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Efficient and green approach for the synthesis of indolyl-pyrimidine derivatives and investigation of biological assays

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The present study reports the synthesis of biologically active chalcones **3a–d** and indolyl-pyrimidine derivatives **5a–d**. The newly synthesized compounds have been characterized using physicochemical techniques, including melting point determination, thin-layer chromatography (TLC), and spectral methods such as infrared (IR) spectroscopy, ^{1}H and ^{13}C NMR, and mass spectrometry. This synthetic approach is advantageous due to its simplicity, cost-effectiveness, and mild reaction conditions. Among the synthesized compounds, compounds **3d** and **5b** exhibit notable radical scavenging activity (RSA) at a concentration of 25 µg/mL, compounds **3a, 3c, 3d, 5b** and **5c** show good RSA at 75 µg/mL. **5c** exhibits good RSA at concentration of 50 µg/mL. Compound **3a** shows best result at a concentration of 75 µg/mL. Compound **3b** demonstrates potent activity with an MIC of 62.5 µg/mL against *Escherichia coli* (MTCC-723) and equipotent activity (125µg/mL) against all other tested bacteria. Compound **3a** shows potent activity (62.5µg/mL) against *Staphylococcus aureus* (ATCC-29513). **3a** exhibits potent activity (62.5 µg/mL) against *Aspergillus niger* (MTCC-281). Compound **3a** exhibits excellent activity against *M. tuberculosis* H37Rv, with an MIC of 3.125 µg/mL, which is comparable to the standard drugs Pyrazinamide (MIC 3.125 µg/mL) and superior to Streptomycin (MIC 6.25 µg/mL). Compounds **3d** and **5c** shows good activity with MIC values of 6.25 µg/mL against *M. tuberculosis* H37Rv.

Keywords: Synthesis, Indolyl-pyrimidine, Antioxidant, Antimicrobial activity, Antitubercular activity

Schiff base (SB), is a versatile compound discovered by chemist Hugo Schiff, it's also termed as imine or azomethine (-C=N-). Shiff base ligands form readily with aldehydes than ketones[1]. Study on SB has been done due to its very flexible character and different structures. SB form stable complexes with metal ions[2,3]. At very high temperature and in the presence of moisture many SB shows catalytic activity in various reactions. SB acts as an important intermediate in many enzymatic reactions which involves the interaction of an enzyme with carbonyl or an amino group of the substrate[4,5]. In the field of organic chemistry, SB shows large number of synthetic uses. It is widely used in organic compounds such as pigment, dyes, catalysts, intermediates and polymer stabilizers[6]. In the recent years, there has been an increased interest in the application of antioxidants to medicinal treatment as information is constantly gathered linking the development of human diseases to oxidative stress. Schiff base are characterized by the imine group which is important in the elucidating them the mechanism of Trans amination and racemzation reactions in biological systems.

Fused pyrimidines continue to attract considerable interest due to their broad spectrum of biological activities and significant practical applications[7]. This is particularly evident from the growing number of reviews and studies focusing on systems where the pyrimidine ring is fused with various heterocyclic frameworks, including pyridopyrimidines, triazolopyrimidines, pyrazolopyrimidines, and furopyrimidines. These fused heterocycles represent an important class of compounds with diverse pharmaceutical and biological properties[7,8]. As a result, the development of versatile and efficient synthetic methods for pyrimidine derivatives remains a prominent area of research. Pyrimidines and their analogs play a critical role in the development of therapeutic agents and agricultural chemicals[9,10]. Numerous pyrimidine derivatives have been reported to exhibit a wide range of biological activities, including antimycobacterial[11], antitumor[12], antiviral[13], anticancer[14], anti-inflammatory[15], and antimicrobial properties[16].

In continuation of our previous research efforts[17–28], the present study reveals that the synthesis of a series

of chalcone and pyrimidine analogs (**3a–d** and **5a–d**). The synthesized compounds were evaluated for their biological activities, with the aim of identifying potential therapeutic agents. These findings may contribute to the development of novel and more effective drug candidates for the treatment of various diseases.

Results and Discussion

Chemistry

An efficient method for the synthesis of 4-(1*H*-indol-3-yl)-pyrimidin-2-amine derivatives was developed via the Claisen–Schmidt condensation reaction. An equimolar mixture of indole-3-carboxaldehyde **(1)** and acetophenone **(2a)** was stirred in 20% ethanolic sodium hydroxide under cold conditions for 1 hour. The resulting mixture was then refluxed on a water bath for 7 hours, leading to the formation of 3-(1*H*-indol-3-yl)-1-phenylprop-2-en-1-one **(3a),** a chalcone intermediate. The synthesized chalcones **(3b–3d)** were subsequently reacted with guanidine hydrochloride in the presence of 20% ethanolic sodium hydroxide under refluxed temperature to afforded the target 4-(1*H*-indol-3-yl)-6-phenylpyrimidin-2-amines **(5a).** The same procedure was applied for the synthesis of the corresponding derivatives **(5b–5d).** The structures of the synthesized compounds **(5a–5d)** were confirmed through spectral analysis, including IR, ^{1}H and ^{13}C NMR**,** and mass spectrometry**.** The synthetic pathway is outlined in Scheme 1, and detailed analytical and spectral data are provided in the Experimental Section.

Biological activities

Antioxidant activities

1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity (RSA)

The results are illustrated in Fig. 1. Among the tested compounds, **3d** & **5b** exhibited notable radical scavenging activity (RSA) of at a concentration of 25 µg/mL. The compounds **3a, 3c, 3d, 5b** & **5c** good RSA at 75 µg/mL. **5c** exhibited good RSA at concentration 50 µg/mL. **3a** showed best result at a concentration of 75 µg/mL. The best result of RSA of compound **3a** may be attributed to the presence of a phenyl ring linked to the indole nucleus. This electron donating effect contributes to the

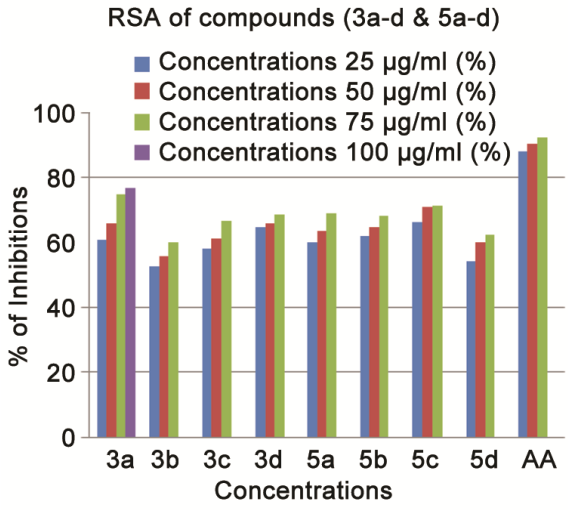
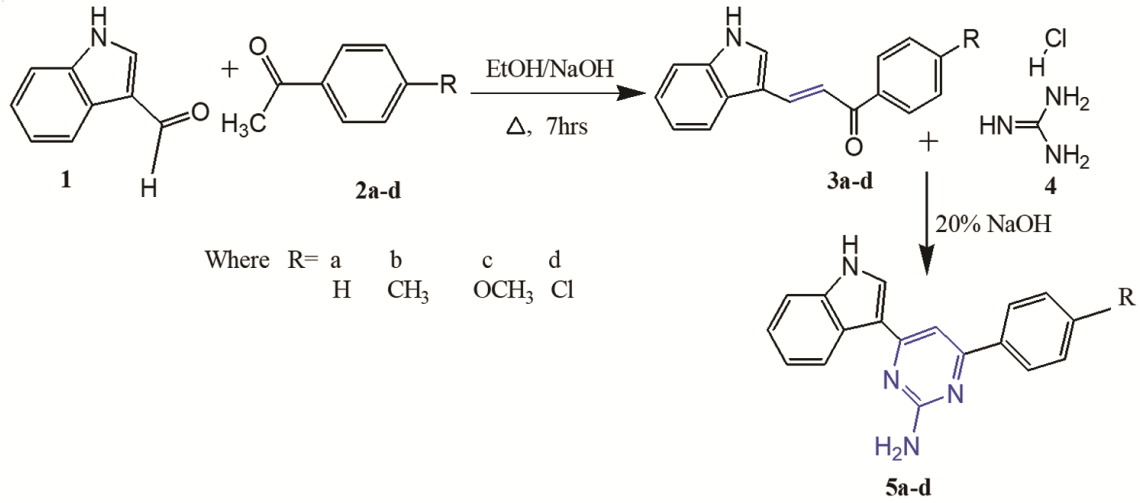


Fig. 1 — RSA of compounds **3a-d** and **5a-d**



Scheme 1 — Schematic route synthetic of compounds **3a-d** and **5a-d**

stabilization of the free radical intermediate formed after hydrogen atom donation to the DPPH radical. Despite these promising results, none of the synthesized compounds exhibited antioxidant activity superior to that of the standard.

Antimicrobial Activity Results

The MIC values indicate that most of the tested compounds exhibited activity in the concentration range of 62.5–500 µg/mL, which is comparatively more potent or equipotent than the standards gentamicin and fluconazole, respectively[19]. The antibacterial screening results are shown in Fig. 2. Compound **3b** demonstrated potent activity with an MIC of 62.5 µg/mL against *Escherichia coli* (MTCC-723) and equipotent activity (125µg/mL) against all other tested bacteria. Compound **3a** showed potent activity (62.5µg/mL) against *Staphylococcus aureus* (ATCC-29513). The potent activity of compound **3b** may be attributed to the presence of a methyl group at the 4-position of the phenyl ring in the chalcone moiety. While compound **3b** showed consistent equipotent activity against all tested bacteria, the remaining compounds in the series exhibited equipotent to lesser activity.

The antifungal screening results, shown in Fig. 3, revealed that compound **3a** exhibited potent activity (62.5 µg/mL) against *Aspergillus niger* (MTCC-281), possibly due to the presence of the phenyl group. The other compounds in the series showed moderate to low antifungal activity. These screening studies demonstrate that the newly synthesized compounds possess promising antibacterial and antifungal properties. Therefore, further investigation of this class of compounds is warranted.

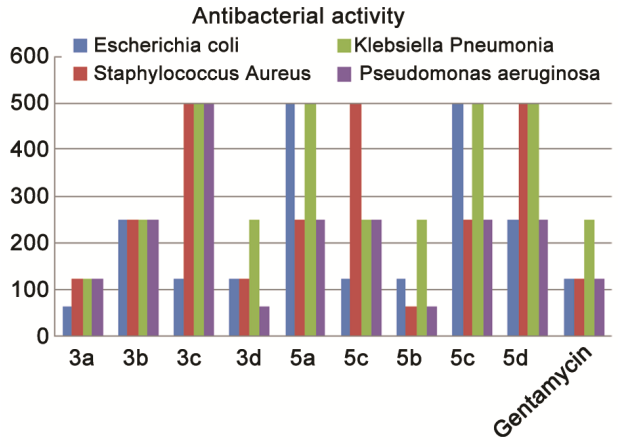


Fig. 2 — Antibacterial Activity of compounds **3a-d** and **5a-d**

Antitubercular activity

The antitubercular activity results are presented in Fig. 4. All compounds **(3a–d** and **5a–c)** were tested for inhibitory activity against *Mycobacterium tuberculosis* H37Rv (ATCC 2794)[20]. The minimum inhibitory concentration (MIC), expressed in µg/mL, was determined for each compound. Compound **3a** exhibited excellent activity against *M. tuberculosis* H37Rv, with an MIC of 3.125 µg/mL, which is comparable to the standard drugs Pyrazinamide (MIC 3.125 µg/mL) and superior to Streptomycin (MIC 6.25 µg/mL). Compounds **3d** and **5c** showed good activity with MIC values of 6.25 µg/mL against *M. tuberculosis* H37Rv. Preliminary structure–activity relationship (SAR) analysis suggests that the presence of a phenyl ring in the chalcone scaffold may contribute to the enhanced antitubercular activity observed in this series of compounds.

Experimental Section

All the reagents were obtained commercially and used by further purification using standard procedures. Melting points were determined by an open capillary method and are uncorrected. The purity of the

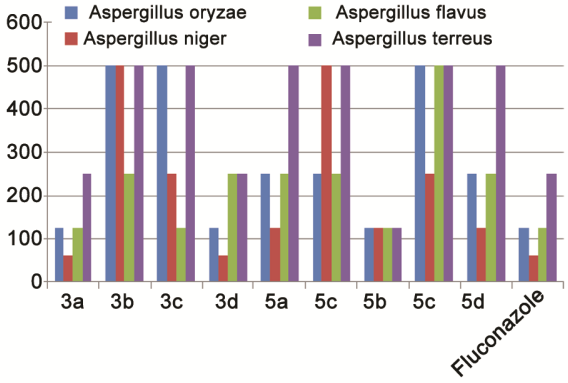


Fig. 3 — Antifungal activity of compounds **3a-d** and **5a-d**

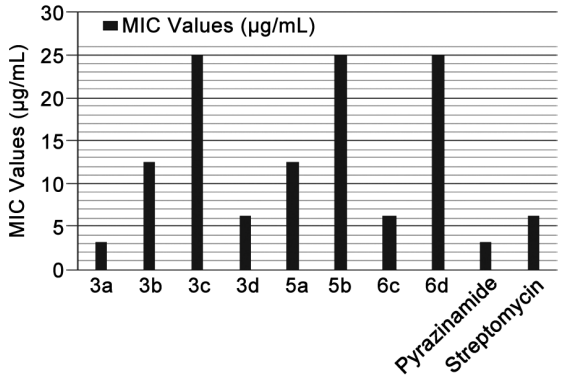


Fig. 4 — Antitubercular activity of compounds **3a-d** and **5a-d**

compounds was checked by thin layer chromatography using silica gel-G coated Al plates (Merck) and spots were visualized by exposing the dry plates in iodine vapors. The IR (KBr pellet) spectra were recorded on a Perkin-Elmer (Spectrum ONE) FT-IR Spectrometer. The ^{1}H and ^{13}C NMR (DMSO-d_6) spectra were recorded with a BRUKER NMR 500 and 125 MHz spectrometers, and the chemical shift values are expressed in ppm (δ scale) using tetramethylsilane as an internal standard. The mass spectral measurements were carried out by Electron Impact method on JEOL GC mate spectrometer at 70 eV. Elemental analyses were performed on flash EA 1112 series elemental analyzer.

General procedure for the synthesis of 3-(1*H*-indol-3-yl)-1-*p*-substitited prop-2-en-1-one, 3a-d

A mixture of indole-3-carboxaldehyde **(1)** (0.01mole) and substituted acetopheones **(2)** (0.01) was taken in a 100 mL conical flask. Than10 mL of ethanol (40%) was added to the reaction mixture and it was stirred for 45 min. TLC was carried out periodically to monitor the progress of the reaction. The mixture was stirred for 1 hr 30 mins which the reaction mixture was left to stand in a refrigerator for 12hrs. Thereafter, 50% of glacial acetic acid was added (in drops) until the supernatant was neutral to litmus. Then the reaction mixture was poured into ice-cold water the solid separate, the separated solid was filtered, washed and dried. The crude product was recrystalized by using ethanol to get pure **3a-d**.

3-(1*H*-Indol-3-yl)-1-phenylprop-2-en-1-one, 3a: Brownish amorphous. Yield 82%. m.p.214-15°C. FT-IR (KBr): 3318 (indole NH), 3055 (Ar -H), 2920 (CH-Aliphatic), 1664 (CO), 1605 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6): δ 10. 3(s, 1H, indole NH),6.20-7.47 (10H, Ar-H), 7.60 (d,1H, -CH =CH-, *J* = 13 Hz), 7.81 (d,1H, -CH =CH-, *J* = 13 Hz); ^{13}C NMR (DMSO-d_6): δ 110.6, 111.3, 119.1, 122.3, 126.6,127.8, 129.4, 129.9, 130.1, 134.4; 135.5,137.4, 145.2, 182.2; MS (EI): *m/z* M^{+} 247. Anal. Calcd for: $C_{17}H_{13}NO$: C, 82.57; H, 5.30; N, 5.66. Found: C, 82.59; H, 5.31; N 5.69%.

3-(1*H*-Indol-3-yl)-1-*p*-tolylprop-2-en-1-one, 3b: Light brownish amorphous. Yield 79%. m.p.228-29°C. FT-IR (KBr): 3320 (indole NH), 3052 (Ar -H), 2928 (CH-Aliphatic), 1662 (CO), 1608 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6): δ 10.2 (s, 1H, indole NH), 6.21-7.49 (9H, Ar-H), 7.61 (d,1H, -CH =CH-, *J* = 13 Hz), 7.79 (d,1H, -CH =CH-, *J* = 13 Hz), 1.2 (s, 1H, CH_3); ^{13}C NMR (DMSO-d_6): δ 29.3, 110.9, 111.32, 119.1, 122.5, 126.7, 127.9, 129.5, 129.5, 130.2, 134.5; 135.7,137.5, 145.3, 182.2; MS (EI): *m/z* M^{+} 261. Anal. Calcd for: $C_{18}H_{15}NO$: C, 82.73; H, 5.79; N, 5.36. Found: C, 82.70; H, 5.80; N, 5.32%.

3-(1*H*-Indol-3-yl)-1-(4-methoxyphenyl)prop-2-en-1-one, 3c: Yellowish amorphous. Yield 76%. m.p.261-62°C. FT-IR (KBr): 3324 (indole NH), 3055 (Ar -H), 2931 (CH-Aliphatic), 1667 (CO), 1609 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6): δ 10.4 (s, 1H, indole NH), 6.18-7.43 (9H, Ar-H), 7.63 (d,1H, -CH =CH-, *J* = 13 Hz), 7.80 (d,1H, -CH =CH-, *J* = 13 Hz), 2.92 (s, 1H, OCH_3); ^{13}C NMR (DMSO-d_6): δ 56.3, 110.8, 111.3, 119.2, 122.6, 126.8, 127.7, 129.5, 129.2, 130.2, 134.2; 135.7,137.4, 145.1, 182.2; MS (EI): *m/z* M^{+} 277. Anal. Calcd for: $C_{18}H_{15}NO_2$: C, 77.96; H, 5.45; N, 5.05. Found: C, 77.92; H, 5.46; N, 5.04%.

1-(4-Chlorophenyl)-3-(1*H*-indol-3-yl)prop-2-en-1-one, 3d: Colourless amorphous. Yield 86%. m.p.274-75°C. FT-IR (KBr): 3326 (indole NH), 3059 (Ar -H), 2932 (CH-Aliphatic), 1669 (CO), 1605 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6): δ 10.3 (s, 1H, indole NH), 6.19-7.41 (9H, Ar-H), 7.62 (d,1H, -CH =CH-, *J* = 13 Hz), 7.81 (d,1H, -CH =CH-, *J* = 13 Hz); ^{13}C NMR (DMSO-d_6): δ 110.9, 111., 119.2, 122., 126.8, 127.0, 129.5, 129.0, 130.1, 134.2; 135.3,137.1, 145.1, 182.0; MS (EI): *m/z* M^{+} 281. Anal. Calcd for: $C_{17}H_{12}ClNO$: C, 72.47; H, 4.29; N, 4.97. Found: C, 72.50; H, 4.27; N, 4.95%.

General procedure for the synthesis of 4-aryl-(1*H*-indol-3-yl)-6-phenylpyrimidin-2-amine, 5a-d

The equimolor mixture of chalcones **3a-d** (0.03 mole) and guanidine hydrochloride **4** (0.03 mol) in a round bottom flask. Then add 20% ethanolic sodium hydroxide (20 mL) as solvent under constant stirring for 3 hours. The reaction mixture was poured into ice-cold water; the solid separated was filtered, washed and dried. The crude product was recrystalized by using ethanol to afforded the target 4-(1H-indol-3-yl)-6-phenylpyrimidin-2-amines **5a-d**.

4-(1*H*-Indol-3-yl)-6-phenylpyrimidin-2-amine, 5a: Light Yellow amorphous. Yield 79%. m.p.242-43°C. FT-IR (KBr): 3405, 3390 (NH_2), 3350 (indole NH), 3058 (Ar -H), 2922 cm^{-1} (CH-Aliphatic), 2211 (CN); ^{1}H NMR (DMSO-d_6): δ 10.2(s, 1H, indole NH),6.9-7.5(10H, Ar-H),6.8(s,1H, CH pyrimidine); ^{13}C NMR (DMSO-d_6): δ 95..2, 112.3, 119.1, 122.2, 127.6,128.8, 128.4, 129.3, 133.1, 131.4; 135.5,160.4,

163.6, 163.7, MS (EI): *m/z* M^+ 291. Anal. Calcd for: $C_{18}H_{14}N_4$: C, 71.98; H, 5.37; N 18.65. Found: C, 72.02; H, 5.39; N 18.67%.

4-(1*H*-Indol-3-yl)-6-*p*-tolylpyrimidin-2-amine, 5b: Yellow amorphous. Yield 83%. m.p.222-227°C. FT-IR (KBr): 3413, 3402 (NH_2), 3359 (indole NH), 3068 (Ar-H), 2934 (CH-Aliphatic) 2209(CN),1483 cm^{-1} (NH-twisting); ^{1}H NMR (DMSO-d_6): δ 10.3 (s, 1H, indole NH),6.6-7.4 (m, 9H, Ar-H), 6.9 (s,1H, CH pyrimidine), 2.2 (s, CH_3); ^{13}C NMR (DMSO-d_6): δ 24.2, 112.4, 112.9, 119.2, 122.3, 127.7, 128.8, 128.9, 129.4, 133.2, 131.4; 135.3, 160.7, 163.5, 163.9; MS (EI): *m/z* M^+ 300.14; $C_{19}H_{16}N_4$. Anal. Calcd for: C, 71.98; H, 5.37; N 18.65. Found: C, 71.98; H, 5.37; N 18.65%.

4-(1*H*-Indol-3-yl)-6-(4-methoxyphenyl)pyrimidin-2-amine, 5c: Yellow amorphous. Yi**eld** 88%. m.p.229-231°C. FT-IR (KBr): 3413, 3402 (NH_2), 3359(indole NH), 3068(Ar-H), 2934 (CH-Aliphatic), 2282(CO),2221(CN),1484 cm^{-1} (NH-twisting); ^{1}H NMR (DMSO-d_6): δ 10.2 (s, 1H, indole NH), 6.9-7.5(m, 9H, Ar-H),6.8 (s,1H, CH pyrimidine); ^{13}C NMR (DMSO-d_6): δ 56.8, 112.4, 112.9, 119.2, 122.3, 127.7, 128.8, 128.9, 129.4, 133.2, 131.4; 135.3, 160.1, 160.7, 163.5, 163.9; MS (EI): *m/z* M^+ 300.14; $C_{19}H_{16}N_4O$. Anal. Calcd for: C, 72.13; H, 5.10; N 17.71. Found: C, 72.10; H, 5.11; N 17.69%.

4-(4-Chlorophenyl)-6-(1*H*-indol-3-yl)pyrimidin-2-amine, 5d: Yellow amorphous. Yi**eld** 87%. m.p.232-235 °C. FT-IR (KBr): 3413, 3402 (NH_2), 3359(indole NH), 3068(Ar-H), 2934 (CH-Aliphatic), 2282(CO),2221(CN),1484 cm^{-1} (NH-twisting); ^{1}H NMR (DMSO-d_6): δ 10.2 (s, 1H, indole NH), 6.9-7.5(m, 9H, Ar-H),6.8 (s,1H, CH pyrimidine); ^{13}C NMR (DMSO-d_6): δ 112.4, 112.9, 119.2, 122.3, 127.7, 128.8, 128.9, 129.4, 133.2, 131.4; 134.6, 135.3, 160.1, 160.7, 163.5, 163.9; MS (EI): *m/z* M^+ 320.78; $C_{18}H_{13}N_4Cl$. Anal. Calcd for: 67.40; H, 4.08; N 17.47. Found: 67.42; H, 4.11; N 17.44%.

Biological Evaluation

Antioxidant Activity

Free radical scavenging activity

Free radical scavenging activity was done by DPPH method[21] with different concentrations of compounds (**3a-d** and **5a-d**) (25µg, 50µg, 75µg, and 100µg/mL) of samples and Ascorbic acid (AA) were taken in different test tubes. The volume was adjusted to 100µL by adding methanol. Five millilitres of 0.1mM methanolic solution of DPPH was added to these test tubes and shaken vigorously. The tubes were allowed to stand at 27°C for 20min. The control was prepared as above without samples. The absorbance of samples was measured at 517nm. Radical scavenging activity was calculated using the following formula:

$$\% \text{ Radical scavenging activity} = [(\text{Control OD} - \text{Sample OD})/ (\text{Control OD})] \times 100$$

In vitro antimicrobial activity

The *in vitro* antimicrobial activity of novel compounds **3a-d** and **5a-d** was carried out by broth micro dilution method in DMF at concentration 500, 250, 125 and 62.5 µg/mL. Muller hinton broth was used as nutrient medium to growth and dilutes the compound suspension for the test bacteria and Saboured Dextrose broth used for fungal nutrition. Inoculums size for test strain was adjusted to 10^8 CFU [Colony Forming Unit] per milliliter by comparing the turbidity. The strain employed for the activity was procured from Department of microbiology, Gulbarga University, Gulbarga[23].

The compounds **3a-d** and **5a-d** were screened for their antibacterial activity against *Escherichia coli* (MTCC-723), *Staphylococcus aureus* (ATCC-29513), *Klebsiella pneumonia* (NCTC-13368) and *Pseudomonas aeruginosa* (MTCC-1688), as well antifungal activity against *Aspergillus oryzae* (MTCC-3567^T), *Aspergillus niger* (MTCC-281), *Aspergillus flavus* (MTCC-1973) and *Aspergillus terreus* (MTCC-1782). DMSO used as a vehicle to get desired concentration of compounds to test upon microbial strains. The lowest concentration which showed no visible growth after spot subculture was considered as MIC for each compound. The standard antibiotics used for comparison in present study were gentamicin for evaluating for antibacterial activity as well as and fluconazole for antifungal activity. The protocols are summarized in Fig. 2 and Fig. 3.

Antitubercular activity using Alamar Blue Dye

The antitubercular activity of compounds **3a-d** and **5a-d** was assessed against *M. tuberculosis* $H37R_V$ strain using micro plate alamar blue dye assay (MABA)[22]. Briefly, 200 µL of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µL of the middle brook 7H9 broth and serial dilution of compounds was made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/mL and compared with standards pyrazinamide 3.125 µg/mL and streptomycin 6.25 µg/mL. Plates

were covered and sealed with parafilm and incubated at 37°C for five days. After this time, 25 µL freshly prepared 1:1 mixture of almar blue reagent and 10% tween-80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC (Minimal inhibition concentration) was defined as lowest drug concentration which prevented the color change from blue to pink. The results are shown in the Fig. 4.

Conclusion

In the present study the chalcone and indolyl-3-pyrimidin-2-one moieties were synthesized using inexpensive method in good yield. The compounds **3d** and **5b** exhibited notable radical scavenging activity (RSA) of at a concentration of 25 µg/mL, compounds **3a, 3c, 3d, 5b** & **5c** good RSA at 75 µg/mL. **5c** exhibited good RSA at concentration 50 µg/mL. **3a** showed best result at a concentration of 75 µg/mL. Compound **3b** demonstrated potent activity with an MIC of 62.5 µg/mL against *Escherichia coli* (MTCC-723) and equipotent activity (125µg/mL) against all other tested bacteria. Compound **3a** showed potent activity (62.5µg/mL) against *Staphylococcus aureus* (ATCC-29513). **3a** exhibited potent activity (62.5 µg/mL) against *Aspergillus niger* (MTCC-281). Compound **3a** exhibited excellent activity against *M. tuberculosis* H37Rv, with an MIC of 3.125 µg/mL, which is comparable to the standard drugs Pyrazinamide (MIC 3.125 µg/mL) and superior to Streptomycin (MIC 6.25 µg/mL). Compounds **3d** and **5c** showed good activity with MIC values of 6.25 µg/mL against *M. tuberculosis* H37Rv. The chalcone and pyrimidine derivatives hold great promise for addressing various diseases and advancing medicinal chemistry in the future.

Disclosure statement

No potential conflict of interest was reported by the author(s).

CRediT authorship contribution statement

Prabhakar Chavan: Writing–original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Writing – review and editing, Visualization, Supervision, Investigation.

Declaration of competing interest

The author declares that the has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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