INTRODUCTION

Isoniazid (isonicotinic acid hydrazide) used as the first-line antibiotic for tuberculosis treatment since 1952. It is produg 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 antitumorogenic activities and showed putative activity against osteoarthritis and granuloma annulare. 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, antiviral, antimalarial, and anti-inflammatory [3-9]. Structural modification of pyrimidine with many moieties was comparatively high as compared with standard drugs. Antifungal activity of many moieties was comparatively high as compared with standard drug Griseofulvin. Where some of the newly synthesized compounds showed good antibacterial 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⁻¹. The 1H-NMR and 13C-NMR spectra were recorded on a Bruker Advance II 400 spectrometer (100 MHz FT-NMR) using tetramethylsilane as the internal standard in deuterochloroform (CDCl₃) and dimethyl sulfoxide (DMSO-d₆) (chemical shifts in ppm). Mass spectra recorded in Waters QTQ 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 1110 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-(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) methyldene]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 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 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 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 quarter of plate of medium sui-

Fig. 1: General synthesis of 2-chloro-N-[4-(substituted phenyl)-6-(substituted aryl) pyrimidin-2-yl] acetamide derivatives. Reagents and conditions: (a) CH3OH, NaOH, stirred at room temperature 2 hrs, (b) guanidine nitrate, CH3ONa, CH3OH, 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) CH3OH, CH3COOH, reflux 2 hrs (b) CH3OH, K2CO3, reflux 4 hrs
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).
1H-NMR (CDCl3, 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 (CDCl3, 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 (CH3). MS (m/z): 609.09 ([M + H]+), 611.09 [M+2], elemental analysis calculated for C30H21BrFN7O2: 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). MS (m/z): 609.09 ([M + H]+), 611.09 [M+2], elemental analysis calculated for C30H21BrFN7O2: 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)

| Component number | M.P.°C  | Yield % |
|------------------|---------|---------|
| 2a               | 210-211 | 56      |
| 2b               | 180-182 | 66      |
| 2c               | 190-195 | 60      |
| 2d               | 155-157 | 56      |
| 2e               | 189-192 | 52      |
| 2f               | 110-115 | 66      |
| 2g               | 139-142 | 61      |
| 2h               | 159-161 | 71      |
| 2i               | 170-174 | 56      |
| 2j               | 165-168 | 59      |
Table 2: In vitro antibacterial activity (MIC, µg/mL) of the synthesized compounds

| Code number | MIC µg/mL |
|-------------|-----------|
| **E. coli** | **P. aeruginosa** | **S. aureus** | **S. pyogenes** |
| Z1          | 200 100 250 100 | 250 200 250 125 | 200 100 250 250 | 250 500 250 200 |
| Z2          | 200 100 250 100 | 250 200 250 125 | 200 100 250 250 | 250 500 250 200 |
| Z3          | 100 125 250 200 | 100 125 200 125 | 100 125 200 125 | 100 125 200 125 |
| Z4          | 500 250 250 125 | 125 250 200 125 | 125 250 200 125 | 125 250 200 125 |
| Z5          | 100 125 250 125 | 50 100 50 50 | 50 100 50 50 | 50 100 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: Microbiological type culture collection.

(E)-2-(3-bromobenzylidene)-1-isonicotinoylhydrazinyl-N-(4-(4-fluorophenyl)-6-(pyridin-2-yl)pyrimidin-2-yl)acetamide $Z_2$
IR: 3423.46, 3351.91 (N-H), 1325.58, 3058.84 (C-H aromatic), 1675.97 (C=O), 1594.72 (N-H bending), 1429.32, 1476.87 (C=C, aromatic), 1536.10, 1522.99, 1218.37, and 1180.87 (C-N), 1067.31 (C-F) 567.86 (mono substituted Br), $^13$C-NMR (CDCl$_3$, 100 MHz): 8.63 (s, 1H, CH of pyrimidine ring); 7.12-7.77 (m, 16H, Ar-H); 8.39 (s, 1H, CH); 5.35 (s, 1H, CONH); 3.85 (s, 2H, CH$_2$). $^13$C-NMR (CDCl$_3$, 100 MHz): 168.54, 163.92 (C=O); 124.81 (CH of pyrimidine ring), 7.31-7.87 (m, 17H, Ar-H); 8.38 (s, 1H, CH); 5.35 (s, 1H, CH$_2$). $^13$C-NMR (CDCl$_3$, 100 MHz): 168.51, 162.69 (C=O); 146.95-107.08 (aromatic ring); 55.41 (CH$_2$). MS (m/z): 609.09 [+M + H$^+$], 610.69 [M$^+$+2], elemental analysis calculated for C$_{39}$H$_{28}$BrN$_3$O$_2$: C 59.03, H 3.47, N 16.06; found: C 59.15, H 3.39, N 16.10.

(E)-2-(3-bromobenzylidene)-1-isonicotinoylhydrazinyl-N-(4-(3-bromophenyl)-6-(4-fluorophenyl)pyrimidin-2-yl)acetamide $Z_2$
IR: 3472.56, 3303.45 (N-H), 3056.70, 1651.75 (C=O), 1591.78 (N-H bending), 1428.37, 1472.37 (C=C, aromatic), 1308.26, 1234.07, 1165.55, and 1140.44 (C-N). $^1$H-NMR (CDCl$_3$, 400 MHz): 7.31-8.77 (m, 16, H, Ar-H); 8.39 (s, 1H, CH); 5.35 (s, 1H, CH$_2$). $^13$C-NMR (CDCl$_3$, 100 MHz): 168.54, 156.20 (Pyrimidine); 149.99-143.77 (aromatic ring); 55.41 (CH$_2$). MS (m/z): 607.12 [+M + H$^+$], 608.12 [M$^+$+1], 607.13 [M$^+$+2], elemental analysis calculated for C$_{34}$H$_{20}$BrN$_3$O$_2$: C 59.27, H 3.30, N 16.17; found: C 59.24, H 3.30, N 16.17.

(E)-2-(3-bromobenzylidene)-1-isonicotinoylhydrazinyl-N-(4-(pyridin-3-yl)-6-(p-tolyl)pyrimidin-2-yl)acetamide $Z_2$
IR: 3341.42 (N-H), 3063.99, 3033.38 (C-H aromatic), 1673.22 (C=O), 1553.21 (N-H bending), 1433.36, 1472.37 (C=C, aromatic), 1267.26, 1218.37, 1184.49, and 1140.64 (C-N). $^1$H-NMR (CDCl$_3$, 400 MHz): 6.80 (s, 2H, C=O); 5.73 (6H, Ar-H); 7.31-8.77 (m, 17H, Ar-H); 8.38 (s, 1H, CH); 5.35 (s, 1H, CH$_2$). $^13$C-NMR (CDCl$_3$, 100 MHz): 168.51, 162.69 (C=O); 146.95-107.08 (aromatic ring); 55.41 (CH$_2$). MS (m/z): 642.90 ([M + H$^+$], 643.88 [M$^+$+1], 644.09 [M$^+$+2], elemental analysis calculated for C$_{30}$H$_{20}$BrN$_3$O$_2$: C 57.83, H 3.29, N 13.05; found: C 57.81, H 3.33, N 13.03.

(E)-2-(3-bromobenzylidene)-1-isonicotinoylhydrazinyl-N-(4-(pyridin-2-yl)-6-(p-toly1)pyrimidin-2-yl)acetamide $Z_2$
IR: 3352.46 (N-H), 3056.39, 3022.38 (C-H aromatic). $^1$H-NMR (CDCl$_3$, 400 MHz): 7.167.22 (C=O), 1541.21 (N-H bending), 1413.36, 1423.38 (C=C, aromatic), 1256.26, 1234.73, 1123.49 (C-N), 567.76 (mono substituted Br). $^13$C-NMR (CDCl$_3$, 100 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); 3.24 (s, 3H, CH$_3$). $^13$C-NMR (CDCl$_3$, 100 MHz): 168.35, 165.46 (C=O). MS (m/z): 162.76 (pyrimidine), 150.03, 147.91-101.39; 55.43 (CH$_2$, 21.34 (CH$_3$). MS (m/z): 605.03 (+M$^+$), 606.13 [M$^+$+1], 607.12 [M$^+$+2], elemental analysis calculated for C$_{37}$H$_{21}$BrN$_3$O$_2$: C 63.19, H 3.99, N 16.17; found: C 63.14, H 3.97 N 16.11.
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 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 antiviral; zidovudine and stavudine as anti-HIV, trimethoprim, sulfamethazine, 5-fluorouracil as anticancer; idoxuridine and trifluridine as antiviral; toxoflavin and fervenulin as antibiotics. In this study, pyrimidine clutered 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 clutered with pyrimidine moiety the resulted compounds revealed good activities. Compounds 2_{A} and 2_{C} with fluorine group and 2_{C} with a methyl group showed good antibacterial activity. Whereas fluorine group containing compounds 2_{A}-2_{B} and 2_{E} was active toward C. albicans. Only one compound 2_{E} showed good potential toward M. tuberculosis H_{37}RV. All the compounds of this series with fluorine and the methyl group showed good potential as an antimicrobial agent and antituberculosis which also 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_{A} and 2_{C} were equally active against E. coli. Compound 2_{B}, 2_{E}, and 2_{F} showed very good activity toward P. aeruginosa. 2_{B}, 2_{E}, and 2_{F} showed excellent activity toward S. aureus. Only 2_{E} showed good activity against S. pyogenes as compared to standard drug ampicillin. Antifungal activity of compound 2_{A}, 2_{C}, and 2_{E} 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_{37}RV, but mainly more active compounds were 2_{B}, 2_{C}, 2_{E}, 2_{F}, and 2_{G}. Among of them, compound 2_{E} was highly active mostly 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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