

## PYRIMIDINE INCORPORATED SCHIFF BASE OF ISONIAZID WITH THEIR SYNTHESIS, CHARACTERIZATION AND *IN VITRO* BIOLOGICAL EVALUATION

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### ABSTRACT

**Objective:** Versatile biological activities of nitrogen containing heterocycles in medicinal chemistry, mainly pyrimidine and pyridine ring based heterocyclic moieties are very important. Pharmaceutical important of pyrimidine and isoniazid moiety prompted us to synthesize isoniazid clubbed pyrimidine derivatives and evaluated for antimicrobial and antituberculosis activity.

**Method:** 2-(2-(3-bromo benzylidene)-1-isonicotinoyl hydrazinyl)-*N*-(4-(substituted phenyl)-6-(substituted aryl) pyrimidin-2-yl) acetamide  $2_{(A-I)}$  have been synthesized by condensation reaction of 2-chloro-*N*-[4-(substituted phenyl)-6-(substituted aryl) pyrimidin-2-yl] acetamide and *N*'-[(*E*)-(3-bromophenyl) methylidene]pyridine-4-carbohydrazide. All newly synthesized compounds were screened for *in vitro* antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus pyogenes*, antifungal against *Candida albicans*, *Aspergillus niger*, and *Aspergillus clavatus*, and antituberculosis activity against *Mycobacterium tuberculosis* H<sub>37</sub>Rv.

**Results:** Majority of the compounds exhibited good antibacterial, antifungal, and antituberculosis activity. All titled compounds were characterized by spectral analyses (infrared spectroscopy, nuclear magnetic resonance spectroscopy, and mass spectroscopy).

**Conclusion:** 2-(2-(3-bromo benzylidene)-1-isonicotinoyl hydrazinyl)-*N*-(4-(substituted phenyl)-6-(substituted aryl) pyrimidin-2-yl) acetamide  $2_{(A-I)}$  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.

**Keywords:** Pyrimidine, Pyridine, Isoniazid, Antimicrobial, Antituberculosis.

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### 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 annulare. Nicotinic acid (Niacin or vitamin B<sub>3</sub>) 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, antiviral, 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* H<sub>37</sub>Rv.

In this study, newly synthesized 2-(2-(3-bromo benzylidene) -1-isonicotinoyl hydrazinyl) -*N*-(4-(substituted phenyl) -6-(substituted aryl) pyrimidin-2-yl) acetamide  $2_{(A-I)}$  were identified and characterized by spectral analysis (IR, nuclear magnetic resonance [NMR], and mass) and elemental analysis. Antibacterial studies were performed toward *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus pyogenes*, antifungal activities against *Candida albicans*, *Aspergillus niger*, and *Aspergillus clavatus*. The majority of the compounds were showed a

high degree of evaluation toward Gram-positive and Gram-negative bacteria as compared to standard drug ampicillin. Antifungal activity of many moieties was comparatively high as compared with standard drug Griseofulvin. Where some of the newly synthesized compounds showed good antitubercular activity.

### 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<sup>-1</sup>. The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were recorded on a Bruker Advance II 400 spectrometer (100 MHz FT-NMR) using tetramethylsilane as the internal standard in deuteriochloroform (CDCl<sub>3</sub>) and dimethyl sulfoxide (DMSO-d<sub>6</sub>) (chemical shifts in δ 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)-3-(substituted phenyl) prop-2-en-1-one derivatives (A)*

α-β 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 toluene:ethyl 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-(4-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 dried, washed water and recrystallized with ethanol to get (C).

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

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

### Synthesis of 2-(2-(3-Bromo benzylidene)-1-isonicotinoyl hydrazinyl)-N-(4-(substituted phenyl)-6-(substituted aryl) pyrimidin-2-yl) acetamide (E)

A synthesized compound (D) (10 mmol) was dissolved in 15 mL methanol, and 3.4 g of  $K_2CO_3$  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 toluene:ethyl acetate (2:8). After completion of the reaction, the mixture was kept at room temperature. After filtration, it was washed with water and crystallized from ethanol to get (E). The crude solid was purified by column chromatography.

## Biological evaluation

### In vitro biological evaluation

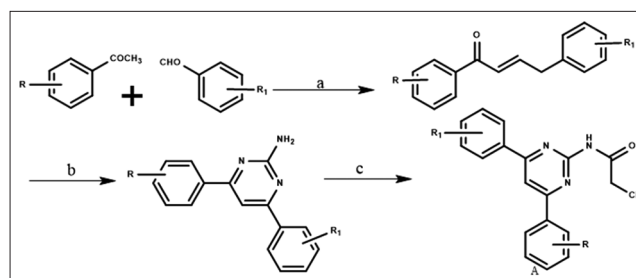
Minimum inhibition concentration method for antimicrobial activity.

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  $10^8$  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 quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37°C 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

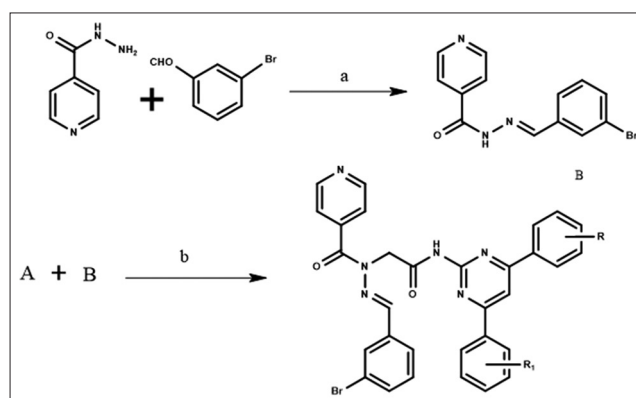
tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 100, 50, 25, 12.5, 6.250, 3.125, and 1.5625 µg/mL concentrations. The highest dilution showing at least 99% inhibition is taken as MIC (Table 1) [27,28].

## 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-(4-substituted aryl) pyrimidin-2-yl] acetamide (A). To prepare final yield, compound (A) condensed with N'-[(E)-(3-bromophenyl)methylidene]pyridine-4-carbohydrazide in the presence of  $K_2CO_3$ , Which yielded (E)-2-(2-(3-Bromo benzylidene)-1-isonicotinoyl hydrazinyl)-N-(4-(substituted phenyl)-6-(substituted aryl) pyrimidin-2-yl) acetamide ( $2_{A_1}$ ). Spectral studies of these compounds proved the structure of derivatives. IR absorption band of compound  $2_{A_1}$ , IR (KBr)  $cm^{-1}$ : 3432.80 (N-H), 3190.58, 3051.74 for aromatic C-H, 1652.88 (-CONH), 1356.10, 1252.29, 1218.37, 1171.87, 1140.44 (C-N), and 573.76 (C-Br).  $^1H$  NMR spectrum of the compound recorded in  $CDCl_3$ , singlet of 1H at 8.62 for CH of pyrimidine ring, 17H of aromatic CH appeared at 7.31-8.77, singlet of 1H at 5.33 for NH, disappeared on D2O exchange and singlet of 2H for  $CH_2$  recorded at 3.85.  $^{13}C$  NMR of the final compound showed at 168.05, 167.78 for C=O, 164.38 for C=N of pyrimidine, 155.3-113.43 for aromatic carbon, and 54.41 for  $CH_2$  confirming the formation of a final compound.

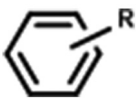
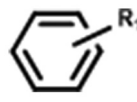
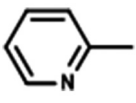
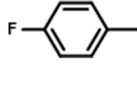
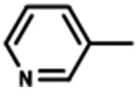
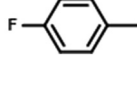
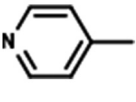
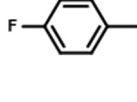
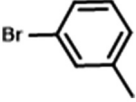
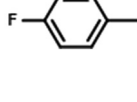
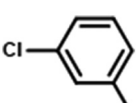
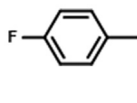
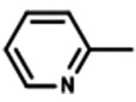
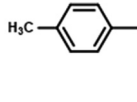
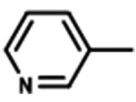
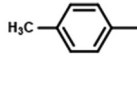
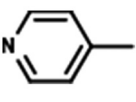
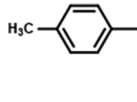
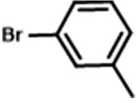
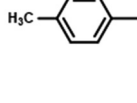
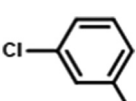
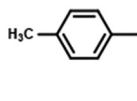


**Fig. 1: General synthesis of 2-chloro-N-[4-(substituted phenyl)-6-(4-substituted phenyl) pyrimidin-2-yl] acetamide derivatives. Reagents and conditions: (a)  $CH_3OH$ , NaOH, stirred at room temperature 2 hrs, (b) guanidine nitrate,  $CH_3ONa$ ,  $CH_3OH$ , reflux 8 hrs (c) dichloromethane, chloroacetyl chloride, and triethylamine**



**Fig. 2: General synthesis of 2-(2-(3-bromo benzylidene)-1-isonicotinoyl hydrazinyl)-N-(4-(substituted phenyl)-6-(substituted aryl) pyrimidin-2-yl) acetamide derivatives. Reagents and conditions: (a)  $CH_3OH$ ,  $CH_3COOH$ , reflux 2 hrs (b)  $CH_3OH$ ,  $K_2CO_3$ , reflux 4 hrs**

Table 1: Physical data of the compounds (2<sub>A-J</sub>)

Component number			M.P.°C	Yield %
2 <sub>A</sub>			210-211	56
2 <sub>B</sub>			180-182	66
2 <sub>C</sub>			190-195	60
2 <sub>D</sub>			155-157	56
2 <sub>E</sub>			189-192	52
2 <sub>F</sub>			110-115	66
2 <sub>G</sub>			139-142	61
2 <sub>H</sub>			159-161	71
2 <sub>I</sub>			170-174	56
2 <sub>J</sub>			165-168	59

Mass spectra of the synthesized compounds showed M<sup>+</sup>/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<sub>A</sub>

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). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 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, CH<sub>2</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 400 MHz): 168.51, 165.62

(C=O); 162.91 (C=N, C<sub>v</sub>, pyrimidine); 155.30; 101.30-149.71; 146.91 (aromatic ring), 55.40 (CH<sub>2</sub>). MS (m/z): 609.09 ([M + H]<sup>+</sup>), 611.09 [M<sup>+</sup>+2], elemental analysis calculated for C<sub>30</sub>H<sub>21</sub>BrFN<sub>7</sub>O<sub>2</sub>: 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<sub>B</sub>

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). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 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);

3.85 (s, 2H, CH<sub>2</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz): 166.24, 165.74 (C=O); 160.20 (Pyrimidine); 149.97-101.75 (aromatic ring); 55.04 (CH<sub>2</sub>). MS (m/z): 609.09 ([M + H]<sup>+</sup>), 611.09 [M<sup>+</sup>+2], elemental analysis calculated for C<sub>30</sub>H<sub>21</sub>BrFN<sub>7</sub>O<sub>2</sub>: C 59.03, H 3.47, N 16.06; found: C 59.05, H 3.40, N 16.04.

(*E*)-2-(2-(3-bromobenzylidene)-1-isonicotinoylhydrazinyl)-N-(4-(4-fluorophenyl)-6-(pyridin-4-yl)pyrimidin-2-yl)acetamide 2<sub>C</sub>

IR: 3423.46, 3351.91 (N-H), 3125.58, 3057.84, (C-H aromatic), 1657.98 (-CONH), 1549.72 (N-H bending), 1429.32, 1476.87 (C=C, aromatic), 1356.10, 1252.29, 1218.37, and 1180.87 (C-N), 1067.31 (C-F) 567.86 (mono substituted Br). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz): 8.61 (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, CH<sub>2</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz): 168.51, 162.91 (C=O); 160.60 (pyrimidine); 106.20-148.82 (aromatic ring); 55.41 (CH<sub>2</sub>). MS (m/z): 609.09 ([M + H]<sup>+</sup>), 611.09 [M<sup>+</sup>+2], elemental analysis calculated for C<sub>30</sub>H<sub>21</sub>BrFN<sub>7</sub>O<sub>2</sub>: C 59.03, H 3.47, N 16.06; found: C 59.15, H 3.39, N 16.10.

(*E*)-2-(2-(3-bromobenzylidene)-1-isonicotinoylhydrazinyl)-N-(4-(3-bromophenyl)-6-(4-fluorophenyl)pyrimidin-2-yl)acetamide 2<sub>D</sub>

IR: 3472.56, 3303.45 (N-H), 3056.70, (C-H aromatic), 1651.75 (-CONH), 1591.78 (N-H bending), 1428.37, 1472.37 (C=C, aromatic), 1308.26, 1234.07, 1165.55, and 1140.44 (C-N), 1024.41 (C-F) 573.76 (mono substituted Br). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz): 8.61 (s, 1H, CH of pyrimidine ring); 7.31-8.77 (m, 16H, Ar-H); 8.39 (s, 1H, CH); 5.35 (s, 1H, CONH); 3.85 (s, 2H, CH<sub>2</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz): 168.54, 162.92 (C=O); 140.81-107.08; 55.41 (CH<sub>2</sub>). MS (m/z): 688.09 ([M + H]<sup>+</sup>), 690.09 [M<sup>+</sup>+2], elemental analysis calculated for C<sub>31</sub>H<sub>21</sub>Br<sub>2</sub>FN<sub>6</sub>O<sub>2</sub>: C 54.09, H 3.08, N 12.21; found: C 54.11, H 3.09, N 12.23.

(*E*)-2-(2-(3-bromobenzylidene)-1-isonicotinoylhydrazinyl)-N-(4-(3-chlorophenyl)-6-(4-fluorophenyl)pyrimidin-2-yl)acetamide 2<sub>E</sub>

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, 1183.49, and 1140.64 (C-N), 1024.41 (C-F), 688.12 (C-Cl), 573.76 (mono substituted Br). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz): 8.61 (s, 1H, CH of pyrimidine ring); 7.31-8.77 (m, 17H, Ar-H); 8.38 (s, 1H, CH); 5.35 (s, 1H, CONH); 3.85 (s, 2H, CH<sub>2</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz): 168.51, 162.69 (C=O); 146.95-107.08 (aromatic ring); 55.41 (CH<sub>2</sub>). MS (m/z): 642.90 ([M + H]<sup>+</sup>), 643.88 [M<sup>+</sup>+1], 644.09 [M<sup>+</sup>+2], elemental analysis calculated for C<sub>31</sub>H<sub>21</sub>BrClFN<sub>6</sub>O<sub>2</sub>: C 57.83, H 3.29, N 13.05; found: C 57.81, H 3.33, N 13.03.

(*E*)-2-(2-(3-bromobenzylidene)-1-isonicotinoylhydrazinyl)-N-(4-(pyridin-2-yl)-6-(p-tolyl)pyrimidin-2-yl)acetamide 2<sub>F</sub>

IR: 3352.46 (N-H), 3056.39, 3022.38 (C-H aromatic), 2941.41, 2932.4 (C-H), 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). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz): 8.64 (s, 1H, CH of pyrimidine ring); 7.42-8.77 (m, 16H, Ar-H); 8.39 (s, 1H, CH); 5.34 (s, 1H, CONH); 3.85 (s, 2H, CH<sub>2</sub>); 2.34 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz): 168.35, 165.46 (C=O); 162.76 (pyrimidine); 155.03; 149.71-101.39; 55.43 (CH<sub>2</sub>), 21.34 (CH<sub>3</sub>). MS (m/z): 605.03 ([M + H]<sup>+</sup>), 606.13 [M<sup>+</sup>+1], 607.12 [M<sup>+</sup>+2], elemental analysis calculated for C<sub>31</sub>H<sub>24</sub>BrN<sub>7</sub>O<sub>2</sub>: C 61.39, H 3.99, N 16.17; found: C 61.34, H 3.97 N 16.11.

(*E*)-2-(2-(3-bromobenzylidene)-1-isonicotinoylhydrazinyl)-N-(4-(pyridin-3-yl)-6-(p-tolyl)pyrimidin-2-yl)acetamide 2<sub>G</sub>

IR: 3431.13, 3331.42 (N-H), 3032.39, (C-H aromatic), 2932.50 (C-H), 1671.21 (-CONH), 1539.21(N-H bend), 1412.46, 1441.37, 1461.37 (C=C, aromatic), 1353.33, 1274.26, 1218.37, 1183.49, 1140.44 (C-N), 573.76 (mono substituted Br). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz): 8.57 (s, 1H, CH of pyrimidine ring), 7.12-9.25 (m, 16H, Ar-H); 8.36 (s, 1H, CH); 5.23 (s, 1H, CONH), 3.85 (s, 2H, CH<sub>2</sub>); 2.34 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100

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

Code number	MIC µg/mL			
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. pyogenes</i>
2 <sub>A</sub>	200	100	250	100
2 <sub>B</sub>	250	200	250	125
2 <sub>C</sub>	200	100	500	200
2 <sub>D</sub>	100	125	500	250
2 <sub>E</sub>	250	200	250	250
2 <sub>F</sub>	250	500	500	200
2 <sub>G</sub>	500	250	250	125
2 <sub>H</sub>	100	125	200	125
2 <sub>I</sub>	500	100	125	200
2 <sub>J</sub>	125	250	200	125
Ampicillin	100	100	250	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	25	25	50	50

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

MHz): 165.18; 163.93 (C=O); 149.99-123.23; 55.34 (CH<sub>2</sub>), 21.14 (CH<sub>3</sub>). MS (m/z): 605.13 ([M + H]<sup>+</sup>), 606.12 [M<sup>+</sup>+1], 607.13 [M<sup>+</sup>+2], elemental analysis calculated for C<sub>31</sub>H<sub>24</sub>BrN<sub>7</sub>O<sub>2</sub>: C 61.39, H 3.99, N 16.17; found: C 61.40, H 3.98 N 16.19.

(*E*)-2-(2-(3-bromobenzylidene)-1-isonicotinoylhydrazinyl)-N-(4-(pyridin-4-yl)-6-(p-tolyl)pyrimidin-2-yl)acetamide 2<sub>H</sub>

IR: 3423.86 (N-H), 3032.19, (C-H aromatic), 1678.21 (-CONH), 1532.21(N-H bend), 1412.46, 1451.37, (C=C, aromatic), 1353.33, 1274.26, 1218.37, 1176.23, 1148.21 (C-N), 571.76 (mono substituted Br). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz): 8.62 (s, 1H, CH of pyrimidine ring), 7.59-8.77 (m, 16H, Ar-H); 8.39 (s, 1H, CH); 5.31 (s, 1H, CONH); 3.85 (s, 2H, CH<sub>2</sub>); 2.34 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz): 168.25, 165.66; 162.76 (C=O); 157.73 (Pyrimidine); 149.71-106.23 (aromatic ring); 55.43 (CH<sub>2</sub>), 21.33 (CH<sub>3</sub>). MS (m/z): 605.13 ([M + H]<sup>+</sup>), 606.11 [M<sup>+</sup>+1], 607.12 [M<sup>+</sup>+2], elemental analysis calculated for C<sub>31</sub>H<sub>24</sub>BrN<sub>7</sub>O<sub>2</sub>: C 61.39, H 3.99, N 16.17; found: C 61.41, H 3.97 N 16.18.

(*E*)-2-(2-(3-bromobenzylidene)-1-isonicotinoylhydrazinyl)-N-(4-(3-bromophenyl)-6-(p-tolyl)pyrimidin-2-yl)acetamide 2<sub>I</sub>

IR: 3472.56, 3303.45 (N-H), 3199.37, 3060.39, 3032.38 (C-H aromatic), 2951.44, 2922.4 (C-H), 1683.22 (-CONH), 1546.21 (N-H bend), 1412.36, 1428.37, 1472.37 (C=C, aromatic), 1353.33, 1274.26, 1218.37, 1183.49, 1140.44, 1189.57 (C-N), 573.76 (mono substituted Br). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz): 7.42-8.77 (m, 17H, Ar-H); 8.39 (s, 1H, CH); 5.34 (s, 1H, CONH); 3.85 (s, 2H, CH<sub>2</sub>); 2.34 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz): 168.55, 165.76 (C=O); 160.26, 156.03 (pyrimidine); 149.71-107.09 (aromatic ring); 55.43 (CH<sub>2</sub>), 21.53 (CH<sub>3</sub>). MS (m/z): 684.03 ([M + H]<sup>+</sup>), 686.03 [M<sup>+</sup>+2], elemental analysis calculated for C<sub>32</sub>H<sub>24</sub>Br<sub>2</sub>N<sub>6</sub>O<sub>2</sub>: C 56.16, H 3.53, N 12.28; found: C 56.24, H 3.55, N 12.24.

(*E*)-2-(2-(3-bromobenzylidene)-1-isonicotinoylhydrazinyl)-N-(4-(3-chlorophenyl)-6-(p-tolyl)pyrimidin-2-yl)acetamide 2<sub>J</sub>

IR: 3472.56, 3303.45 (N-H), 3199.37, 3060.39, 3032.38 (C-H aromatic), 2951.44, 2922.4 (C-H), 1679.22 (-CONH), 1534.21 (N-H bend), 1412.36, 1428.34, 1471.37 (C=C, aromatic), 1351.33, 1264.26, 1218.37, 1183.49, 1140.44 (C-N), 834.23 (C-Cl) 581.76 (mono substituted Br). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz): 7.15-8.77 (m, 17H, Ar-H); 8.39 (s, 1H, CH); 5.34 (s, 1H, CONH); 3.85 (s, 2H, CH<sub>2</sub>); 2.34 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz): 168.89, 165.67 (C=O); 160.61, 156.03 (pyrimidine); 149.71-107.80 (aromatic ring); 55.43 (CH<sub>2</sub>), 21.3 (CH<sub>3</sub>). MS (m/z): 638.08 ([M + H]<sup>+</sup>), 639.08 [M<sup>+</sup>+1], 640.03, elemental analysis calculated for C<sub>32</sub>H<sub>24</sub>BrClN<sub>6</sub>O<sub>2</sub>: C 60.06, H 3.78, N 13.13; found: C 60.04, H 3.75, N 13.10.

Table 3: *In vitro* antifungal and antitubercular activity (MIC, µg/mL) of compounds

Code number	Minimal fungicidal concentration (µg/mL)			MIC µg/mL <i>M. tuberculosis</i> H <sub>37</sub> RV
	<i>C. albicans</i>	<i>A. niger</i>	<i>A. clavatus</i>	
2 <sub>A</sub>	500	1000	1000	250
2 <sub>B</sub>	1000	500	1000	500
2 <sub>C</sub>	1000	>1000	>1000	250
2 <sub>D</sub>	500	1000	>1000	500
2 <sub>E</sub>	500	>1000	>1000	1000
2 <sub>F</sub>	1000	>1000	>1000	1000
2 <sub>G</sub>	1000	500	1000	250
2 <sub>H</sub>	>1000	250	500	250
2 <sub>I</sub>	>1000	200	250	1000
2 <sub>J</sub>	>1000	200	500	62.5
Griseofulvin	500	100	100	-
Nystatin	100	100	100	-
Rifampicin	-	-	-	40
Isoniazid	-	-	-	0.20

MIC: Minimal inhibitory concentration, *C. albicans*: *Candida albicans* (MTCC no. 227), *A. niger*: *Aspergillus niger* (MTCC no. 282), *A. clavatus*: *Aspergillus clavatus* (MTCC no. 1323), *M. tuberculosis*: *Mycobacterium tuberculosis* 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 micro dilution 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 antibacterial 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 antiviral; zidovudine and stavudine as anti-HIV, trimethoprim, sulfamethazine, and sulfadiazine as antibacterial; sulfadoxine as antimalarial and antibacterial; minoxidil and prazosin as antihypertensive; barbiturates, e.g., phenobarbitone as sedative, hypnotics and anticonvulsant; propylthiouracil as antithyroid; thonzylamine as H1-antihistamine; toxoflavin and ferverulin as antibiotics. In this study, pyrimidine clubbed Schiff base of isoniazid have been taken for dual evaluations antimicrobial as well as antituberculosis. Isoniazid itself used as first-line antituberculosis drug. When the Schiff of isoniazid clubbed with pyrimidine moiety the resulted compounds revealed good activities. Compounds 2<sub>A,E</sub> with fluorine group and 2<sub>G,I</sub> with a methyl group showed good antibacterial activity. Whereas fluorine group containing compounds 2<sub>A'</sub>, 2<sub>D'</sub>, and 2<sub>E</sub> was active toward *C. albicans*. Only one compound 2<sub>J</sub> showed good potential toward *M. tuberculosis* H<sub>37</sub>RV. All the compounds of this series with fluorine and the methyl group showed good potential as an antimicrobial agent and antituberculosis also which open door for future investigation. The results of all compounds against antibacterial activity were displayed in Table 2. Antifungal and antituberculosis activity results of the all compounds were displayed in Table 3. All synthesized compounds conducted at 250 µg/mL, 500 µg/mL, and 1000 µg/mL for antituberculosis activity.

### CONCLUSION

Most of the synthesized compounds showed good antibacterial activity against Gram-positive as well as Gram-negative bacteria.

Compounds 2<sub>D</sub> and 2<sub>H</sub> were equally active against *E. coli*. Compound 2<sub>A'</sub>, 2<sub>C'</sub>, and 2<sub>I</sub> showed very good activity toward *P. aeruginosa*. 2<sub>A'</sub>, 2<sub>B'</sub>, 2<sub>E'</sub>, 2<sub>G'</sub>, 2<sub>H'</sub>, 2<sub>I'</sub>, and 2<sub>J</sub> showed excellent activity toward *S. aureus*. Only 2<sub>A</sub> showed good activity against *S. pyogenes* as compared to standard drug ampicillin. Antifungal activity of compound 2<sub>A'</sub>, 2<sub>D'</sub>, and 2<sub>E</sub> equal against *C. albicans* compared with standard drug Griseofulvin. Other compounds were moderately sensitive toward *A. niger* and *A. clavatus*. All the compounds were active against *Mycobacterium tuberculosis* H<sub>37</sub>RV, but mainly more active compounds were 2<sub>A'</sub>, 2<sub>B'</sub>, 2<sub>C'</sub>, 2<sub>D'</sub>, 2<sub>G'</sub>, 2<sub>H'</sub>, and 2<sub>J</sub>. Among of them, compound 2<sub>J</sub> was highly active moiety as compared to another compound. The final conclusion of this series is that this class of molecules certainly holds great promise toward the pursuit to discover the novel class of antimicrobial and antituberculosis agents.

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