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Design, Synthesis and Anti-Tubercular Activity of Isoniazid Derivatives

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ABSTRACT

The aim of this study was to develop novel isoniazid derivatives, which can be utilized as anti-tubercular agents. The compounds were synthesized by reaction between the hydrazine unit of isoniazid with an appropriate lipophilic moiety. The compounds obtained were characterized on the basis of their melting points, R_f value, Fourier Transform Infra Red (FTIR) Spectra, ^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR) Spectra, ^{13}C- Nuclear Magnetic Resonance (^{13}C-NMR) Spectra, Elemetal Analysis, and the Mass spectra. The novel compounds were assessed for their anti-tubercular activity by reported method against four strains of the *Mycobacterium*. The minimum inhibitory concentration of the active compounds was also determined. The anti-tubercular activity data revealed that most of the compounds have higher MIC values as compared to the standard drug isoniazid. Two compounds, namely **3b** and **3c**, exhibited very good anti-tubercular activity against all four strains of the *Mycobacterium*, which was comparable to the standard drug isoniazid. It has been concluded that the incorporation of a lipophilic chain at the position-2 of the pyridazine ring along with a halogen group, preferably a fluorine atom, at the p-position of the phenyl ring in this series of compounds increase the anti-tubercular activity of the compounds. However, this assumption cannot be generalized because in the present study the anti-tubercular activity was assessed against four strains of *Mycobacterium*. Accordingly, further studies are recommended to assess the anti-tubercular activity of these two compounds, **3b** and **3c**, against other strains of the *Mycobacterium*.

Keywords: Design, Synthesis, *Mycobacterium tuberculosis,* Anti-tubercular Activity, Isoniazid Derivatives.

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INTRODUCTION

Tuberculosis, caused by *Mycobacterium tuberculosis*, has become a worldwide pandemic disease [1]. According to the global tuberculosis report of 2017 of the World Health organization, tuberculosis is the 9th leading cause of mortality in the world and about 10.4 million people were infected with tuberculosis in the year 2016 [2]. The emergence of resistance towards the anti-tubercular drugs has increased the challenges to eradicate tuberculosis worldwide [3,4]. In the last 60 years, only a few drugs have been developed against tuberculosis. Recent approaches to develop better anti-TB drugs include the synthesis of new analogues or modification of the existing molecules with an established activity for shortening and improving tuberculosis treatment. Isoniazid has been widely used since last 60 years as the first line anti-tubercular drug. However, the bacterial resistance to isoniazid therapy has also increased due to its long term use, and abuse. Isoniazid is metabolized by the process of acetylation by the enzyme N-acetyltransferase, which is the major cause of resistance to isoniazid therapy in long term use [5]. It has been suggested that the chemical modification of the hydrazine unit of isoniazid with an appropriate chemical moiety can protect it against the action of the enzyme N-acetyltransferase and improve the clinical curative effects of this drug [6]. It has also been documented that the addition of a lipophilic group into the INH structure may also enhance its permeation into the bacterial cell, which is evident from the existing literature on isoniazide derivatives [7-19]. Accordingly, the aim of the present study was to protect the hydrazine unit of isoniazid from the enzyme N-acetyltransferase by incorporating appropriate lipophilic moiety that can lead to the development of new anti-tubercular candidate with improved anti-tubercular activity, improved metabolic stability against the enzyme N-acetyltransferase, and decreased toxicity.

EXPERIMENTAL

General

Open capillary tube method was used to measure the melting points. Nicolet, 5PC FT-IR spectrometer (Browser Morner, USA) was used to record FTIR spectra (KBr, cm^{-1}). Bruker DRX-300 FT NMR (Bruker, Germany) spectrophotometer was used to record the ^{1}H-NMR (DMSO-d_6, 400 MHz, δ ppm) and ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm) spectra using Tetramethylsilane as internal reference (chemical shift in δ ppm). Jeol-JMS-D-300 mass spectrometer (70 eV, Jeol, Japan) was used to record Mass spectra. Elemental analysis (C, H, and N) was obtained within ± 0.4% of the theoretical values. The purity of the compounds was assessed on silica gel G plates by using iodine vapors (visualizing agent). A mixture of Benzene: Acetone (8:2) was used to determine the R_f value of the compounds.

Synthesis of the compounds 1a-1e

These compounds were obtained according to the literature method [20-22].

Synthesis of the compounds 2a-2j

Equimolar quantities of the appropriate compound of the formula **1a-1e** and propyl bromide or pentyl bromide were stirred at room temperature for about 20 hours in acetone in the presence of K_2CO_3. After reaction completion, the solution was poured in cold water and filtered. The solid residue obtained was washed repeatedly with water. The residue was dried and then recrystallized from ethanol.

Synthesis of the compounds 3a-3j

Equimolar quantities of the appropriate compound of the formula **2a-2j** and isoniazid were refluxed for about 10 hours in acetic acid. After reaction completion, the solution was poured in cold water and filtered. The solid obtained was washed repeatedly with water. The residue was dried and then recrystallized from ethanol.

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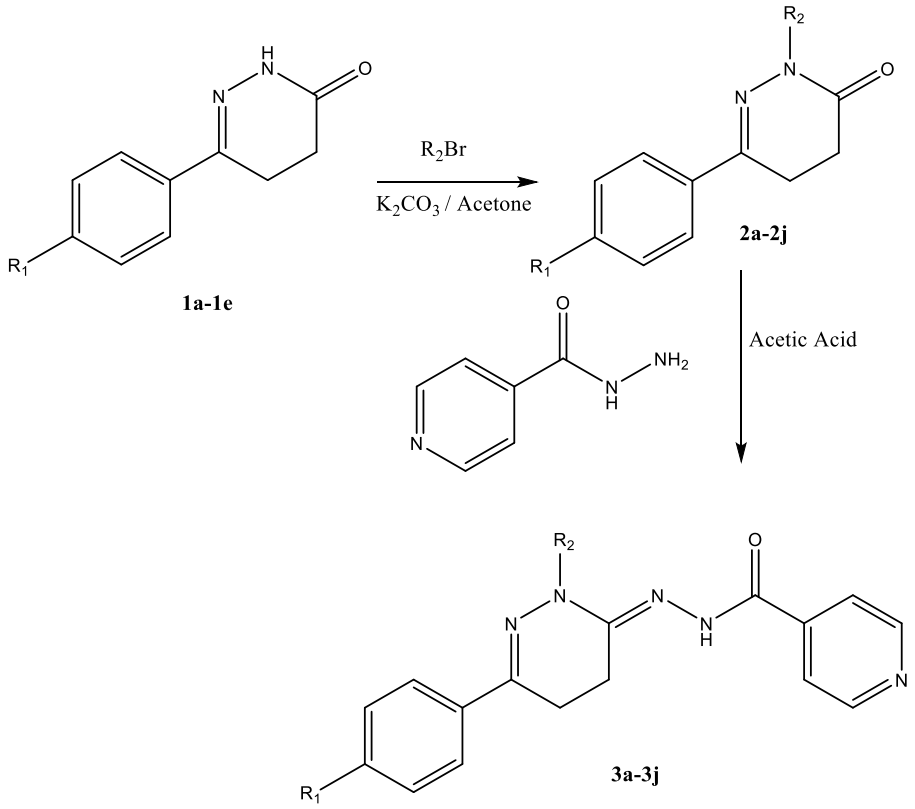
The following reaction scheme depicts the preparation of the isoniazid derivatives.



Reaction Scheme: Preparation of isoniazid derivatives

Anti-tubercular activity

Anti-tubercular activity was done by the reported methods [23,24]. The investigated strains were *Mycobacterium intercellulari* (ATCC 35734), *Mycobacterium cheleneoi* (ATCC 35751), *Mycobacterium xenopi* (ATCC 14470) and *Mycobacterium smegmatis* (ATCC 35797). In brief, the new isoniazid derivatives (**3a-3j**) and isoniazid were solubilized in dimethylsulfoxide to obtain 1 mg/mL concentration. The aliquot of each solution was diluted with 10% agar to get a concentration of about 100 µg/mL. The mixture of the agar medium and the tested compounds was poured into the petri dishes and were allowed to harden. The inoculum was obtained by growing overnight the culture in the Mueller-Hinton broth, which were diluted to about 1:100. The tested microorganisms were streaked and the petri plates were incubated at about 35°C for about 48 hours to assess the growth of the strains in the single concentration. The active derivatives were again diluted and were further tested in the smilar manner, to find their minimum inhibitory concentration (MIC). Blank experiments were also performed.

RESULTS AND DISCUSSION

The starting material (**1a-1e**) were prepared according to the literature methods [20-22]. These compounds were reacted with appropriate alkyl bromide to obtain the compounds of formula (**2a-2j**). The compounds of the formula (**2a-2j**) were reacted with isoniazid to obtain the final compounds (**3a-3j**), which were characterized on the basis of their physical data and the spectral data. The characterization data of the representative compounds are provided below.

6-phenyl-2-propyl-4,5-dihydropyridazin-3(2H)-one (2a)

% Yield: 60; M.P. (°C): 156; R_f Value: 0.75; IR: 1710 (C=O), 1590 (C=N); ^{1}H-NMR: 1.01 (t, 3H, -CH_3), 1.61 (m, 2H, -CH_2-), 2.44 (t, 2H, C-4 of pyridazine), 2.94 (t, 2H, C-5 of pyridazine), 3.19 (t, 2H, -CH_2-N), 7.40-7.69 (m, 5H, Ar-H); ^{13}C-NMR: 12.3, 21.5, 25.5, 33.8, 44.2, 129.3 (2C), 129.9 (2C), 132, 137.5, 147.6, 163.2; Mass (m/z): 216 (M^+); Elemental Analysis (Calculate (Found) for $C_{13}H_{16}N_2O$): C, 72.19 (72.15); H, 7.46 (7.45); N, 12.95 (12.91).

2-pentyl-6-phenyl-4,5-dihydropyridazin-3(2H)-one (2b)

% Yield: 65; M.P. (°C): 166; R_f Value: 0.82; IR: 1705 (C=O), 1595 (C=N); ^{1}H-NMR: 1.03 (t, 3H, -CH_3), 1.29-1.32 (m, 4H, 2-CH_2-), 1.55 (m, 2H, -CH_2-), 2.45 (t, 2H, C-4 of pyridazine), 2.93 (t, 2H, C-5 of pyridazine), 3.19 (t, 2H, -CH_2-N), 7.42-7.67 (m, 5H, Ar-H); ^{13}C-NMR: 15.2, 23.5, 25.5, 26.2, 27, 33.9, 42, 129.3 (2C), 129.9 (2C), 132, 137.5, 147.6, 163.2; Mass (m/z): 244 (M^+); Elemental Analysis (Calculate (Found) for $C_{15}H_{20}N_2O$): C, 73.74 (73.72); H, 8.25 (8.23); N, 11.47 (11.45).

6-(4-Fluorophenyl)-2-pentyl-4,5-dihydropyridazin-3(2H)-one (2c)

% Yield: 60; M.P. (°C): 178; R_f Value: 0.88; IR: 1705 (C=O), 1590 (C=N); ^{1}H-NMR: 1.02 (t, 3H, -CH_3), 1.28-1.29 (m, 4H, 2-CH_2-), 1.55 (m, 2H, -CH_2-), 2.46 (t, 2H, C-4 of pyridazine), 2.96 (t, 2H, C-5 of pyridazine), 3.2 (t, 2H, -CH_2-N), 7.45-7.65 (m, 4H, Ar-H); ^{13}C-NMR: 15.2, 23.5, 25.5, 26.2, 30, 33.9, 42, 116.7 (2C), 130.6 (2C), 133.1, 147.6, 163.2, 166.3; Mass (m/z): 262 (M^+); Elemental Analysis (Calculate (Found) for $C_{15}H_{19}FN_2O$): C, 68.68 (68.65); H, 7.30 (7.28); N, 10.68 (10.65).

6-(4-chlorophenyl)-2-pentyl-4,5-dihydropyridazin-3(2H)-one (2d)

% Yield: 55; M.P. (°C): 169; R_f Value: 0.84; IR: 1710 (C=O), 1590 (C=N); ^{1}H-NMR: 1.02 (t, 3H, -CH_3), 1.28-1.30 (m, 4H, 2-CH_2-), 1.54 (m, 2H, -CH_2-), 2.45 (t, 2H, C-4 of pyridazine), 2.96 (t, 2H, C-5 of pyridazine), 3.19 (t, 2H, -CH_2-N), 7.62-7.84 (m, 4H, Ar-H); ^{13}C-NMR: 15.2, 23.5 (2C), 26.2, 30, 33.9, 42, 129.3 (2C), 130 (2C), 135.5, 137.7, 147.5, 163.2; Mass (m/z): 278 (M^+); Elemental Analysis (Calculate (Found) for $C_{15}H_{19}ClN_2O$): C, 64.63 (64.62); H, 6.87 (6.85); N, 10.05 (10.01).

N'-(6-phenyl-2-propyl-4,5-dihydropyridazin-3(2H)-ylidene)isonicotinohydrazide (3a)

% Yield: 45; M.P. (°C): 177; R_f Value: 0.77; IR: 3340 (N-H), 1690 (C=O), 1580 (C=N); ^{1}H-NMR: 1.12 (t, 3H, -CH_3), 1.59 (m, 2H, -$\underline{CH_2}$-CH_3), 2.58 (t, 2H, C-4 of pyridazine), 2.96 (t, 2H, C-5 of pyridazine), 3.34 (t, 2H, -CH_2-N), 7.42-7.80 (m, 7H, Ar-H), 8.79 (d, 2H, C2 & C6 H of pyridine), 10.55 (s, 1H, NH); ^{13}C-NMR: 12.3, 16.2, 22.1, 27, 44.5, 122.8 (2C), 129.3 (2C), 129.9 (2C), 132.1, 137.5, 141.9, 147.6, 148.2, 149.9 (2C), 163.5; Mass (m/z): 335 (M^+, 100.0%); Elemental Analysis (Calculate (Found) for $C_{19}H_{21}N_5O$): C, 68.04 (68); H, 6.31 (6.28); N, 20.88 (20.86).

N'-(2-pentyl-6-phenyl-4,5-dihydropyridazin-3(2H)-ylidene)isonicotinohydrazide (3b)

% Yield: 55; M.P. (°C): 165; R_f Value: 0.71; IR: 3340 (N-H), 1685 (C=O), 1585 (C=N); ^{1}H-NMR: 1.04 (t, 3H, -CH_3), 1.27-1.29 (m, 4H, 2-CH_2-), 1.57 (m, 2H, -CH_2-), 2.57 (t, 2H, C-4 of pyridazine), 2.93 (t, 2H, C-5 of pyridazine), 3.34 (t, 2H, -CH_2-N), 7.39-7.81 (m, 7H, Ar-H), 8.77 (d, 2H, C2 & C6 H of pyridine), 10.56 (s, 1H, NH); ^{13}C-NMR: 15.1, 16.1, 23.4, 26.8, 27, 29.9, 42.3, 122.8 (2C), 129.3 (2C), 129.9 (2C), 132, 137.4, 141.8, 147.5, 148.2, 149.9 (2C), 163.5; Mass (m/z): 363 (M^+, 100.0%); Elemental Analysis (Calculate (Found) for $C_{21}H_{25}N_5O$): C, 69.40 (69.35); H, 6.93 (6.90); N, 19.27 (19.25).

N'-(6-(4-fluorophenyl)-2-pentyl-4,5-dihydropyridazin-3(2H)-ylidene)isonicotinohydrazide (3c)

% Yield: 60; M.P. (°C): 189; R_f Value: 0.76; IR: 3345 (N-H), 1690 (C=O), 1590 (C=N); ^{1}H-NMR: 1.04 (t, 3H, -CH_3), 1.27-1.29 (m, 4H, 2-CH_2-), 1.57 (m, 2H, -CH_2-), 2.57 (t, 2H, C-4 of pyridazine), 2.93 (t, 2H, C-5 of pyridazine), 3.34 (t, 2H, -CH_2-N), 7.36-7.82 (m, 6H, Ar-H), 8.78 (d, 2H, C2 & C6 H of pyridine), 10.56 (s, 1H, NH); ^{13}C-NMR: 15.1, 16.1, 23.4, 26.8, 27, 30, 42.3, 116.7 (2C), 122.8 (2C), 130.5 (2C), 133.2, 141.8, 147.5, 148.2, 149.5 (2C), 163.5, 165.1; Mass (m/z): 381 (M^+, 100.0%); Elemental Analysis (Calculate (Found) for $C_{21}H_{24}FN_5O$): C, 66.12 (66.10); H, 6.34 (6.30); N, 18.36 (18.35).

N'-(6-(4-chlorophenyl)-2-pentyl-4,5-dihydropyridazin-3(2H)-ylidene)isonicotinohydrazide (3d)

% Yield: 50; M.P. (°C): 210; R_f Value: 0.83; IR: 3335 (N-H), 1685 (C=O), 1575 (C=N); ^{1}H-NMR: 1.05 (t, 3H, -CH_3), 1.28-1.29 (m, 4H, 2-CH_2-), 1.58 (m, 2H, -CH_2-), 2.58 (t, 2H, C-4 of pyridazine), 2.92 (t, 2H, C-5 of pyridazine), 3.35 (t, 2H, -CH_2-N), 7.62-7.82 (m, 6H, Ar-H), 8.78 (d, 2H, C2 & C6 H of pyridine), 10.57 (s, 1H, NH); ^{13}C-NMR: 15.1, 16.1, 23.4, 26.8, 27, 30, 42.3, 122.8 (2C), 129.2 (2C), 129.9 (2C), 135.5, 137.6, 141.9, 147.7, 148.1, 149.5 (2C), 163.4; Mass (m/z): 397 (M^+, 100.0%); Elemental Analysis (Calculate (Found) for $C_{21}H_{24}ClN_5O$): C, 63.39 (63.38); H, 6.08 (6.05); N, 17.60 (17.55).

N'-(6-(4-chlorophenyl)-2-propyl-4,5-dihydropyridazin-3(2H)-ylidene)isonicotinohydrazide (3e)

% Yield: 55; M.P. (°C): 201; R_f Value: 0.64; IR: 3350 (N-H), 1695 (C=O), 1585 (C=N); ^{1}H-NMR: 1.05 (t, 3H, -CH_3), 1.55 (m, 2H, -CH_2-CH_3), 2.56 (t, 2H, C-4 of pyridazine), 2.92 (t, 2H, C-5 of pyridazine), 3.35 (t, 2H, -CH_2-N), 7.62-7.98 (m, 6H, Ar-H), 8.79 (d, 2H, C2 & C6 H of pyridine), 10.57 (s, 1H, NH); ^{13}C-NMR: 12.3, 15.1, 22, 26.9, 44.5, 122.8 (2C), 129.3 (2C), 130 (2C), 135.6, 137.7, 141.9, 147.6, 148.2, 149.5 (2C), 163.5; Mass (m/z): 369 (M^+, 100.0%); Elemental Analysis (Calculate (Found) for $C_{19}H_{20}ClN_5O$): C, 61.70 (61.68); H, 5.45 (5.43); N, 18.94 (18.92).

N'-(6-(4-fluorophenyl)-2-propyl-4,5-dihydropyridazin-3(2H)-ylidene)isonicotinohydrazide (3f)

% Yield: 40; M.P. (°C): 180; R_f Value: 0.68; IR: 3345 (N-H), 1690 (C=O), 1580 (C=N); ^{1}H-NMR: 1.05 (t, 3H, -CH_3), 1.56 (m, 2H, -$\underline{CH_2}$-CH_3), 2.57 (t, 2H, C-4 of pyridazine), 2.92 (t, 2H, C-5 of pyridazine), 3.34 (t, 2H, -CH_2-N), 7.36-7.82 (m, 6H, Ar-H), 8.78 (d, 2H, C2 & C6 H of pyridine), 10.55 (s, 1H, NH); ^{13}C-NMR: 12.2, 16.1, 22.1, 27, 44.5, 116.7 (2C), 122.8 (2C), 130 (2C), 133.2, 141.9, 147.6, 148.2, 149.5 (2C), 163.5, 165.3; Mass (m/z): 353 (M^+, 100.0%); Elemental Analysis (Calculate (Found) for $C_{19}H_{20}FN_5O$): C, 64.58 (64.55); H, 5.70 (5.65); N, 19.82 (19.80).

N'-(6-(4-bromophenyl)-2-propyl-4,5-dihydropyridazin-3(2H)-ylidene)isonicotinohydrazide (3g)

% Yield: 50; M.P. (°C): 195; R_f Value: 0.88; IR: 3350 (N-H), 1695 (C=O), 1590 (C=N); ^{1}H-NMR: 1.05 (t, 3H, -CH_3), 1.58 (m, 2H, -CH_2-CH_3), 2.58 (t, 2H, C-4 of pyridazine), 2.92 (t, 2H, C-5 of pyridazine), 3.35 (t, 2H, -CH_2-N), 7.64-7.82 (m, 6H, Ar-H), 8.79 (d, 2H, C2 & C6 H of pyridine), 10.57 (s, 1H, NH); ^{13}C-NMR: 12.3, 16.2, 22, 27, 44.5, 122.8 (2C), 126.5, 129.7 (2C), 132.7 (2C), 136.6, 141.8, 147.5, 148.1, 149.5 (2C), 163.5; Mass (m/z): 413 (M^+, 100.0%); Elemental Analysis (Calculate (Found) for $C_{19}H_{20}BrN_5O$): C, 55.08 (55); H, 4.87 (4.85); N, 16.90 (16.85).

N'-(6-(4-ethylphenyl)-2-propyl-4,5-dihydropyridazin-3(2H)-ylidene)isonicotinohydrazide (3h)

% Yield: 55; M.P. (°C): 184; R_f Value: 0.75; IR: 3335 (N-H), 1685 (C=O), 1580 (C=N); ^{1}H-NMR: 1.05 (t, 3H, -CH_3), 1.23 (t, 3H, -CH_3), 1.58 (m, 2H, -CH_2-CH_3), 2.58 (t, 2H, C-4 of pyridazine), 2.72 (q, 2H, -CH_2-Ph) 2.92 (t, 2H, C-5 of pyridazine), 3.35 (t, 2H, -CH_2-N), 7.38-7.80 (m, 6H, Ar-H), 8.77 (d, 2H, C2 & C6 H of pyridine), 10.57 (s, 1H, NH); ^{13}C-NMR: 12.3, 15.6, 16.2, 22, 27, 29.3, 44.5, 122.8 (2C), 128.1 (2C), 128.9 (2C), 134.7, 141.9, 147.6 (2C), 148.2, 149.5 (2C), 163.5; Mass (m/z): 363 (M^+, 100.0%); Elemental Analysis (Calculate (Found) for $C_{21}H_{25}N_5O$): C, 69.40 (69.35); H, 6.93 (6.90); N, 19.27 (19.25).

N'-(6-(4-ethylphenyl)-2-pentyl-4,5-dihydropyridazin-3(2H)-ylidene)isonicotinohydrazide (3i)

% Yield: 60; M.P. (°C): 165; R_f Value: 0.66; IR: 3340 (N-H), 1680 (C=O), 1575 (C=N); ^{1}H-NMR: 1.05 (t, 3H, -CH_3), 1.22-1.29 (m, 7H, -CH_3, 2-CH_2-), 1.58 (m, 2H, -CH_2-), 2.58 (t, 2H, C-4 of pyridazine), 2.72 (q, 2H, -CH_2-Ph) 2.92 (t, 2H, C-5 of pyridazine), 3.35 (t, 2H, -CH_2-N), 7.31-7.82 (m, 6H, Ar-H), 8.78 (d, 2H, C2 & C6 H of pyridine), 10.57 (s, 1H, NH); ^{13}C-NMR: 15.1, 15.5, 16.2, 23.5, 26.8, 26.9, 29.2, 29.9, 42.3, 122.8 (2C), 128.2 (2C), 128.9 (2C), 134.7, 141.9, 147.6 (2C), 148.2, 149.5 (2C), 163.5; Mass (m/z): 391 (M^+, 100.0%); Elemental Analysis (Calculate (Found) for $C_{23}H_{29}N_5O$): C, 70.56 (70.50); H, 7.47 (7.45); N, 17.89 (17.85).

N'-(6-(4-bromophenyl)-2-pentyl-4,5-dihydropyridazin-3(2H)-ylidene)isonicotinohydrazide (3j)

% Yield: 50; M.P. (°C): 191; R_f Value: 0.79; IR: 3345 (N-H), 1695 (C=O), 1585 (C=N); ^{1}H-NMR: 1.06 (t, 3H, -CH_3), 1.28-1.29 (m, 4H, 2-CH_2-), 1.58 (m, 2H, -CH_2-), 2.58 (t, 2H, C-4 of pyridazine), 2.92 (t, 2H, C-5 of pyridazine),

3.35 (t, 2H, -CH_2-N), 7.64-7.80 (m, 6H, Ar-H), 8.78 (d, 2H, C2 & C6 H of pyridine), 10.55 (s, 1H, NH); ^{13}C-NMR δ ppm: 15.1, 16.1, 23.4, 26.7, 26.9, 29.9, 42.2, 122.7 (2C), 126.4, 129.6 (2C), 132.7 (2C), 136.4, 141.8, 147.5, 148.1, 149.5 (2C), 163.5; Mass (m/z): 441 (M^+, 100.0%); Elemental Analysis (Calculate (Found) for $C_{21}H_{24}BrN_5O$): C, 57.02 (57); H, 5.47 (5.45); N, 15.83 (15.80).

Anti-tubercular activity

Anti-tubercular activity was done by the reported method [23,24] against *M. intercellulari* (ATCC 35734), *M. cheleneoi* (ATCC 35751), *M. xenopi* (ATCC 14470) and *M. smegmatis* (ATCC 35797). The minimum inhibitory concentration was determined by the serial dilution method. The data of the anti-tubercular activity are provided in the Table 1.

Table 1: *In vitro* anti-tubercular activities of the compounds (3a-3j)

Compound	Minimum Inhibitory Concentration (µg/mL)*			
	M. intercellulari	***M. cheleneoi***	***M. xenopi***	***M. smegmatis***
3a	75	> 75	100	75
3b	25	25	25	25
3c	12.5	12.5	25	12.5
3d	25	12.5	12.5	12.5
3e	> 100	75	100	> 100
3f	50	50	75	75
3g	75	> 75	100	75
3h	75	75	100	75
3i	75	50	75	75
3j	75	> 75	100	75
Isoniazid	12.5	12.5	12.5	12.5

** $p < 0.05$*

The anti-tubercular activity data revealed that most of the compounds have higher MIC values as compared to the standard drug isoniazid. However, two compounds, namely **3b** and **3c**, exhibited very good anti-tubercular activity against all four strains of the *Mycobacterium*, which was comparable to the standard drug isoniazid. The compound **3c** almost displayed equipotent anti-tubercular activity when compared to isoniazid and the compound **3b** also exhibited good actitubercular activity against all four strains. Recent studies have stated that isoniazid derivatives having lipophilic moieties are emerging as potent anti-tubercular agents [6]. Structurally, the compounds **3b** and **3c** possess a pentyl side chain at position-2 of the pyridazine ring, which might be increasing the lipophilicity of these compounds. These compounds also possess a halogen at the para position of the phenyl ring. It is also expected that these halogen atoms, fluorine and chlorine, are also responsible for the good anti-tubercular activity of these two compounds. Apart from the compounds **3b** and **3c**, other compounds also have a pentyl side chain and propyl side chain. However, those compounds did not provide comparable anti-tubercular activities, when compared to the standard drug isoniazid. Accordingly, it can be assumed that the incorporation of a lipophilic chain at the position-2 of the pyridazine ring along with a halogen group, preferably a fluorine atom, at the p-position of the phenyl ring in this series of compounds increase the anti-tubercular activity of the compounds. Similar type of observations with respect to the lipophilic substituents have been published [6,23]. It is also assumed that the replacement of the pentyl side chain and the propyl side chain in the compounds of the present study with another more lipophilic side chain or group, for example hexyl side chain, along with a halogen substituted phenyl ring will provide better anti-tubercular agents. Accordingly, the authors recommend to perform these changes in the structure of the compounds of the present studies.

CONCLUSION

The anti-tubercular activity data revealed that the compound **3c** almost displayed equipotent anti-tubercular activity when compared to isoniazid and the compound **3b** also exhibited good actitubercular activity against all four strains of the Mycobacterium. There is a possibility that the lipophilic pentyl chain along

with the halogen atom in the structures of these compounds are responsible for the potency of these two compounds. However, this assumption cannot be generalized because in the present study the anti-tubercular activity was assessed against four strains of *Mycobacterium*. Accordingly, further studies are recommended to assess the anti-tubercular activity of these two compounds, **3b** and **3c**, against other strains of the *Mycobacterium*.

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REFERENCES

[1] Eldehna WM, Fares M, Abdel-Aziz MM, Abdel-Aziz HA. Molecules 2015; 20:8800-8815.
[2] World Health Organization. Global Tuberculosis Report 2017, available at http://www.who.int/tb/publications/global_report/en/; Accessed on September 22, 2018.
[3] Sureram S, Senadeera SPD, Hongmanee P, Mahidol C, Ruchirawat S, Kittakoop P. Bioorg Med Chem Lett 2012; 22:2902-2905.
[4] Ganihigama DU, Sureram S, Sangher S, Hongmanee P, Aree T, Mahidol C, Ruchirawat S, Kittakoop P. Eur J Med Chem 2015; 89:1-12.
[5] Sandy J, Mushtaq A, Kawamura A, Sinclair J, Sim E, Noble M. J Mol Biol 2002; 318:1071-1083.
[6] Hu YQ, Zhang S, Zhao F, Gao C, Feng LS, Lv ZS, Xu Z, Wu X. Eur J Med Chem 2017; 16:255-267.
[7] Beena DK, Khare G, Kidwai S, Tyagi AK, Singh R, Rawat DS. Eur J Med Chem 2014; 81:301-313.
[8] Boechat N, Ferreira VF, Ferreira SB, Ferreira MLG, Silva FCD, Bastos MM, Costa MS, Lourenco MCS, Pinto AC, Krettli AU, Aguiar AC, Teixeira BM, Silva NV, Martins PRC, Bezerra FAFM, Camilo AS, Silva GP, Costa CCP. J Med Chem 2011; 54:5988-5999.
[9] Aragade P, Palkar M, Ronad P, Satyanarayana D. Med Chem Res 2013; 22:2279-2283.
[10] Nayak N, Ramprasad J, Dalimba U. Bioorg Med Chem Lett 2015; 25:5540-5545.
[11] Shaharyar M, Siddiqui AA, Ali MA, Sriram D, Yogeeswari P. Bioorg Med Chem Lett 2006; 16:3947-3949.
[12] Hearn MJ, Chen MF, Terrot MS, Webster ER, Cynamon MH, J Heterocyc Chem 2010; 47:707-712.
[13] Martins F, Santos S, Ventura C, Elvas-Leitão R, Santos L, Vitorino S, Reis M, Miranda V, Correia HF, Aires-de-SousaJ, Kovalishyn V, Latino DARS, Ramos J, Viveiros M. Eur J Med Chem 2014; 81:119-138.
[14] Patel RV, Keum YS, Park SW. Mini Rev Med Chem 2014; 14:768-789.
[15] Lele AC, Raju A, Ray MK, Rajan MGR, Degani MS. Curr Res Drug Discov 2014; 1:45-50.
[16] Joshi SD, More UA, Parkale D, Aminabhavi TM, Gadad AK, Nadagouda MN, Jawarkar R. Med Chem Res 2015; 24:3892-3911.
[17] Xu Z, Zhang S, Gao C, Zhao F, Lv ZS, Feng LS. Chin Chem Lett 2017; 2:159-167.
[18] Pitta E, Rogacki MK, Balabon O, Huss S, Cunningham F, Lopez-Roman EM, Joossens J, Augustyns K, Ballell L, Bates RH, Van der Veken P. J Med Chem 2016; 59:6709-6728.
[19] Fernandes GFS, Souza PC, Marino LB, Chegaev K, Guglielmo S, Lazzarato L, Fruttero R, Chung MC, Pavan FR, Santos JL. Eur J Med Chem 2016; 123:523-531.
[20] Husain A, Sarafroz M, Ahuja P. Acta Pol Pharm 2008; 65(5):527-534.
[21] Husain A. Acta Pol Pharm 2009; 66(5):513-521.
[22] Imran M, Nayeem N. Orient J Chem 2016; 32(1):267-274.
[23] Bhat MA. Acta Pol Pharm Drug Res 2014; 71(5):763-770.
[24] Canetti G, Rist N, Grosset J. Rev Tuberc Pneumol (Paris) 1963; 27:217-222.