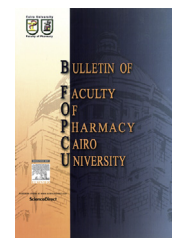




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ORIGINAL ARTICLE

Synthesis and anti-mycobacterial evaluation of some new isonicotinyldiazide analogues



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Abstract The synthesis of some new 3,4-disubstituted thiazolyldieneisonicotinyldiazide derivatives **3a–k**, 2-substituted thiazolidinyldiazide derivatives **4a–d** and pyrrolyldiazide derivatives **5**, **6** and **7** is described. The resulted compounds are evaluated for their *in vitro* antitubercular activity. The minimum inhibitory concentration (MIC) of compound **3g** showed comparable *in vitro* activity to isoniazid against *Mycobacterium tuberculosis* H37Ra 7131 strain in concentration 9.77 µg/mL.

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1. Introduction

Tuberculosis (TB) is a common and often deadly infectious disease caused by various strains of mycobacterium, mainly *Mycobacterium tuberculosis*. Members of *M. tuberculosis* complex are *M. tuberculosis*, *M. africanum*, *M. bovis* and the Bacillus Calmette-Guérin strain, *M. microti*, *M. canettii*, *M. caprae*, *M. pinnipedii*, and *M. mungi*. The species (*M. avium* complex, *M. gordonae*, *M. kansasii*, *M. simiae*, *M. chelonae*, *M. fortuitum*, etc.) other than *M. tuberculosis* complex does not cause tuberculosis in humans, TB usually attacks the lungs but can also affect other parts of the body. It was considered to be a disease of poverty for many years due to its rare occurrence in developed countries.¹ However, recently more people in the developed world are contracting TB because their immune

systems are compromised by immunosuppressive drugs, substances abuse, or AIDS.^{2–5} Several decades ago, effective anti-TB drugs were launched and one could hardly find a TB case demonstrated at medical universities. But the return of TB was declared by the World Health Organization (WHO) as a global emergency compared to a hypothetical third world war with 9 million TB cases and two million deaths reported each year; about one third of the world's population is already infected with *M. tuberculosis*.⁶ Moreover, recent advances in diagnostics, drugs, and vaccines and enhanced implementation of existing interventions have increased the prospects for improved clinical care and global tuberculosis control.⁷

According to the 13th Annual Tuberculosis Report of the WHO published on the world TB day on March 24, 2009, there were 9.27 million new cases of TB estimated worldwide.^{4,8} The terms associated with drug resistance in tuberculosis are very important. The stages of tuberculosis in terms of developing resistance are as follows: drug-sensitive tuberculosis, mono-drug resistant tuberculosis, poly-drug resistant tuberculosis, multi-drug resistant (MDR) tuberculosis, extensively drug resistant (XDR) tuberculosis, and extremely drug resistant (XXDR) tuberculosis. MDR-TB is defined as resistance to

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isoniazid and rifampicin, with or without resistance to other first-line anti-TB drugs. XDR-TB is defined as resistance to at least isoniazid and rifampicin from the first-line anti-tuberculosis drugs (the definition of MDR-TB) in addition to resistance to any fluoroquinolone, and to at least one of the three injectable second-line anti-tuberculosis drugs (kanamycin, capreomycin and amikacin). Multidrug Resistant Tuberculosis (MDR-TB) proved resistant to at least rifampicin and isoniazid, the two most frequently used anti-TB agents.^{5,9,10} Current treatment programmes to combat TB are under threat due to the emergence of multi-drug and extensively-drug resistant TB. As part of our efforts towards the discovery of new anti-tubercular leads, a number of potent tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxamide (THPP) and *N*-benzyl-6',7'-dihydrospiro [piperidine-4,4'-thieno[3,2-*c*]pyran] (Spiro) analogues were recently identified against *M. tuberculosis* and *Mycobacterium bovis* BCG through a high-throughput whole-cell screening campaign.¹¹

TB treatment strategy is tedious, long and has several side effects. The currently applied classical drugs used to treat TB include broad and narrow spectrum agents and different combinations targeting different types of TB. Such drugs are traditionally divided into two lines. First line drugs include fundamental chemotherapeutics of choice, like isoniazid and streptomycin. They are highly effective, but very susceptible to resistant strains and must be administered for 6–9 months. When these treatments fail, second line TB drugs are used. Second line tuberculostatics are the most commonly used. However, these drugs have far lower efficacy and require even longer administration periods up to 18–24.¹²

Isoniazid (Fig. 1) is one of the active and successful agents used to treat TB. It was considered as a starting point in the search for new active derivatives and analogues such as hydrazones which have been reported as active anti-TB drugs (Fig. 2).^{13–16}

Nevertheless, the isoniazid therapy has two major drawbacks. The first one is its hepatotoxic effects. Isoniazid is metabolized in the liver mainly by acetylation and dehydrazination. The *N*-acetylhydrazine metabolite is believed to be responsible for the hepatotoxic effects. The second is its deactivation by the action of *N*-aryl aminoacyltransferases (NATs). These enzymes are found in both mycobacteria as well as mammalian hosts, they deactivate isoniazid by acetylation reaction at N² (terminal amino group) which is a step in the metabolism of isonicotinic acid hydrazide (INH). The literature survey demonstrated the synthesis of isoniazid derivatives such as hydrazones and Schiff's bases, where N² is blocked towards acetylation by NATs.¹⁷ These derivatives are more effective and less hepatotoxic than isoniazid.¹⁸

On the other hand, several heterocyclic compounds were explored in the search for a reliable starting platform for anti-TB drug development such as pyrrole derivatives. The recently synthesized pyrrole LL-3858 (Sudoterb) (Fig. 3)

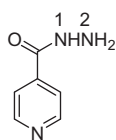


Figure 1 Isoniazid.

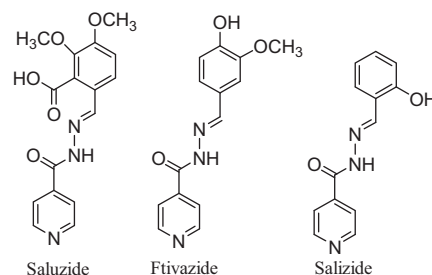


Figure 2 Some hydrazones of isoniazid.

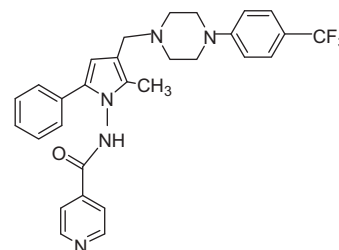


Figure 3 LL-3858 (Sudoterb).

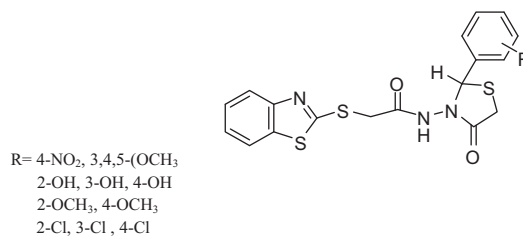


Figure 4 Some literature cited antituberculosis thiazolidine analogue.

showed higher bactericidal activity compared to isoniazid. Sudoterb is active against *M. tuberculosis* strains that are resistant to available anti-TB drugs.^{19,20}

Moreover, some drugs comprising a variety of ring systems exhibited bactericidal activity such as thiazolidine derivatives (Fig. 4).^{21,22}

Guided by these findings, our rationale is to prepare new potent and safe isoniazid derivatives. Our strategy to construct new agents of isoniazid is based on the incorporation of various heterocyclic rings that possess anti-TB activity such as pyrrole and thiazolidine derivatives in the core structure of isoniazid with the aim of increasing their activity through blocking position NH₂ of the hydrazide group of isoniazid.

2. Materials and methods

2.1. Chemistry

Melting points were determined on Griffin apparatus and the values given are uncorrected. IR spectra were determined on Shimadzu IR 435 spectrophotometer (KBr, cm⁻¹). ¹H NMR

spectra were recorded on Varian Gemini 200 and/or 300 MHz spectrophotometer, 75 MHz for ^{13}C using TMS as internal standard. Chemical shift values are recorded in ppm on δ scale, at the Micro analytical centre, Cairo University, Egypt. Mass spectra were recorded on a Hewlett Packard 5988 spectrometer, at the Micro analytical centre, Cairo University, Egypt. Elemental analyses were carried out at the Micro analytical centre, Cairo University, Egypt. Progress of the reactions was monitored using TLC sheets pre-coated with UV fluorescent silica gel Merck 60F 254 using acetone/benzene (1:9) and were visualized using UV lamp. Log *P* was calculated using Chem. Draw Ultra 9.0 program. All chemicals were obtained from Aldrich, Fluka, or Merck chemicals.

2.1.1. *N'*-[3,4-Disubstitutedthiazol-2(3*H*)-ylidene]isonicotinohydrazide (3a–k)

A mixture of the appropriate **2a–e** (0.01 mol) and the selected phenacyl bromide (0.01 mol) in 99% ethanol and chloroform mixture (1:1) (20 mL) was heated under reflux for 3 h. The reaction mixture was concentrated under vacuum to half its volume, cooled and the formed precipitate was filtered, dried and recrystallized from benzene/petroleum ether.

2.1.1.1. *N'*-(4-(4-Bromophenyl)-3-ethylthiazol-2(3*H*)-ylidene)isonicotinohydrazide (3a). Yield 62%; mp 186–187 °C (benzene/petroleum ether); MS (EI) *m/z* (% rel. Int.): 402 (M+, 48.10%), 404 (M+2, 54.30%); IR (KBr) cm^{-1} : 3444 (NH), 2978, 2947 (CH aliphatic), 1666 (C=O); ^1H NMR (DMSO-*d*₆) δ (ppm) (300 MHz): 0.94 (t, 3H, *J* = 7.2 Hz, $-\text{CH}_2-\text{CH}_3$), 3.59 (q, 2H, *J* = 7.2 Hz, $-\text{CH}_2-\text{CH}_3$), 6.80 (s, 1H, =CH of thiazole), 7.25–7.77 (m, 2H, Ar-H), 7.87 (d, 2H, *J* = 6 Hz, pyridine H-3, H-5), 8.08–8.91 (m, 2H, Ar-H), 8.93 (d, 2H, *J* = 6 Hz, pyridine H-2, H-6), 11.70 (s, 1H, NH, D₂O exchangeable); Anal. Calcd for C₁₇H₁₅BrN₄OS, Mwt. (403.3): C, 50.63; H, 3.75; N, 13.89; Found; C, 50.54; H, 3.65; N, 13.80.

2.1.1.2. *N'*-(3-Allyl-4-(4-bromophenyl)thiazol-2(3*H*)-ylidene)isonicotinohydrazide (3b). Yield 57%; mp 156–157 °C (benzene/petroleum ether); IR (KBr) cm^{-1} : 3394 (NH), 2997, 2931 (CH aliphatic), 1647 (C=O); ^1H NMR (DMSO-*d*₆) δ (ppm) (300 MHz): 4.38 (d, 2H, N-CH₂), 4.92–5.15 (m, 2H, $\text{CH}_2=\text{CH}$ of allyl), 5.78–5.80 (m, 1H, $\text{CH}=\text{CH}_2$ of allyl), 6.38 (s, 1H, =CH of thiazole), 7.40–7.43 (m, 2H, Ar-H), 7.67–7.70 (m, 2H, Ar-H), 7.75 (d, 2H, *J* = 6 Hz, pyridine H-3, H-5), 8.72 (d, 2H, *J* = 6 Hz, pyridine H-2, H-6), 11.02 (s, 1H, NH, D₂O exchangeable); Anal. Calcd for C₁₈H₁₅BrN₄OS, Mwt. (415.31): C, 52.06; H, 3.64; N, 13.49; Found; C, 52.11; H, 3.55; N, 13.71.

2.1.1.3. *N'*-(4-(4-Bromophenyl)-3-phenylthiazol-2(3*H*)-ylidene)isonicotinohydrazide (3c). Yield 59%; mp 262–267 °C; (benzene/petroleum ether); MS (EI) *m/z* (%): 450 (M+, 94.68%), 452 (M+2, 98.01%); IR (KBr) cm^{-1} : 3441 (NH), 1693 (C=O); ^1H NMR (DMSO-*d*₆) δ (ppm) (300 MHz): 6.62 (s, 1H, =CH thiazole), 7.13–7.15 (m, 5H, Ar-H), 7.26–7.46 (m, 4H, Ar-H), 7.72 (d, 2H, *J* = 6 Hz, pyridine H-3, H-5), 8.71 (d, 2H, *J* = 6 Hz, pyridine H-2, H-6), 10.91 (s, 1H, NH, D₂O exchangeable); ^{13}C NMR (75 MHz, DMSO-*d*₆ TMS): 163.54 (C=O), 147.54 (C=N), (140.97, 139.16) (C_{2,6} pyridine), 135.05 (C-Br), 134.12 (C), 133.94 (C), 133.73 (C), 131.38 (C),

130.26 (C), 130.13 (C), 128.83 (C), 128.25 (C), 127.91 (C), 127.24 (C), 126.10 (C), 125.53 (C), (123.84, 122.80) (C_{3,5} pyridine), 120.86 (C), 101.91 (=CH thiazole); Anal. Calcd for C₂₁H₁₅BrN₄OS, Mwt. (451.34): C, 55.88; H, 3.35; N, 12.41; Found; C, 55.49; H, 3.31; N, 12.09.

2.1.1.4. *N'*-(4-(4-Bromophenyl)-3-*p*-tolylthiazol-2(3*H*)-ylidene)isonicotinohydrazide (3d). Yield 63%; mp 125–127 °C (benzene/petroleum ether); MS (EI) *m/z* (%): 464 (M+, 57.88%), 466 (M+2, 61.50%); IR (KBr) cm^{-1} : 3420 (NH), 2920 (CH aliphatic), 1689 (C=O); ^1H NMR (DMSO-*d*₆) δ (ppm) (300 MHz): 2.28 (s, 3H, CH₃), 6.70 (s, 1H, =CH thiazole), 7.14–7.23 (m, 4H, Ar-H), 7.46–7.50 (m, 4H, Ar-H), 7.85 (d, 2H, *J* = 6 Hz, pyridine H-3, H-5), 8.79 (d, 2H, *J* = 6 Hz, pyridine H-2, H-6), 11.20 (s, 1H, NH, D₂O exchangeable); Anal. Calcd for C₂₂H₁₇BrN₄OS, Mwt. (465.37): C, 56.78; H, 3.68; N, 12.04; Found; C, 56.92; H, 3.65; N, 12.43.

2.1.1.5. *N'*-(4-(4-Chlorophenyl)-3-ethylthiazol-2(3*H*)-ylidene)isonicotinohydrazide (3e). Yield 58%; mp 210–211 °C (benzene/petroleum ether); MS (EI) *m/z* (%): 358 (M+, 96.69%), 360 (M+2, 37.28%); IR (KBr) cm^{-1} : 3414 (NH), 2978, 2947 (CH aliphatic), 1665 (C=O); ^1H NMR (DMSO-*d*₆) δ (ppm) (200 MHz): 0.96 (t, 3H, *J* = 7.2 Hz, CH₂–2H, Ar-H), 6.74 (s, 1H, =CH thiazole), 7.63 (d, 2H, *J* = 6 Hz, pyridine H-3, H-5), 8.89 (d, 2H, *J* = 6 Hz, pyridine H-2, H-6), 8.97–9.06 (m, 2H, Ar-H), 11.77 (s, 1H, NH, D₂O exchangeable); Anal. Calcd for C₁₇H₁₅ClN₄OS, Mwt. (358.85): C, 56.90; H, 4.21; N, 15.61; Found; C, 56.55; H, 4.54; N, 15.23.

2.1.1.6. *N'*-(3-Allyl-4-(4-chlorophenyl)thiazol-2(3*H*)-ylidene)isonicotinohydrazide (3f). Yield 52%; mp 200–203 °C (benzene/petroleum ether); MS (EI) *m/z* (%): 369 (M–1, 6.47%), 370 (M+, 32.19%), 372 (M+2, 12.67.50%); IR (KBr) cm^{-1} : 3417 (NH), 2924 (CH aliphatic), 1685 (C=O); ^1H NMR (DMSO-*d*₆) δ (ppm) (300 MHz): 4.35 (d, 2H, N-CH₂), 4.85–5.21 (m, 2H, CH=CH₂), 5.40–5.85 (m, 1H, CH=CH₂), 6.68 (s, 1H, =CH thiazole), 7.18–7.57 (m, 2H, Ar-H), 7.82 (d, 2H, *J* = 6 Hz, pyridine H-3, H-5), 8.88 (d, 2H, *J* = 6 Hz, pyridine H-2, H-6), 8.94–8.96 (m, 2H, Ar-H), 11.72 (s, 1H, NH, D₂O exchangeable); ^{13}C NMR (75 MHz, DMSO-*d*₆ TMS): 202.60 (C=O), 151.26 (C=N), (147.63, 146.27) (C_{2,6} pyridine), 140.80 (C), 137.39 (C), 134.07 (C), 132.85 (C-Cl), 131.14 (C), 128.89 (C), (128.46, 128.15) (C_{3,5} pyridine), 126.03 (C) 122.84 (C), 121.38 (C), 116.18 (C), 91.22 (=CH thiazole), 42.73 (CH₂); Anal. Calcd for C₁₈H₁₅ClN₄OS, Mwt. (370.86): C, 58.30; H, 4.08; N, 15.11; Found; C, 58.61; H, 4.06; N, 15.09.

2.1.1.7. *N'*-(4-(4-Chlorophenyl)-3-phenylthiazol-2(3*H*)-ylidene)isonicotinohydrazide (3g). Yield 51%; mp 243–245 °C (benzene/petroleum ether); MS (EI) *m/z* (%): 406 (M+, 100%), 408 (M+2, 35.89%); IR (KBr) cm^{-1} : 3421 (NH), 1670 (C=O); ^1H NMR (DMSO-*d*₆) δ (ppm) (300 MHz): 6.84 (s, 1H, =CH of thiazole), 7.21–7.47 (m, 2H, Ar-H), 7.61 (d, 2H, *J* = 6 Hz, pyridine H-3, H-5), 8.05 (d, 2H, *J* = 6 Hz, pyridine H-2, H-6), 8.85–8.95 (m, 2H, Ar-H), 11.50 (s, 1H, NH, D₂O exchangeable); ^{13}C NMR (75 MHz, DMSO-*d*₆ TMS): 168.77 (C=O), 160.57, 150.13 (C=N), (140.77, 138.25) (C_{2,6} pyridine), 137.16 (C), 131.22 (C-Cl), 130.11 (C), 129.74 (C), 128.97 (C), (128.13, 128.57) (C_{3,5} pyridine), 127.97 (C) 121.84 (C),

121.10(C), 120.86(C), 120.83(C), 120.81(C), 120.73(C), 120.62(C), 99.92 (=CH thiazole). Anal. Calcd for $C_{21}H_{15}ClN_4OS$, Mwt. (406.89): C, 61.99; H, 3.72; N, 13.77; Found; C, 61.71; H, 3.76; N, 13.65.

2.1.1.8. *N'-(4-(4-Chlorophenyl)-3-p-tolylthiazol-2(3H)-ylidene)isonicotinohydrazide (3h)*. Yield 51%; mp 225–227 °C (benzene/petroleum ether); MS (EI) m/z (%): 420 (M+, 50.50%), 422 (M+2, 19.60%), IR (KBr) cm^{-1} : 3421 (NH), 2947, 2920 (CH aliphatic), 1697 (C=O); 1H NMR (DMSO- d_6) δ (ppm) (300 MHz): 2.28 (s, 3H, CH₃), 6.60 (s, 1H, =CH thiazole), 7.12–7.18 (m, 4H, Ar-H), 7.20–7.22 (m, 2H, Ar-H), 7.32–7.35 (m, 2H, Ar-H), 7.72 (d, 2H, $J = 6$ Hz, pyridine H-3, H-5), 8.72 (d, 2H, $J = 6$ Hz, pyridine H-2, H-6), 10.96 (s, 1H, NH, D₂O exchangeable); ^{13}C NMR (75 MHz, DMSO- d_6 , TMS): 186.88 (C=O), 179.91 (C=N), 158.66, (148.35, 146.97) (C_{2,6} pyridine), 142.21(C), 138.94(C), 138.05(C), 133.67(C), 132.24(C), 131.84(C-Cl), 131.25(C), 130.83(C), 129.71(C), 129.33(C), 128.49(C), 127.77(C), 125.61(C), (122.91, 120.77)(C_{3,5} pyridine), 92.73(=CH thiazole), 20.54(CH₃); Anal. Calcd for $C_{22}H_{17}ClN_4OS$, Mwt. (420.91): C, 62.78; H, 4.07; N, 13.31; Found; C, 62.71; H, 4.11; N, 13.54.

2.1.1.9. *N'-(3-Ethyl-4-(3-nitrophenyl)thiazol-2(3H)-ylidene)isonicotinohydrazide (3i)*. Yield 51%; mp 216–217 °C; (benzene/petroleum ether); MS (EI) m/z (%): 369 (M+, 100%) IR (KBr) cm^{-1} : 3421 (NH), 2978, 2908 (CH aliphatic), 1705 (C=O), 1530 (NO₂); 1H NMR (DMSO- d_6) δ (ppm) (300 MHz): 1.15 (t, 3H, $J = 7.2$ Hz, $-CH_2-CH_3$), 3.80 (q, 2H, $J = 7.2$ Hz, $-CH_2-CH_3$), 6.49 (s, 1H, =CH of thiazole), 7.76–7.84, (m, 3H, Ar-H), 7.96 (d, 2H, $J = 6$ Hz, pyridine H-3, H-5), 8.27–8.36 (m, 1H, Ar-H), 8.73 (d, 2H, $J = 6$ Hz, pyridine H-2, H-6), 11.03 (s, 1H, NH, D₂O exchangeable); ^{13}C NMR, (75 MHz, DMSO- d_6 , TMS): 168.10(C=O), 161.10(C=N), (150.40, 147.86)(C_{2,6} pyridine), 140.92(C) 137.92(C), 134.98(C), 131.82(C), 130.50(C), 123.95(C), 123.21(C), 121.20, (120.70,120.60)(C_{3,5} pyridine), 8, 99.72(=C thiazole), 40.52(CH₂), 12.72(CH₂); Anal. Calcd for $C_{17}H_{15}N_5O_3S$, Mwt. (369.04): C, 55.27; H, 4.09; N, 18.96; Found; C, 55.08; H, 4.31; N, 18.76.

2.1.1.10. *N'-(3-Allyl-4-(3-nitrophenyl)thiazol-2(3H)-ylidene)isonicotinohydrazide (3j)*. Yield 60%; mp 230–231 °C (benzene/petroleum ether); MS (EI) m/z (%): 381 (M+, 16.01%); IR (KBr) cm^{-1} : 3421, 3244 (NH), 2981 (CH aliphatic), 1639(C=O), 1531 (NO₂); 1H NMR (DMSO- d_6) δ (ppm) (300 MHz): 4.07 (d, 2H, N-CH₂), 4.93–5.24 (m, 2H, =CH₂), 5.75–5.96 (m, 1H, CH=), 6.65 (s, 1H, =CH thiazole), 7.70–7.89 (m, 3H, Ar-H), 7.94 (d, 2H, $j = 6$ Hz, pyridine H-3, H-5), 7.95–8.11 (m, 1H, Ar-H), 8.80 (d, 2H, $J = 6$ Hz, pyridine H-2, H-6), 12.02 (s, 1H, NH, D₂O exchangeable); Anal. Calcd for $C_{18}H_{15}N_5O_3S$, Mwt. (381.41): C, 56.68; H, 3.96; N, 18.36; Found; C, 56.43; H, 3.95; N, 18.44.

2.1.1.11. *N'-(4-(3-Nitrophenyl)-3-p-tolylthiazol-2(3H)-ylidene)isonicotinohydrazide (3k)*. Yield 60%; mp 100–101 °C (benzene/petroleum ether); MS (EI) m/z (%): 431 (M+, 23.65%), 432 (M+1, 7.38%), 433 (M+2, 3.14%); IR (KBr) cm^{-1} : 3421 (NH), 2920 (CH aliphatic), 1666 (C=O), 1527 (NO₂); 1H NMR (DMSO- d_6) δ (ppm) (300 MHz): 2.28 (s, 3H, CH₃), 6.83 (s, 1H, =CH thiazole), 7.19–7.59 (m, 3H, Ar-H), 7.72 (d, 2H, $J = 6$ Hz, pyridine H-3, H-5), 8.05–8.12

(m, 1H, Ar-H), 8.73 (d, 2H, $J = 6$ Hz, pyridine H-2, H-6), 11.02 (s, 1H, NH, D₂O exchangeable); Anal. Calcd for $C_{22}H_{17}N_5O_3S$, Mwt. (431.47): C, 61.24; H, 3.97; N, 16.23; Found; C, 61.31; H, 3.88; N, 16.09.

2.1.2. *N-[2-(Substituted imino)-4,5-dioxo[1,3]thiazolidin-3-yl]isonicotinamide (4a-d)*

A mixture of the appropriate **2a**, **b**, **d** and **e** (0.01 mol) anhydrous potassium carbonate (2.8 g, 0.02 mol) and oxalyl chloride (1.9 g, 0.015 mol) in dry benzene (20 mL) was heated under reflux with stirring for 7 h. The formed precipitate was filtered while hot, washed twice with water (10 mL), dried and recrystallized from chloroform/petroleum ether.

2.1.2.1. *N-(2-(Ethylimino)-4,5-dioxothiazolidin-3-yl)isonicotinamide (4a)*. Yield 60%; mp 150–152 °C (chloroform/petroleum ether); MS (EI) m/z (%): 278 (M+, 5.20%) IR (KBr) cm^{-1} : 3402 (NH), 2931 (CH aliphatic), 1789, 1765, 1708 (3C=O); 1H NMR (DMSO- d_6) δ (ppm) (300 MHz): 1.05 (t, 3H, $J = 7.2$ Hz, $-CH_2-CH_3$), 3.48 (q, 2H, $J = 7.2$ Hz, $-CH_2-CH_3$), 8.08 (d, 2H, $J = 6$ Hz, pyridine H-3, H-5), 8.91 (d, 2H, $J = 6$ Hz, pyridine H-2, H-6), 12.30 (s, 1H, NH, D₂O exchangeable); ^{13}C NMR (75 MHz, DMSO- d_6 , TMS): 178.91(C=O), 165.15(C=O), 163.67(C=O), 153.94(C=N), (149.29, 147.45)(C_{2,6} pyridine), 145.23(C), (124.22, 122.21)(C_{3,5} pyridine), 37.49(CH₂), 14.33(CH₃); Anal. Calcd for $C_{11}H_{10}N_4O_3S$, Mwt. (278.29): C, 47.48; H, 3.62; N, 20.13; Found; C, 47.59; H, 3.41; N, 20.44.

2.1.2.2. *N-(2-(Allylimino)-4,5-dioxothiazolidin-3-yl)isonicotinamide (4b)*. Yield 60%; mp 203–205 °C; (chloroform/petroleum ether); MS (EI) m/z (%): 293 (M+3H, 0.92%); IR (KBr) cm^{-1} : 3263 (NH), 2924 (CH aliphatic), 1720, 1705, 1678 (3C=O); 1H NMR (DMSO- d_6) δ (ppm) (300 MHz): 4.10 (d, 2H, N-CH₂), 5.03–5.16 (m, 2H, =CH₂), 5.78–5.89 (m, 1H, CH=), 7.81 (d, 2H, $J = 6$ Hz, pyridine H-3, H-5), 8.77 (d, 2H, $J = 6$ Hz, pyridine H-2, H-6), 10.64 (s, 1H, NH, D₂O exchangeable); ^{13}C NMR (75 MHz, DMSO- d_6 , TMS): 182.00(C=O), 166.49(C=O), 165.02(C=O), (150.67, 150.24)(C_{2,6} pyridine), 139.98(C=N), 138.54(C), 134.94(C), (123.14, 122.07)(C_{2,5} pyridine), 115.67(C), 46.67(CH₂); Anal. Calcd for $C_{12}H_{10}N_4O_3S$, Mwt. (290.3): C, 49.65; H, 3.47; N, 19.30; Found; C, 49.66; H, 3.61; N, 19.05.

2.1.2.3. *N-(4,5-Dioxo-2-(phenylimino)thiazolidin-3-yl)isonicotinamide (4c)*. Yield 60%; mp 160–162 °C; (chloroform/petroleum ether); MS (EI) m/z (%): 341 (M+H, 41.50%); IR (KBr) cm^{-1} : 3421 (NH), 2912 (CH aliphatic), 1789, 1724, 1693 (3C=O); 1H NMR (DMSO- d_6) δ (ppm) (300 MHz): 2.33 (s, 3H, CH₃), 7.03–7.46 (m, 4H, Ar-H), 7.88 (d, 2H, $J = 6$ Hz, pyridine H-3, H-5), 8.85 (d, 2H, $J = 6$ Hz, pyridine H-2, H-6), 12.25 (s, 1H, NH, D₂O exchangeable); ^{13}C NMR (75 MHz, DMSO- d_6 , TMS): 184.00(C=O), 174.10(C=O), 165.62(C=O), 153.80(C=N), (150.00, 148.85)(C_{2,6} pyridine), 139.80(C), 138.97(C), 131.85(C), 128.98(C), 128.22, 125.19, 123.52, (121.85, 121.01)(C_{3,5} pyridine), 20.67(CH₃) Anal. Calcd for $C_{16}H_{12}N_4O_3S$, Mwt. (340.36): C, 56.46; H, 3.55; N, 16.46; Found; C, 56.81; H, 3.90; N, 16.54.

2.1.2.4. *N-(4,5-Dioxo-2-(p-tolylimino)thiazolidin-3-yl)isonicotinamide (4d)*. Yield 63%; mp 190–192 °C; (chloroform/petro-

leum ether); IR (KBr) cm^{-1} : 3417 (NH), 2912 (CH aliphatic), 1793, 1716, 1697 (3C=O); ^1H NMR (DMSO- d_6) δ (ppm) (300 MHz): 2.35 (s, 3H, CH₃), 6.86–7.39 (m, 4H, Ar-H), 7.98 (d, 2H, $J = 6$ Hz, pyridine H-3, H-5), 8.90 (d, 2H, $J = 6$ Hz, pyridine H-2, H-6), 12.40 (s, 1H, NH, D₂O exchangeable); ^{13}C NMR (75 MHz, DMSO- d_6 , TMS): 179.21(C=O), 163.78(C=O), 153.93(C=O), 152.44(C=N), (149.91, 148.97)(C_{2,6} pyridine), 139.76(C) 139.39(C), 138.42(C), 129.72(C), 129.43(C), 127.95(C), 123.47(C), (121.96, 120.77)(C_{3,5} pyridine) 20.76(CH₃); Anal. Calcd for C₁₆H₁₂N₄O₃S Mwt. (340.36): C, 56.46; H, 3.55; N, 16.46; Found; C, 56.82; H, 3.81; N, 16.79.

2.1.3. General procedure of compounds 5, 6 and 7

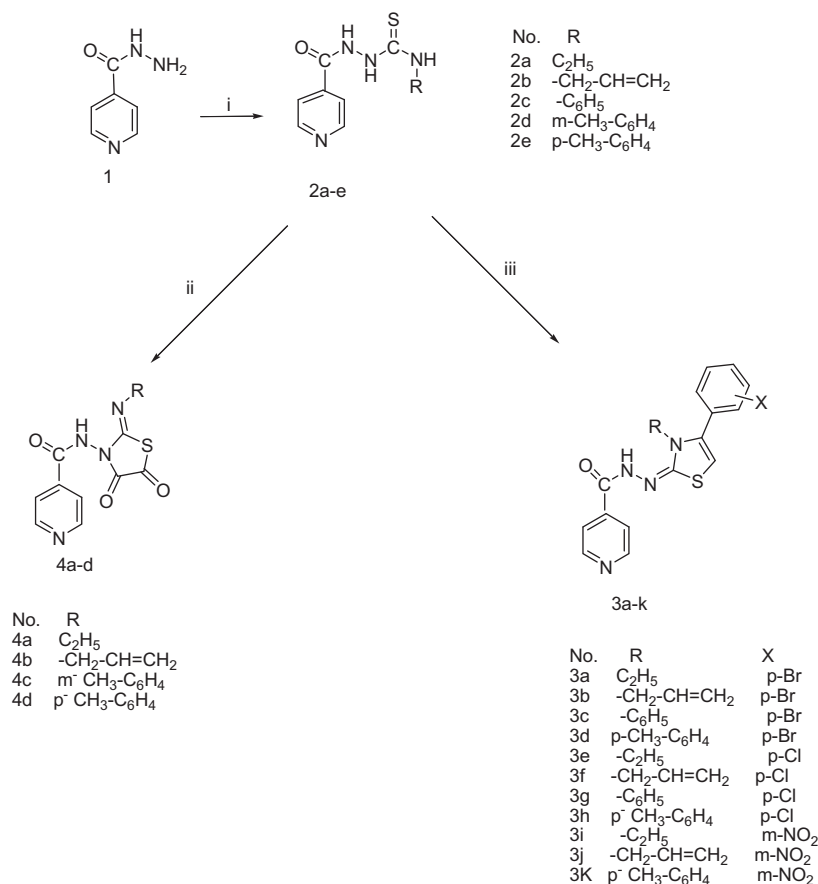
A mixture of **1** (1.37, 0.01 mol), 2-acetyl- γ -butyrolactone or the selected anhydride (0.01 mol) and glacial acetic acid (10 mL) with few drops of acetic anhydride was heated under reflux for 13 h. The reaction mixture was cooled and poured onto ice-cold water (20 mL). The formed precipitate was filtered, washed twice with water (20 mL), dried and recrystallized from methanol.

2.1.3.1. *N*-(3-Acetyl-2-oxopyrrolidin-1-yl)isonicotinamide (**5**). Yield 43%; mp 150–152 °C; (methanol), MS (EI) m/z (%): 247 (M⁺, 0.09%); IR (KBr) cm^{-1} : 3429 (NH), 2924, 2854

Table 1 MIC of tested compounds **3a–c**, **3f–k**, **4a–d**, **5**, **6** and **7**.

Compound	MIC (mg/mL)	Log P
3a	39	4.27
3b	19.5	4.63
3c	78	5.76
3f	39	4.36
3g	9.77	5.49
3h	19.5	5.49
3i	78	2.76
3j	78	2.97
3k	39	3.60
4a	19.5	0.76
4b	19.5	0.42
4c	19.5	2.57
4d	78	2.57
5	78	−1.05
6	19.5	−0.73
7	39	0.74
Isoniazid	1.22	−0.60

(CH aliphatic), 1735, 1705, 1658 (3C=O); ^1H NMR (DMSO- d_6) δ (ppm) (300 MHz): 1.98 (s, 3H, CH₃), 2.05–2.07

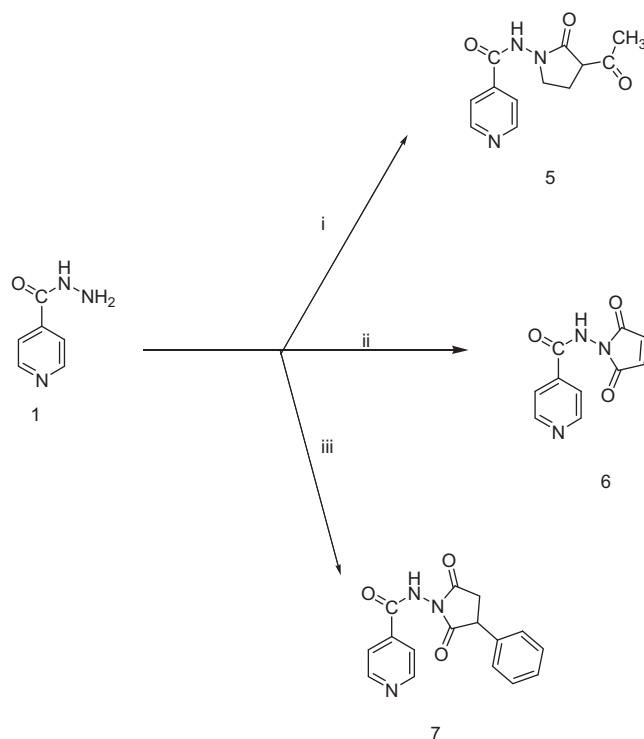


Reagents and conditions: i= RCNS, ethanol, reflux 4h.

ii= (COCl)₂, dry benzene, reflux 3h.

iii= substituted phenacylbromide, ethanol/methanol, reflux 7h.

Scheme 1 Synthesis of target compounds **3a–k** and **4a–d**.



Reagents and condition

i= 2-acetyl-3-butyrrolactone, gl.acetic acid/acetic anhydride, reflux 13h.

ii= maleic anhydride, gl.acetic acid/acetic anhydride, reflux 13h.

iii= phenyl succinic anhydride, gl.acetic acid/acetic anhydride, reflux 13h.

Scheme 2 Synthesis of target compounds **5**, **6** and **7**.

(m, 2H, $\text{CH}_2\text{—CH}$), 2.49 (t, 1H, $\text{CH}_2\text{—CH}$), 3.97 (t, 2H, $\text{CH}_2\text{—N}$), 7.80 (d, 2H, $J = 6$ Hz, pyridine H-3, H-5), 8.78 (d, 2H, $J = 6$ Hz, pyridine H-2, H-6), 11.90 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $\text{C}_{12}\text{H}_{13}\text{N}_3\text{O}_3$, Mwt. (247.25): C, 58.29; H, 5.30; N, 16.99; Found; C, 58.33; H, 5.60; N, 16.92.

2.1.3.2. *N*-(2,5-Dioxo-2H-pyrrol-1(5H)-yl)isonicotinamide (**6**). Yield 50%; mp 205–207 °C; (methanol). IR (KBr) cm^{-1} : 3429 (NH), 3097 (CH aromatic), 1730, 1689, 1651 ($3\text{C}=\text{O}$); ^1H NMR ($\text{DMSO-}d_6$) δ (ppm) (300 MHz): 6.63 (s, 2H, $\text{CH}=\text{CH}$), 7.79 (d, 2H, $J = 6$ Hz, pyridine H-3, H-5), 8.79 (d, 2H, $J = 6$ Hz, pyridine H-2, H-6), 10.94 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $\text{C}_{10}\text{H}_7\text{N}_3\text{O}_3$, Mwt. (217.18): C, 55.30; H, 3.25; N, 19.35; Found; C, 55.66; H, 3.15; N, 19.41.

2.1.3.3. *N*-(2,5-Dioxo-3-phenylpyrrolidin-1-yl)isonicotinamide (**7**). Yield 59%; mp 230–231 °C; (methanol). MS (EI) m/z (%): 295 (M^+ , 4.93%) IR (KBr) cm^{-1} : 3429 (NH), 2974, 2858 (CH aliphatic), 1732, 1681, 1662 ($3\text{C}=\text{O}$); ^1H NMR ($\text{DMSO-}d_6$) δ (ppm) (300 MHz): 2.87, 3.31 (2d, 2H, CH_2), 3.48–3.51 (2d, 1H, CH-ph), 7.30–7.36 (m, 5H, Ar-H), 7.81 (d, 2H, $J = 6$ Hz, pyridine H-3, H-5), 8.80 (d, 2H, $J = 6$ Hz, pyridine H-2, H-6), 11.60 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, $\text{DMSO-}d_6$, TMS): 74.77($\text{C}=\text{O}$), 173.00($\text{C}=\text{O}$), 164.47($\text{C}=\text{O}$), (150.53, 150.38), ($\text{C}_{2,6}$ pyridine),

139.35(C), 128.86(C), 127.75(C), 127.64(C), 122.76(C), 121.38(C), (120.90, 120.83), ($\text{C}_{3,5}$ pyridine), 120.77(C), 43.80(CH), 35.59(CH_2); Anal. Calcd for $\text{C}_{16}\text{H}_{13}\text{N}_3\text{O}_3$, Mwt. (295.29): C, 65.08; H, 4.44; N, 14.23; Found; C, 65.06; H, 4.30; N, 14.01.

2.2. Antituberculous activity

The minimum inhibitory concentration (MIC) was recorded as the highest dilution of a compound inhibiting bacterial growth. MIC of the selected compounds against *M. tuberculosis* H37Ra 7131 strain was evaluated in liquid medium by the broth dilution method according to the modified method of Heifets.^{28,23}

2.2.1. Preparation of stock solutions of the selected compounds

Stock solutions of the compounds were prepared by dissolving 104 mg of each compound in dimethylsulphoxide (1 mL). Further dilutions were performed in Middlebrook broth 7H9 medium.

2.2.2. Preparation of inoculum

Inoculum was prepared by scrapping colonies of 21 days-old culture of the bacilli from the surface of solid drug-free Middlebrook agar 7H10 medium. The bacterial mass was transferred

to a sterile 16 × 25 mm, screw capped tube containing glass beads and 4 mL of Middle brook 7H9 medium. The mixture was homogenized for 2 min. and allowed to stand undisturbed for 30 min. Supernatant suspension was withdrawn and adjusted to the turbidity equivalent to 0.5 McFarland standard using Middle brook 7H9 medium.

2.2.3. Broth dilution

In sterile screw capped tubes, two fold serial dilutions of each compound were prepared in Middle brook broth 7H9 medium with a final volume of 1 mL. Each tube was inoculated with 0.05 mL of prepared bacterial suspension. A positive growth control was prepared by inoculating 0.05 mL of bacterial suspension into a tube containing Middle brook 7H9 broth with no drugs. A positive control for antimycobacterial activity was included using a solution of isoniazid prepared at the same concentration and inoculated the same way as the compounds under test. Tubes were incubated for three weeks at 37 °C in a moisturized incubator and were examined for bacterial growth after 21 days. Results were the mean of two independent experiments and the data are represented in Table 1.

3. Results and discussion

3.1. Chemistry

The thiosemicarbazide derivatives **2a–e** which are considered as the key intermediates in Schemes 1 and 2 were prepared according to reported procedures.^{23–25} The synthesis of compounds **3a–k** was achieved through the efficient synthetic route outlined in Scheme 1. Cyclocondensation reaction of the thiosemicarbazides **2a–e** with the appropriate phenacyl bromide afforded the corresponding thiazolidine **3a–k**.^{25,26} Cyclization of **2a–e** using oxalyl chloride yielded the thiazolidinedione derivatives **4a–d**.^{6,26,27} (Scheme 1). Scheme 2 involves the synthesis of the pyrrolyl derivatives **5**, **6** and **7**.^{27,28} Reaction of isoniazid with either a number of acid anhydrides such as maleic acid anhydride, phenyl succinic anhydride or 2-acetyl- γ -butyrolactone seemed to be a convenient route to fulfill this aim. Structures of the synthesized compounds were established on the basis of elemental analysis and spectral data (IR, ¹³C NMR, ¹H NMR and Mass spectra).

In general, IR spectra of compounds **3a,b,d–f,h–k** showed the appearance of absorption band at 2978–2920 cm⁻¹ indicating aliphatic substituents whereas compounds **4a–d** revealed the presence of carbonyl groups of thiazolidinedione ring ranging from 1793 to 1705 cm⁻¹. Furthermore, IR spectra of pyrrolidinones **5**, **6** and **7** demonstrated extra carbonyl band ranging from 1730 to 1705 cm⁻¹. On the other hand, ¹H NMR of compounds **3a–k** showed a singlet signal of the thiazole ring at 6.31–6.87 ppm.

3.2. Antimycobacterial activity

3.2.1. Preliminary in vitro antitubercular screening

MIC of the selected compounds **3a–c**, **3f–k**, **4a–d**, **5**, **6** and **7** were subjected to antimycobacterial evaluation against *M. tuberculosis* H37Ra 7131 strain in liquid medium by the broth dilution method according to modified method of Heifets.^{28,23}

4. Conclusion

The objective of the present study was to construct new agents of isoniazid based on the incorporation of various heterocyclic rings that possess anti-TB activity such as pyrrole and thiazolidine derivatives in the core structure of isoniazid with the aim of increasing their activity.

Results showed that compound **3g**, a thiazolidine derivative, has comparable activity to that of isoniazid while compounds **3b**, **3h**, **4a**, **4b**, **4c**, **3a** and **3f** exhibited moderate anti-TB activity. In addition, compounds **3c**, **3i**, **3j** and **4d** demonstrated poor anti-TB activity. On the other hand, the pyrrolyl derivative **6** was found to be more active than compounds **5** and **7** yet it is less active than the thiazolidine derivatives. Log *P* are calculated for the tested compounds using Chem. Draw Ultra 9.0 program. This revealed that compounds with high Log *P* value have low MIC. This indicates that increasing the lipophilic moieties in the structure leads to an increase in Log *P* values. Other tested compounds showed lower activity with higher MIC ranging from 19.5 to 78 μ g/mL. The mechanism of action of newly synthesized nicotinamide adenine dinucleotide compounds may resemble that of isoniazid which is the peroxidative activation of isoniazid by the mycobacterial enzyme (*KatG*) *M. tuberculosis* catalase–peroxidase enzyme that generates reactive species forming adducts with (NAD⁺) the oxidized form of the *NAD* coenzyme nicotinamide adenine dinucleotide and (NADP⁺) *NAD* is also converted into nicotinamide adenine dinucleotide phosphate (*NADP*); which are potent inhibitors of lipid and nucleic acid biosynthetic enzymes.²⁹

5. Conflict of interest

The authors declare no conflict of interest.

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