INTRODUCTION

Isoniazid (isonicotinic acid hydrazide) used as the first-line antibiotic for tuberculosis treatment since 1952. It is prodrug which is activated by Mycobacterium tuberculosis catalase-peroxidase (Kat G) is a bifunctional hemoprotein [1]. The primary target of isoniazid (INH) is the enoyl-ACP reductase enzyme (InhA) from M. tuberculosis. Isoniazid is a derivative of nicotinic acid, which possesses antibacterial, antioxidant, anti-inflammatory, and anticarcinogenic activities and showed putative activity against osteoarthritis and granuloma annularis. Nicotinic acid (nicotinamide) or vitamin B3 derivatives are also very important starting material to prepare bioactive moieties [2]. Likewise, pyrimidine also appeared as an important pharmacophore used as building blocks of numerous natural compounds and found in vitamins (thiamine, riboflavin, and folic acid), lipopolysaccharides, DNA, and RNA. The synthetic drugs such as HIV drugs, barbiturates, anticancer agents, antimicrobial, antimalarial, and anti-inflammatory [3-9]. Structural modification of pyrimidine with different functional groups or other heterocycles may improve clinical outcomes [10-19]. Due to this importance of isoniazid (nicotinic acid derivatives) and pyrimidine nucleus, we thought to synthesize isoniazid clubbed pyrimidine derivatives and evaluated in vitro antibacterial activity against several kinds of bacteria, fungi, and M. tuberculosis H₃⁷Rv.

In this study, newly synthesized 2-(2-(3-bromo benzylidene)-1-isonicotinoyl hydrazinyl)-N-(4-(substituted phenyl)-6-(substituted aryl) pyrimidin-2-yl) acetamide 2$_{a1}$ showed good antitubercular activity. Where some of the newly synthesized compounds showed good antimicrobial activity and comparatively good antituberculosis activity. Hence, all the compounds of this series considered for future investigation mainly in area of antibacterial, antifungal study.

METHODS

The chemicals used in the synthesis were purchased from commercial sources were of analytical grade and were used without further purification. Laboratory Chemicals were supplied by Sigma-Aldrich and Fisher Scientific Ltd. Melting points were determined by the open tube capillary method and are uncorrected. The purity of the compounds was determined by thin layer chromatography (TLC) plates (silica gel G) using different eluent system. The IR spectra were obtained on Fourier transform infrared spectroscopy (FT-IR) Infrared spectrophotometer model RZX (Perkin Elmer) and Agilent resolution Pro FT-IR spectrometer using potassium bromide pellets; the frequencies are expressed in cm$^{-1}$. The $^1$H-NMR and $^13$C-NMR spectra were recorded on a Bruker Advance II 400 spectrometer (100 MHz FT-NMR) using tetramethylsilane (TMS) as the internal standard in deuteriochloroform (CDCl$_3$) and dimethyl sulfoxide (DMSO-d$_6$) (chemical shifts in ppm). Mass spectra recorded WATERS Q-T of micro mass (electrospray ionization-MS). Column chromatography was performed on silica gel 60 (0.043-0.06 mm) Merck. Elemental analysis was performed on Carlo Erba 1108 analyzer, and the result was varying within ±0.04% of the calculated values.

General method of synthesis

Synthesis of (2E)-1-(4-(substituted phenyl)) prop-2-en-1-one derivatives (A)

a-$\beta$ unsaturated ketone [20] compounds were prepared by reported method [21-24].
Synthesis of 4-(4-substituted phenyl)-6-(substituted phenyl) pyrimidin-2-amine derivatives (B)

A synthesized compound (A) (10 mmol), guanidine nitrate (15 mmol) and were dissolved in ethanol (10 mL) and added sodium methoxide in methanol (25%, 20 mL) was refluxed for 6-8 hrs. The progress of the reaction was monitored by TLC using tolueneethyl acetate (2.5:7.5), interval of every 30 minutes and after completion of the reaction, the mixture was cooled, diluted with water and filtered. The separated solid compound was washed with water, dried and recrystallized with ethanol to get (B).

Synthesis of 2-chloro-N-[4-(substituted phenyl)-6-(substituted phenyl)pyrimidin-2-yl] acetamide (C)

A synthesized compound (B) (10 mmol) dissolved in dichloromethane (15 mL) and dropwise addition of chloroacetyl chloride (15 mmol). Then added triethylamine (10 mmol). Reaction mass was stirred for 3 hrs. The progress of the reaction was monitored by TLC using toluene: methanol:ethyl acetate (2:3:5), interval of every 30 minutes and after completion of the reaction, the mixture diluted with water and the organic layer was separated. The separated liquid compound was washed, washed water and recrystallized with ethanol to get (C).

Synthesis of N'-([E]-[3-bromophenyl) methylidene]pyridine-4-carboxyhydrazide (D)

This compound was prepared by reported method [25,26].

Synthesis of 2-(2-(3-bromo benzylidene)-1-isonicotinoyl hydrayzynyl)-N-[4-(substituted phenyl)-6-(substituted aryl) pyrimidin-2-yl] aceticamide (E)

A synthesized compound (D) (10 mmol) dissolved in 15 mL methanol, and 3.4 g of K2CO3 was added. The reaction mixture was refluxed on a water bath. A dropping funnel was fitted to the round bottom flask, and in the dropping funnel, a solution of synthesized compound (C) (10 mmol) in 20 mL methanol was taken. A slow addition of this solution was done. The reaction mixture was refluxed in water bath at 80°C for 4 hrs. The reaction was monitored by TLC using tolueneethyl acetate (2-8). After completion of the reaction, the mixture was kept at room temperature. After filtration, it was washed with water and crystallized with ethanol to get (E). The crude solid was purified by column chromatography.

Biological evaluation

In vitro biological evaluation

Minimum inhibition concentration method for antimicrobial activity.

For nutrient medium Mueller–Hinton broth was used as to grow and dilute the drug suspension for the test. Size of inoculum for test strain was adjusted to 10⁶ colony forming unit. DMSO was used for negative control and as diluents to get the desired concentration of drugs to test on standard bacterial strains. For primary and secondary screening serial dilutions were prepared. The control tube containing no antibiotic was immediately subcultured (before inoculation) by spreading a loopful evenly over a plate of medium suitable for the growth of the test organism and put for incubation at 37°C for 24 hrs. The minimum inhibitory concentration (MIC) of the control organism was read to check the accuracy of the drug concentrations.

The standard drug used in this method was ampicillin, chloramphenicol, ciprofloxacin, griseofulvin, and nystatin. The lowest concentration inhibiting the growth of the organism was recorded as the MIC. The amount of growth of the control tube before incubation (which represents the original inoculum) was compared. The test included a second set of the same dilutions inoculated with an organism of known sensitivity. Each synthesized drug was diluted obtaining 1000 µg/mL concentration, as a stock solution. In primary screening 500, 250, and 125 µg/mL concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 100, 50, 25, 12.5, 6.250, 3.125, and 1.5625 µg/mL concentrations. The highest dilution showing at least 99% inhibition is taken as MIC (Table 1) [27,28].

RESULTS

Synthetic route for title compounds given in Figs. 1 and 2. The pyrimidine ring system was prepared by 4-(4-substituted phenyl)-6-(substituted aryl) pyrimidine-2-amine. This compound was key intermediate required for the synthesis of title compounds. This compound further reacted with chloroacetyl chloride and given 2-chloro-N-[4-(substituted phenyl)-6-(substituted aryl) pyrimidin-2-yl] acetamide. To prepare final yield, compound (A) condensed with N'-([E]-[3-bromophenyl)m ethylidene]pyridine-4-carboxyhydrazide in the presence of K2CO3. Which yielded (E)-2-(2-(3-Bromo benzylidene)-1-isonicotinoyl hydrayzynyl)-N-[4-(substituted phenyl)-6-(substituted aryl) pyrimidin-2-yl] acetamide (Z). Spectral studies of these compounds proved the structure of derivatives. IR absorption band of compound Z, IR (KBr) cm−1: 1171.87, 1140.44 (C-N), and 573.76 (C-Br). 1H NMR spectrum of the compound recorded in CDCl3, singlet of H at 8.62 for NH, which yielded 2. H was assigned due to aromatic proton at 7.31-8.77, singlet of 1H at 5.33 for NH, which disappeared on D2O exchange and singlet of 2H for CH recorded at 3.85. 13C NMR of the final compound showed at 166.05, 167.28 for aromatic C-H. 1652.88 (-CONH), 1356.10, 1252.29, 1218.37, 1171.87, 1140.44 (C-N), and 573.76 (C-Br).
Mass spectra of the synthesized compounds showed M+/M+1 peak, confirmed their molecular formula. The analytical data of synthesized compounds are as follows.

(E)-2-(2-(3-bromobenzylidene)-1-isonicotinoylhydrazinyl)-N-(4-(4-fluorophenyl)-6-(pyridin-2-yl)pyrimidin-2-yl)acetamide 2
IR: 3432.80 (N-H), 3190.58, 3051.74, (C-H aromatic), 1652.88 (-CONH), 1544.72 (N-H bending), 1429.32, 1476.17 (C=C aromatic), 1356.10, 1252.29, 1218.37, 1171.87, 1140.44 (C-N), 1063.01 (C-F) 573.76 (mono substituted Br).

h-NMR (CDCl$_3$, 400 MHz): 8.62 (s, 1H, CH of pyrimidine ring); 7.31-8.77 (m, 16H, Ar-H); 8.39 (s, 1H, CH); 5.33 (s, 1H, CONH); 3.85 (s, 2H, CH2). 13C-NMR (CDCl$_3$, 400 MHz): 168.51, 165.62 (C=O); 162.91 (C=N, pyrimidine); 155.30; 101.30-149.71; 146.91 (aromatic ring), 55.40 (CH$_2$). MS (m/z): 609.09 ([M + H]$^+$), 611.09 [M$^+$2], elemental analysis calculated for C$_{30}$H$_{21}$BrFN$_7$O$_2$: C 59.03, H 3.47, N 16.06; found: C 59.04, H 3.45, N 16.04.

(E)-2-(2-(3-bromobenzylidene)-1-isonicotinoylhydrazinyl)-N-(4-(4-fluorophenyl)-6-(pyridin-3-yl)pyrimidin-2-yl)acetamide 2
IR: 3430.70 (N-H), 3190.58, 3041.44, (C-H aromatic), 1657.18 (-CONH), 1544.72 (N-H bending), 1429.32, 1476.17 (C=C aromatic), 1356.10, 1252.29, 1218.37, and 1170.87 (C-N), 1061.31 (C-F) 577.66 (mono substituted Br).

h-NMR (CDCl$_3$, 400 MHz): 8.66 (s, 1H, CH of pyrimidine ring); 6.68-8.78 (m, 16H, Ar-H); 8.45 (s, 1H, CH); 5.63 (s, 1H, CONH); 5.33 (s, 1H, CONH). MS (m/z): 611.09 ([M + H]$^+$), 613.09 [M$^+$2], elemental analysis calculated for C$_{30}$H$_{21}$BrFN$_7$O$_2$: C 59.03, H 3.47, N 16.06; found: C 59.04, H 3.45, N 16.04.

Table 1: Physical data of the compounds (2a–j)

<table>
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<th>Component number</th>
<th>M.P. °C</th>
<th>Yield %</th>
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<tbody>
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<td>56</td>
</tr>
<tr>
<td>2b</td>
<td>180-182</td>
<td>66</td>
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<td>2c</td>
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<td>2d</td>
<td>155-157</td>
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<td>2e</td>
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<tr>
<td>2f</td>
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<td>139-142</td>
<td>61</td>
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<tr>
<td>2h</td>
<td>159-161</td>
<td>71</td>
</tr>
<tr>
<td>2i</td>
<td>170-174</td>
<td>56</td>
</tr>
<tr>
<td>2j</td>
<td>165-168</td>
<td>59</td>
</tr>
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</table>
(E)-2-(2-bromobenzylidene)-1-isonicotinoylhydrazinyl-N-(4-(4-fluorophenyl)-6-(pyridin-4-yl)pyrimidin-2-yl)acetamide $Z_2$.

IR: 3472.56, 3303.45 (N-H), 3056.70, 1651.75 (CONH), 1591.78 (N-H bending), 1428.37, 1472.37 (C=C, aromatic), 1308.26, 1234.07, 1166.55, and 1140.44 (C=N), 1024.41 (C-F) 573.76 (mono substituted Br). $^1$H-NMR (CDCl$_3$, 400 MHz): 8.61 (1H, CH of pyrimidine ring); 7.31-8.77 (m, 16H, Ar-H); 8.39 (1H, CH); 5.35 (1H, CONH). 3.85 (2H, CH$_2$). $^1$C-NMR (CDCl$_3$, 100 MHz): 168.54, 162.92 (C=O); 140.81-107.08; 55.41 (CH$_3$). MS (m/z): 688.09 [M + H$^+$], 690.09 [M$^+$2], elemental analysis calculated for C$_{38}$H$_{28}$Br$_2$FN$_2$O$_2$: C 54.09, H 3.08, N 12.21; found: C 54.11, H 3.19, N 12.23.

(E)-2-(2-bromobenzylidene)-1-isonicotinoylhydrazinyl-N-(4-(4-bromomethyl)-4-fluorophenyl) pyrimidin-2-yl acetamide $Z_2$.

IR: 3341.42 (N-H), 3060.39, 3033.38 (C=H aromatic), 1673.22 (CONH), 1553.21 (N-H bending), 1433.36, 1472.37 (C=C, aromatic), 1267.26, 1218.37, 1184.39, and 1140.64 (C=N), 1024.41 (C-F). $^1$H-NMR (CDCl$_3$, 400 MHz): 8.61 (1H, CH of pyrimidine ring); 7.31-8.77 (m, 17H, Ar-H); 8.38 (1H, CH); 5.35 (1H, CONH). 3.85 (2H, CH$_2$). $^1$C-NMR (CDCl$_3$, 100 MHz): 168.51, 162.69 (C=O); 1468.95-107.08 (aromatic ring); 55.41 (CH$_3$). MS (m/z): 642.90 [M + H$^+$], 643.88 [M$^+$+1], 644.09 [M$^+$2], elemental analysis calculated for C$_{38}$H$_{28}$Br$_2$FN$_2$O$_2$: C 57.68, 57.83, 57.78, 57.39, 3.29, 13.05; found: C 57.81, H 3.33, N 13.03.

(E)-2-(2-bromobenzylidene)-1-isonicotinoylhydrazinyl-N-(4-(pyridin-2-yl)-6-(pyridin-2-yl)pyrimidin-2-yl)acetamide $Z_2$.

IR: 3352.46 (N-H), 3056.39, 3032.38 (C=H aromatic), 2941.41, 2923.44, 1671.22 (CONH), 1541.21 (N-H bending), 1413.36, 1423.38 (C=C, aromatic), 1256.26, 1234.37, 1123.49 (C-N), 567.76 (mono substituted Br). $^1$H-NMR (CDCl$_3$, 400 MHz): 8.64 (1H, CH of pyrimidine ring); 7.42-8.77 (m, 16H, Ar-H); 8.39 (1H, CH); 5.34 (1H, CONH). 3.85 (2H, CH$_2$). 2.34 (3H, CH$_3$). $^1$C-NMR (CDCl$_3$, 100 MHz): 168.35, 165.46 (CO$_2$); 162.76 (pyridine); 157.94; 135.01-110.39; 55.43 (CH$_3$). MS (m/z): 660.05 [M + H$^+$], 661.03 [M$^+$+1], 662.12 [M$^+$2], elemental analysis calculated for C$_{35}$H$_{28}$Br$_2$N$_2$O$_2$: C 61.39, H 3.99, N 16.17; found: C 61.34, H 3.97 N 16.11.

(E)-2-(2-bromobenzylidene)-1-isonicotinoylhydrazinyl-N-(4-(pyridin-3-yl)-6-(pyridin-2-yl)pyrimidin-2-yl)acetamide $Z_2$.

IR: 3431.13, 3331.42 (NH), 3032.39, 2932.50 (C=H aromatic), 2932.50 (C=H), 1671.21 (CONH), 1539.21 (N-H bend), 1441.24, 1441.37, 1441.37 (C=C, aromatic), 1353.33, 1274.26, 1218.37, 1183.49, and 1140.44 (C=N), 573.76 (mono substituted Br). $^1$H-NMR (CDCl$_3$, 400 MHz): 8.57 (1H, CH of pyrimidine ring); 7.12-7.25 (m, 16H, Ar-H); 8.36 (1H, CH); 5.23 (3H, CONH). 3.85 (2H, CH$_2$); 2.34 (3H, CH$_3$). $^1$C-NMR (CDCl$_3$, 100 MHz): 165.18; 163.93 (CO$_2$); 149.99-123.23; 55.34 (CH$_3$). 21.14 (CH$_3$). MS (m/z): 605.13 [M + H$^+$], 606.12 [M$^+$+1], 607.13 [M$^+$2], elemental analysis calculated for C$_{35}$H$_{28}$Br$_2$N$_2$O$_2$: C 61.39, H 3.99, N 16.17; found: C 61.40, H 3.98 N 16.19.

Table 2: In vitro antibacterial activity (MIC, µg/mL) of the synthesized compounds

<table>
<thead>
<tr>
<th>Code number</th>
<th>MIC µg/mL</th>
</tr>
</thead>
<tbody>
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<td><strong>E. coli</strong></td>
<td><strong>P. aeruginosa</strong></td>
</tr>
<tr>
<td>$Z_2^*$</td>
<td>2</td>
</tr>
<tr>
<td>$Z_2^{*+}$</td>
<td>250</td>
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<tr>
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<tr>
<td>Ampicillin</td>
<td>100</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>25</td>
</tr>
</tbody>
</table>

**MIC**: Minimal inhibitory concentration. **E. coli**: Escherichia coli (MTCC no. 442). **P. aeruginosa**: Pseudomonas aeruginosa (MTCC no. 441). **S. aureus**: Staphylococcus aureus (MTCC no. 96). **S. pyogenes**: Staphylococcus pyogenes (MTCC no. 443). **MTCC**: Microbial type culture collection.
In vitro antimicrobial and antituberculosis activity

All the newly synthesized compounds were screened for their antimicrobial activity. This activity was determined by the broth microdilution method according to the National Committee for Clinical Laboratory Standards [29,30]. For antibacterial activity, we used *S. aureus* microbial type culture collection (MTCC 96) and *S. pyogenes* (MTCC 443) as Gram-positive, *E. coli* (MTCC 442) and *P. aeruginosa* (MTCC 441) as Gram-negative strains using ampicillin, chloramphenicol, and ciprofloxacin as a standard antibiotic drug. Antifungal activity was screened for three different fungal species *C. albicans* (MTCC 227), *A. niger* (MTCC 282), and *A. clavatus* (MTCC 1323). Griseofulvin and nystatin used as a standard antifungal drug. The strains were procured from Institute of Microbial Technology, Chandigarh.

**DISCUSSION**

Pyrimidine derivatives have been very well known in medicinal chemistry for their therapeutic applications. The presence of a pyrimidine base in thymine, cytosine and uracil, which are the essential binding blocks of nucleic acids, DNA and RNA is one possible reason for their activity. The literature indicated that compounds having pyrimidine nucleus possess a broad range of biological activities. Like 5-fluorouracil as anticancer; idoxuridine and trifluridine as antigviral; zidovudine and stavudine as anti-HIV, trimethoprim, sulfamethazine, and sulfadiazine as antibacterial; sulfadoxine as antimalarial and isoniazid itself used as antituberculosis agents. Isoniazid has been found to block mycobacterial DNA synthesis, which initially leads to the death of the cell. Later, it is converted to its active metabolite, isoniazid-N-hydroxylamine, which inhibits the production of mycolic acids, the major structural component of the cell wall. Isoniazid is therefore a potent antitubercular agent.

**REFERENCES**

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