 (C-6), 171.7 (C-7), 170.3 (C-10), 63.0 (C-13), 39.0 (C-11), 28.4 (C-16), 12.6 (C17). MS (ESI) m/z = 252.2 $[M + 1]^+$. Analysis: calcd. for $C_{18}H_{27}N_3O_2S$ (251.3): C 61.86, H 7.79, N 12.0, S 9.17%; found: C 61.76, H 7.8, N 12.02, S 9.18%.

N-(2-heptyl-4-oxothiazolidin-3-yl)isonicotinamide (3b)

Yield: 70%; semisolid. FT-IR (ν, cm^{-1}): 3300 (NH, str.), 3000 (C-H, str.), 1710 (C=O, str., thiazolidinone), 1680 (C=O, str.). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 0.7 (3H, t, CH_3), 1.8 (12H, m,

CH_2), 3.7 (2H, s, -CH_2, thiazolidinone), 4.8 (1H, t, CH, thiazolidinone), 7.8 (2H, d, *J* = 4 Hz, CH pyridyl), 8.7 (2H, d, *J* = 4 Hz, CH pyridyl), 11.1 (1H, s, -NH, D_2O exch.). ^{13}C NMR (125.76 MHz, DMSO-d_6, δ, ppm): 168.7 (C-1), 164.2 (C-3), 139.4 (C-4), 150.0 (C-5), 121.8 (C-6), 171.2 (C-7), 170.2 (C-10), 62.3 (C-13), 34.2 (C-11), 28.5 (C-16), 23.9 (C-17), 22.0 (C-18, 19, 20, 21), 13.7 (C-22). MS (ESI) m/z = 321.1 $[M]^+$. Analysis: calcd. for $C_{18}H_{27}N_3O_2S$ (321.4): C 61.86, H 7.79, N 12.0, S 9.17%; found: C 61.76, H 7.8, N 12.02, S 9.18%.

N-(2-nonyl-4-oxothiazolidin-3-yl)isonicotinamide (3c)

Yield: 70%; semisolid. FT-IR (*v*, cm^{-1}): 3300 (NH, str.), 3000 (C-H, str.), 1700 (C=O, str., thiazolidinone), 1670 (C=O, str.). ^1H NMR (500 MHz, DMSO-d_6, *δ,* ppm): 0.8 (3H, t, CH_3), 1.2 (16 H, m, CH_2), 3.7 (2H, s, -CH_2, thiazolidinone), 4.87 (1H, t, CH, thiazolidinone), 7.3 (2H, d, *J* = 4 Hz, CH pyridyl), 8.8 (2H, d, *J* = 4 Hz, CH pyridyl), 11.7 (1H, s, -NH, D_2O exch.). ^{13}C NMR (125.76 MHz, DMSO-d_6, δ, ppm): 164.0 (C-1), 161.1 (C-3), 155.7 (C-5), 153.7 (C-4), 150.4 (C-6), 174.4 (C-7), 168.9 (C-10), 62.9 (C-13), 48.5 (C-11), 34.5 (C-16), 31.2 (C-17), 28.9 (C-18), 27.9 (C-18), 23.9 (C19), 22.0 (C-20), 18.5 (C-21), 13.8 (C-22), 12.6 (C-23). MS (ESI) *m/z* = 349.1 $[M]^+$. Analysis: calcd. for $C_{18}H_{27}N_3O_2S$ (349.4): C 61.86, H 7.79, N 12.0, S 9.17%; found: C 61.76, H 7.8, N 12.02, S 9.18%.

N-(2-decyl-4-oxothiazolidin-3-yl)isonicotinamide (3d)

Yield: 75%; semisolid. FT-IR (*v*, cm^{-1}): 3320 (NH, str.), 3010 (C-H, str.), 1700 (C=O, str., thiazolidinone), 1680 (C=O, str.).^1H NMR (500 MHz, DMSO-d_6, δ, ppm): 0.8 (3H, t, CH_3), 1.2 (18 H, m, CH_2), 3.6 (2H, s, -CH_2, thiazolidinone), 4.87 (1H, t, CH, thiazolidinone), 7.8 (2H, d, *J* = 4 Hz, CH pyridyl), 8.8 (2H, d, *J* = 4 Hz, CH pyridyl), 11.7 (1H, s, -NH, D_2O exch.). ^{13}C NMR (125.76 MHz, DMSO-d_6, δ, ppm): 149.8 (C-1,3), 122.8 (C-4), 140.9 (C-5), 122.8 (C-6), 164.9 (C-7), 168.5 (C-10), 36.1 (C-11), 54.3 (C-13), 35.5 (C-16), 23.2 (C-17), 29.0 (C-18), 29.7 (C-19, 20), 29.4 (C-21), 31.9 (C-22), 22.8 (C-23),14.1 (C-24). MS (ESI) m/z = 363.5 $[M]^+$. Analysis: calcd. for $C_{19}H_{29}N_3O_2S$ (363.5): C 62.78, H 8.04, N 11.5, S 8.82%; found: C 62.80, H 8.05, N 11.52, S 8.80%.

N-(4-oxo-2-undecylthiazolidin-3-yl)isonicotinamide (3e)

Yield: 80%; semisolid. FT-IR (*v*, cm^{-1}): 3330 (NH, str.), 3010 (C-H, str.), 1710 (C=O, str., thiazolidinone), 1680 (C=O, str.). ^1H NMR (500 MHz, DMSO-d_6, δ, ppm): 0.8 (3H, t, CH_3), 1.2 (20 H, m, CH_2), 3.6 (2H, s, -CH_2, thiazolidinone), 4.86 (1H, t, CH, thiazolidinone), 7.8 (2H, d, *J* = 4 Hz, CH pyridyl), 8.8 (2H, d, *J* = 4 Hz, CH pyridyl), 11.1 (1H, s, -NH, D_2O exch.). ^{13}C NMR (125.76 MHz, DMSO-d_6, δ. ppm): 149.5 (C-1,3), 122.0 (C-4), 140.5 (C-5), 122.0 (C-6), 164.4 (C-7), 168.0 (C-10), 36.0 (C-11), 54.1 (C-13), 35.5 (C-16), 23.1 (C-17), 29.0 (C-18), 29.5 (C-19, 20, 21), 29.1 (C-22), 31.5 (C-23), 22.8 (C-24), 14.1 (C-25). MS (ESI) m/z = 377.5 $[M]^+$. Analysis: calcd. for $C_{20}H_{31}N_3O_2S$ (377.5): C 63.6, H 8.28, N 11.13, S 8.49%; found: C 62.58, H 8.06, N 11.52, S 8.80%.

N-acetyl-4-(5-ethyl-1,3,4-oxadiazol-2-yl)pyridine (4a)

A mixture of Schiff base of INH **2a** (0.1 mol) and anhydrous acetic anhydride (10 mL) was refluxed for 4 h. After completion of the reaction, the excessive acetic anhydride was distilled off at reduced pressure; the residue was poured into ice cooled water and stirred for 30 min. The solid product was filtered and recrystallized from ethanol to give final compound (**4a**). The other 1,3,4-oxadiazole derivatives (**4b**–**e**) were synthesized similarly.

Yield: 65%; semisolid. FT-IR (*v*, cm^{-1}): 1564 (C=N), 1670 (C=O), 1620, 1369, 1173, 1097, 1014 (oxadiazole nucleus). ^1H NMR (500 MHz, DMSO-d_6, δ, ppm): 0.7 (3H, t, CH_3), 1.9 (3H, s, $COCH_3$), 2.2 (2H, m, CH_2), 6.3 (1H, s, -CH, oxadiazole), 7.8 (2H, d, *J* = 4 Hz, CH pyridyl), 8.7 (2H, d, *J* = 4 Hz, CH pyridyl). ^{13}C NMR (125.76 MHz, DMSO-d_6, δ, ppm): 168.3 (C-12), 155.0 (C-7), 149.5 (C-1, 3), 138.4 (C-5), 124.1 (C-4, 6), 73.4 (C-9), 29.5 (C-15), 23.4 (C-14), 5.0 (C-16). MS (ESI) m/z = 219.1 $[M]^+$. Analysis: calcd. for $C_{11}H_{13}N_3O_2$ (219.2): C 60.26, H 5.98, N 19.17%; found: C 60.02, H 5.60, N 19.11%.

N-acetyl-4-(5-heptyl-1,3,4-oxadiazol-2-yl)pyridine (4b)

Yield: 67%; semisolid. FT-IR (*v*, cm^{-1}): 1560 (C=N), 1680 (C=O), 1630, 1470, 1350, 1011 (oxadiazole nucleus). ^1H NMR (500 MHz, DMSO-d_6, δ, ppm): 0.85 (3H, t, CH_3), 1.2 (10H, m, CH_2), 1.9 (3H, s, $COCH_3$), 2.5 (2H, m, CH_2), 6.3 (1H, s, -CH, oxadiazole), 7.8 (2H, d, *J* = 4 Hz, CH pyridyl), 8.7 (2H, d, *J* = 4 Hz, CH pyridyl). ^{13}C NMR (125.76 MHz, DMSO-d_6, δ, ppm): 166.9 (C-12), 150.6 (C-7), 138.0 (C-1, 3), 131.5 (C-5), 120.0 (C-4, 6), 93.2 (C-9), 32.5 (C-15), 31.2 (C-14), 28.9 (C-16), 22.0 (C-17), 21.1 (C-18), 20.1 (C-19), 19.0 (C-20), 13.9 (C-21). MS (ESI) m/z = 288.2 $[M]^+$. Analysis: calcd.

for $C_{16}H_{23}N_3O_2$ (288.3): C 66.41, H 8.01, N 14.52%; found: C 66.66, H 8.04, N 14.55%.

N-acetyl-4-(5-nonyl-1,3,4-oxadiazol-2-yl)pyridine (4c)

Yield: 80%; semisolid. FT-IR (ν, cm^{-1}): 1564 (C=N), 1670 (C=O), 1640, 1473, 1350, 1011 (oxadiazole nucleus). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 0.82 (3H, t, CH_3), 1.2 (14H, m, CH_2), 1.9 (3H, s, $COCH_3$), 2.5 (2H, m, CH_2), 6.9 (1H, s, -CH, oxadiazole), 7.8 (2H, d, J = 4 Hz, CH pyridyl), 8.8 (2H, d, J = 4 Hz, CH pyridyl). ^{13}C NMR (125.76 MHz, DMSO-d_6, δ, ppm): 169.9 (C-12), 168.4 (C-7), 167.9 (C-1), 163.3 (C-3), 153.3 (C-5), 150.0 (C-6), 139.4 (C-4), 138.6 (C-9), 31.2 (C-15), 29.4 (C-14), 28.7 (C-16), 28.4 (C-17), 22.0 (C-18, 22), 20.3 (C-20), 18.0 (C-21), 13.8 (C-22), 10.7 (C-23). MS (ESI) m/z = 317.1 $[M]^+$. Analysis: calcd. for $C_{18}H_{27}N_3O_2$ (317.4): C 68.11, H 8.57, N 13.24%; found: C 68.37, H 8.55, N 13.28%.

N-acetyl-4-(5-decyl-1,3,4-oxadiazol-2-yl)pyridine (4d)

Yield: 75%; m.p.:178–180°C. FT-IR (ν, cm^{-1}): 1564 (C=N), 1680 (C=O), 1643, 1470, 1350, 1010 (oxadiazole nucleus). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 1.0 (3H, t, CH_3), 1.2 (16H, m, CH_2), 1.9 (3H, s, $COCH_3$), 2.5 (2H, m, CH_2), 6.3 (1H, s, -CH, oxadiazole), 7.8 (2H, d, J = 4 Hz, CH pyridyl), 8.8 (2H, d, J = 4 Hz, CH pyridyl). ^{13}C NMR (125.76 MHz, DMSO-d_6, δ, ppm): 172.0 (C-12), 166.1 (C-7), 150.8 (C-1, 3), 138.1 (C-5), 122.7 (C-4, 6), 119.9 (C-9), 56.0 (C-15), 20.9 (C-14), 18.4 (C-16, 17, 18, 19), 18.0 (C-20, 21, 22, 23), 10.6 (C-24). MS (ESI) m/z = 331.0 $[M]^+$. Analysis: calcd. for $C_{19}H_{29}N_3O_2$ (331.4): C 68.85, H 8.82, N 12.68%; found: C 68.65, H 8.80, N 12.63%.

N-acetyl-4-(5-undecyl-1,3,4-oxadiazol-2-yl)pyridine (4e)

Yield: 70%; m.p.: 68–70°C. FT-IR (ν, cm^{-1}): 1560 (C=N), 1670 (C=O), 1630, 1373, 1150, 1011 (oxadiazole nucleus). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 0.7 (3H, t, CH_3), 1.2 (18H, m, CH_2), 1.9 (3H, s, $COCH_3$), 2.2 (2H, m, CH_2), 6.3 (1H, s, -CH, oxadiazole), 7.7 (2H, d, J = 4 Hz, CH pyridyl), 8.7 (2H, d, J = 4 Hz, CH pyridyl). ^{13}C NMR (125.76 MHz, DMSO-d_6, δ, ppm): 173.9 (C-12), 170.7 (C-7), 169.3 (C1), 166.9 (C-3), 163.9 (C-5), 157.7 (C-4), 153.2 (C-6), 150.5 (C-9), 33.6 (C-15), 32.4 (C-14), 28.4 (C-16), 22.7 (C-17), 21.9 (C-18), 20.0 (C-19, 20, 21 ,22, 23, 24), 13.7 (C-25). MS (ESI) m/z = 345.2 $[M]^+$. Analysis: calcd. for $C_{20}H_{31}N_3O_2$ (345.4): C 69.53, H 9.04, N 12.16%; found: C 69.80, H 9.07, N 12.11.

Anti-mycobacterial activity

Anti-mycobacterial activity was performed at the Research Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The tested *Mycobacterium* strains are *M. intercellulari* (ATCC 35734), *M. xenopi* (ATCC 14470), *M. cheleneoi* (ATCC 35751) and *M. smegmatis* (ATCC 35797) using Rist and Grosset proportion method, agar dilution method (28). The synthesized compounds (**3a–e**), (**4a–e**) and INH were dissolved in 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 to 4 mm and allowed to harden. The inocula were prepared by growing overnight culture in Mueller-Hinton broth. The cultures were diluted 1 : 100. The 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 the investigated compounds was also carried out.

RESULTS AND DISCUSSION

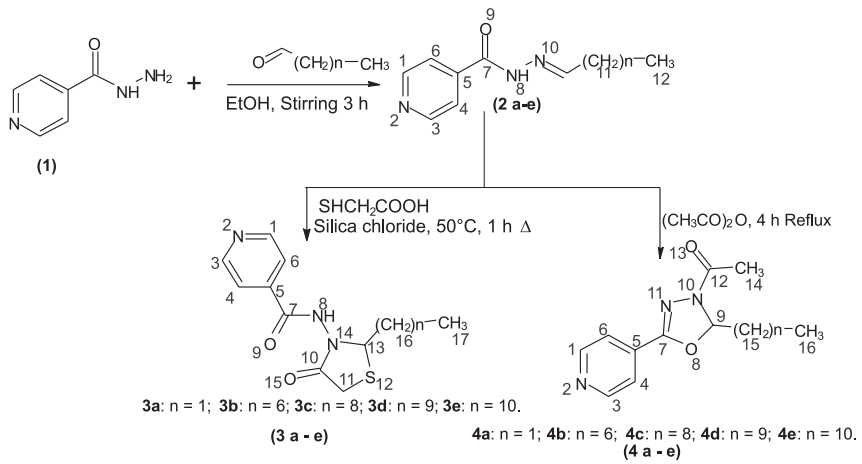
4-Thiazolidinone (**3a–e**) and 1,3,4-oxadiazole (**4a–e**) derivatives of INH were synthesized by reaction of Schiff bases (**2a–e**) with thioglycolic acid in the presence of chlorinated silica and anhydrous acetic anhydride, respectively, to obtain anti-mycobacterial agents in good yield. The condensation was carried by using equimolar amount of the Schiff bases, mercaptoacetic acid, and 25 mmol % of silica chloride. The reaction was monitored by thin layer chromatography (TLC) and was found to reach completion in 4 h giving 60–80% yields of the 4-thiazolidinones (29).

1,3,4 -Oxadiazoles (**4a–e**) were obtained by refluxing Schiff bases (**2a–e**) in excess of anhydrous acetic anhydride for 4 h. The excess of acetic anhydride was distilled off to obtain pure compounds with 65–80% yields. The purity of the synthesized compounds was checked by thin layer chromatography (TLC) and elemental analysis. The structure of the compounds were identified and confirmed by spectral data. The IR spectra of 4-thiazolidinone derivatives (**3a–e**) exhibited in each case, a band in the region of 3300–3200 cm^{-1} due to NH stretching, 3100–3000 cm^{-1} due to CH stretching, 1700–1710

Table 1. Lipophilicity (Clog *P)* and *in vitro* antimycobacterial activities of compounds (**3a–e**) and (**4a–e**).

Compound No.	C log P[a]	MIC (μg/mL)			
		M. intercellulari	*M. xenopi*	*M. cheleneoi*	*M. smegmatis*
3a	0.63	>100	>100	>100	>100
3b	2.03	50	50	50	50
3c	3.09	25	25	25	25
3d	3.62	25	25	25	25
3e	4.16	6.0	6.0	6.0	6.0
4a	0.22	>100	>100	>100	>100
4b	2.88	>100	>100	>100	>100
4c	3.94	25	25	25	25
4d	4.47	25	25	25	25
4e	5.00	6.0	6.0	6.0	6.0
Isoniazid	0.67	12.5	12.5	12.5	12.5

[a]C log P was calculated using software Chem Office 6.0.



Scheme 1. Synthesis of 4-thiazolidinone (**3a–e**) and 1,3,4-oxadiazole (**4a–e**) derivatives of isoniazid.

cm^{-1} due to carbonyl of thiazolidinone and 1680 cm^{-1} due to carbonyl absorption, whereas the IR spectra of 1,3,4-oxadiazole derivatives (**4a–e**) exhibited in each case, a band in the region 1564–1560 cm^{-1} due to C=N stretching, 1680–1670 cm^{-1} due to carbonyl absorption. In the ^1H NMR spectra, the signals of the synthesized compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. The spectra of the 4-thiazolidinone derivatives (**3a–e**) showed the characteristic D_2O exchangeable NH protons at δ 11.1–11.7 ppm, CH_2 (thiazolidinone) protons at δ 3.6–3.7 ppm, CH (thiazolidinone) proton at δ 4.8–5.0 ppm in addition to aromatic protons at δ 7.3 ppm with *J* value of 4 Hz, and δ 8.8 ppm with *J* value of 4 Hz. The spectra of 1,3,4-oxadiazole derivatives (**4a–e**) showed the characteristic $COCH_3$ protons at δ 1.9 ppm, CH (1,3,4-oxadiazole) proton at 6.3–6.9 ppm in addition to pyridyl protons at δ 7.8 ppm with *J* value of 4 Hz, and δ 8.8 ppm with *J* value of 4 Hz. The mass spec-

tra of the compounds showed the molecular ion peaks $[M]^+$ and $[M + 1]^+$. The elemental analysis of CHN and S were within ± 0.4% and 0.3%, respectively, of the theoretical values.

Anti-mycobacterial activity

The synthesized compounds (**3a–e**) and (**4a–e**) were evaluated for their anti-mycobacterial activity *in vitro* against four *Mycobacterium* strains: *Mycobacterium intercellulari* (ATCC 35734), *Mycobacterium xenopi* (ATCC 14470), *Mycobacterium cheleneo* (ATCC 35751) and *Mycobacterium 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 (30). 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-mycobacterial activity of the tested compounds along with the standard drug for comparison are given in Table 1. Compound **3e**, N-(4-oxo-2-undecylthiazolidin-3-yl)isonicotinamide and **4e**, N-acetyl-4-(5-undecyl-1,3,4-oxadiazol-2-yl)pyridine presented significant growth inhibition against all strains of *Mycobacterium* with minimum inhibitory concentration (MIC) 6.0 µg/mL, with the highest C log P value of 4.1 and 5.0, respectively. Compounds **3c**, **3d**, **4c** and **4d** showed growth inhibition against all strains of *Mycobacterium* with minimum inhibitory concentration (MIC) 25 µg/mL. Compound **3b** also showed growth inhibition against all strains of *Mycobacterium* with MIC 50 µg/mL, with C log P value of 2.03. Compound **3a**, **4a** and **4b** revealed no activity on the tested strains up to the concentration of 100 µg/mL. The active compounds **3e** and **4e** were found to be more potent than first line anti-tubercular drug INH under investigation conditions. Lipophilicity of the drug molecules may make them more capable of penetrating various biomemebranes, consequently improving their permeation properties towards microbial cell membranes (31). Correlation between lipophilicity and anti TB has been reported (32). Lipophilicity of the synthesized compounds expressed in the term of their C log P values, is shown in Table 1. All the compounds presented lipophilicity higher than that of INH except **3a** and **4a**. Another raised hypothesis explores the possibility that compounds **3b**, **3c**, **3d**, **4c** and **4d** could be acting as INH prodrugs. According to Scior and Garces-Eisele, the pharmacological role of INH derivatives (isonicotinoyl hydrazones, hydrazides, and amides) must be considered as bio-reversible prodrugs of INH or isonicotinic acid. Worse activities showed by these kinds of structures can be explained by the compounds with a structural gain of stability against prodrug hydrolysis (33). None of them showed more potency than INH against the *Mycobacterium*. Although oxadiazole nucleus is very stable to acid hydrolysis, it has been reported that it may be chemically hydrolyzed with a strong base and heating leading thus to the generation of acyl-INH, which are very likely to be completely hydrolyzed to INH (34). It was reported that 5-low alkyl homologues (methyl, ethyl and propyl-1,3,4-oxadiazol-2-yl)pyridines showed a low tuberculostatic *in vitro* effect (35). Apparently, it is necessary to increase the steric hindrance at position 5 of oxadiazole moiety in order to improve the biological activity of these derivatives. It also implies that lipophilicity plays an important role in the bioactivity of these compounds.

CONCLUSION

4-Thiazolidinone (**3a–e**) and 1,3,4-oxadiazole (**4a–e**) derivatives of INH were synthesized and evaluated for their *in vitro* anti-mycobacterial activity against four *Mycobacterium* strains: *M. intercellulari* (ATCC 35743), *M. xenopi* (ATCC 14470), *M. cheleneo* (ATCC 35751) and *M. smegmatis* (ATCC 35797). The present results highlight the importance of lipophilicity of these compounds to present good anti-mycobacterial activity. The high activity of compounds **3e** and **4e** makes them suitable hits for additional *in vitro* and *in vivo* evaluations, in order to develop new anti-mycobacterial drugs or prodrugs with potential use in the tuberculosis treatment. Further studies in this area are in progress in our laboratory.

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