INTRODUCTION

The evidences over drug resistance, narrow spectrum and side effects of commercially available antimicrobials intended present study to carry out synthesis and antimicrobial evaluation of some novel pyrrolopyrimidine analogues. Current study involved synthesis of some novel 4-[2-(substituted benzylidene)hydrazinyl]-7H-pyrrolo[2,3-d]pyrimidines (2a-j) followed by evaluation of their antimicrobial potential against bacterial and fungal strains. The synthesis of new pyrrolopyrimidine analogues 2a-j, was carried out in two distinct stages. In first step, azo compound 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine was synthesized by reacting hydrazine hydrate with 4-chloropyrrolopyrimidine, whereas in second step, novel imino analogues of 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidines (2a-j) were synthesized by treating with various substituted benzaldehydes. All the synthesized compounds were subjected to characterization based on IR, 1H NMR and CHN analysis data. The synthesized compounds were also evaluated for their antibacterial and antifungal potential using disc diffusion method. Results of present study revealed that tested compounds possess significant inhibitory potential against tested bacterial and fungal strains. Present study concludes that novel compounds 2a-j possess high antibacterial and antifungal potential, however they must be further evaluated preclinically and clinically for their therapeutic significance.

Keywords: Pyrrolopyrimidinedihydrazide, Hydrazine hydrate, Dimethyl formamide, Potassium carbonate.
[2,3-\textit{d}]pyrimidine analogues, followed by evaluation of their antimicrobial and antifungal activity against \textit{Staphylococcus aureus}, \textit{Bacillus subtilis}, \textit{Pseudomonas aeruginosa}, \textit{Escherichia coli} and \textit{Candida albicans}.

EXPERIMENTAL

All the chemicals used were of the highest possible purity and were of the analytical reagent grade (AR). They comprised 2/3/4-iodobenzaldehyde, 3,4/3,5-diodobenzaldehyde, 2,3/2,4/3,4/3,5-dibromobenzaldehyde, 4-chloro-7\textit{H}-pyrrolo[2,3-\textit{d}]pyrimidine and hydrazine hydrate. Melting points were measured using open capillary tube method and are uncorrected. Characterization of synthesized compounds was done using IR spectra on Jasco FTIR spectrometer at ranging from 4000-400 cm\textsuperscript{-1}; and proton magnetic resonance spectra recorded on Bruker DPX 300 using DMSO as solvent, on \(\delta\) value scale in ppm with TMS as standard. Purity of synthesized compounds and reactions were monitored by TLC experiment using aluminum sheets and silica gel 60 F\textsubscript{254} (Merck, India) with methanol: acetone (9.5:0.5) solvent mixture in SPRECTROLINE\textsuperscript{®} CM-26 UV viewing chamber [27,28].

Synthesis of 4-[2-(substituted benzylidene)hydrazinyl]-7\textit{H}-pyrrolo[2,3-\textit{d}]pyrimidines (2a-j): Compounds 2a-j were synthesized as per the standard protocol with minor modification [29,30], briefly a mixture of 4-hydrazinyl-7\textit{H}-pyrrolo[2,3-\textit{d}]pyrimidine (0.01 mol) and 2-iodobenzaldehyde (0.01 mol) in ethanol were refluxed for 7 h at 60-70 °C. The resultant crude was filtered, washed with ethanol and diethyl ether and finally vacuum dried to offer pure compound 2a. Following the similar protocol, other compounds 2b-j were also synthesized (Scheme-I).

4-[2-(2-Iodobenzylidene)hydrazinyl]-7\textit{H}-pyrrolo[2,3-\textit{d}]pyrimidine (2a): White crystals, yield: 76.98 %; m.p.: 195 °C; FT-IR (KBr, \(v\text{max}, \text{cm}^{-1}\)): 3365 (N-H), 3065 (=C-H), 1592 (C=N), 1486, 1447 (C=C), 578 (C-I); \(^1\text{H}\) NMR (DMSO-\(\delta\)6, ppm): 12.90 (s, 1H, NH), 8.36 (s, 1H, NH-Ar), 8.57 (s, 1H, N=CH), 7.56-8.01 (m, 6H, Ar-H); Anal. calcd. (found) % for C\textsubscript{5}H\textsubscript{10}N\textsubscript{5}I\textsubscript{2}: C, 31.93 (31.88), H, 1.85 (1.84), N, 14.32 (14.28), I, 51.90 (51.86).

4-[2-(3-Iodobenzylidene)hydrazinyl]-7\textit{H}-pyrrolo[2,3-\textit{d}]pyrimidine (2b): White crystals, yield: 77.62 %, m.p.: 199 °C; FT-IR (KBr, \(v\text{max}, \text{cm}^{-1}\)): 3369 (N-H), 3067 (=C-H), 1589 (C=N), 1487, 1449 (C=C), 488 (C-I); \(^1\text{H}\) NMR (DMSO-\(\delta\)6, ppm): 12.86 (s, 1H, NH), 8.33 (s, 1H, NH-Ar), 8.55 (s, 1H, N=CH), 7.56-8.13 (m, 5H, Ar-H); Anal. calcd. (found) % for C\textsubscript{5}H\textsubscript{10}N\textsubscript{5}Br\textsubscript{2}: C, 39.52 (39.49), H, 2.30 (2.21); N, 17.73 (17.29); Br, 40.45 (40.41).

4-[2-(3,5-Diiodobenzylidene)hydrazinyl]-7\textit{H}-pyrrolo[2,3-\textit{d}]pyrimidine (2c): White crystals, yield: 76.98 %; m.p.: 201 °C; FT-IR (KBr, \(v\text{max}, \text{cm}^{-1}\)): 3368 (N-H), 3064 (=C-H), 1591 (C=N), 1483, 1444 (C=C), 485 (C-I); \(^1\text{H}\) NMR (DMSO-\(\delta\)6, ppm): 12.86 (s, 1H, NH), 8.35 (s, 1H, NH-Ar), 8.57 (s, 1H, N=CH), 7.56-8.00 (m, 6H, Ar-H); Anal. calcd. (found) % for C\textsubscript{5}H\textsubscript{10}N\textsubscript{5}I\textsubscript{2}: C, 31.93 (31.90), H, 1.85 (1.81), N, 14.32 (14.28), I, 51.90 (51.83).

4-[2-(3,4-Diiodobenzylidene)hydrazinyl]-7\textit{H}-pyrrolo[2,3-\textit{d}]pyrimidine (2d): White crystals, yield: 81.06 %; m.p.: 210 °C; FT-IR (KBr, \(v\text{max}, \text{cm}^{-1}\)): 3398 (N-H), 3063 (C-Br), 1588 (C=N), 1491, 1451 (C=C), 487 (C-Br); \(^1\text{H}\) NMR (DMSO-\(\delta\)6, ppm): 12.89 (s, 1H, NH), 8.36 (s, 1H, NH-Ar), 8.56 (s, 1H, N=CH), 7.56-8.00 (m, 5H, Ar-H); Anal. calcd. (found) % for C\textsubscript{5}H\textsubscript{10}N\textsubscript{5}I\textsubscript{2}: C, 31.93 (31.88), H, 1.85 (1.84), N, 14.32 (14.25), I, 51.90 (51.86).

4-[2-(2,3-Dibromobenzylidene)hydrazinyl]-7\textit{H}-pyrrolo[2,3-\textit{d}]pyrimidine (2e): White crystals, yield: 81.70 %; m.p.: 205 °C; FT-IR (KBr, \(v\text{max}, \text{cm}^{-1}\)): 3369 (N-H), 3065 (C-Br), 1592 (C=N), 1487, 1449 (C=C), 469 (C-Br); \(^1\text{H}\) NMR (DMSO-\(\delta\)6, ppm): 12.86 (s, 1H, NH), 8.33 (s, 1H, NH-Ar), 8.55 (s, 1H, N=CH), 7.56-8.13 (m, 5H, Ar-H); Anal. calcd. (found) % for C\textsubscript{5}H\textsubscript{10}N\textsubscript{5}Br\textsubscript{2}: C, 39.52 (39.80); H, 2.30 (2.21); N, 17.73 (17.29); Br, 40.45 (40.41).

4-[2-(2,4-Dibromobenzylidene)hydrazinyl]-7\textit{H}-pyrrolo[2,3-\textit{d}]pyrimidine (2f): White crystals, yield: 80.71 %, m.p.: 209 °C; FT-IR (KBr, \(v\text{max}, \text{cm}^{-1}\)): 3369 (N-H), 3065 (C-Br), 1590 (C=N), 1489, 1451 (C=C), 649 (C-Br); \(^1\text{H}\) NMR (DMSO-\(\delta\)6, ppm): 12.86 (s, 1H, NH), 8.33 (s, 1H, NH-Ar), 8.55 (s, 1H, N=CH), 7.56-8.13 (m, 5H, Ar-H); Anal. calcd. (found) % for C\textsubscript{5}H\textsubscript{10}N\textsubscript{5}Br\textsubscript{2}: C, 39.52 (39.50); H, 2.30 (2.21); N, 17.73 (17.29); Br, 40.45 (40.41).

\begin{center}
\textbf{Scheme-I: Synthesis of novel 4-hydrazinyl-7\textit{H}-pyrrolo[2,3-\textit{d}]pyrimidine derivatives}
\end{center}

where, \(R = \text{2-iodo (2a); 3-iodo (2b); 4-iodo (2c); 3,5-diiodo (2d); 3,4-diiodo (2e); 2,3-dibromo (2f); 3,5-dibromo (2g); 2,4-dibromo (2h); 3,4-dibromo (2i); 3,5-dibromo (2j)}\)
RESULTS AND DISCUSSION

The problems of drug resistance, narrow spectrum and side effects associated with commercially available antimicrobials to treat various types of bacterial, fungal and viral infections on one hand; and the potential applications of heterocycles with fused ring systems or substituted with halogens, hydrazides and imino groups emphasizes the need for for present study to carry out the synthesis and antimicrobial evaluation of some novel pyrrolopyrimidine analogues. Present study presented 4-[2-(substituted benzylidene)hydrazinyl]-7H-pyrrolo[2,3-d]-pyrimidines (2a-j) using one pot multicomponent reaction.

One-pot multicomponent reactions have emerged as an attractive methodology for the synthesis of diverse organic molecules with varying substitution patterns, owing to their inherent simplicity and rapidity. This makes them an extremely attractive new way in synthesis [33]. The condensation process of 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine was key step in synthesizing the newly prepared 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine derivatives. For this, 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine dissolved in ethanol was added to halo benzaldehyde derivatives dissolved in ethanol. The resulting mixture was agitated under reflux for approximately 7 h at temperature ranged between 60-70 ºC. During this reaction a solid crude was separated, which was then dried in a vacuum after being filtered, recrystallized, washed multiple times with ethanol and diethyl ether and finally dried out [29]. The purity of synthesized compounds 2a-j was assessed based on the sharp melting point, single spot TLC pattern and elemental analysis.

The structures of newly synthesized compounds 2a-j were confirmed based on the FTIR and 1H NMR data. The newly synthesized compounds 2a-j were characterized based on the spectral data and supported with literature data [22]. The stretching vibration across the 3398-3360 cm⁻¹ range was found FTIR spectra of new synthesized compounds 2a-j. This stretching vibration is one of the numerous low-intensity absorption peaks correlating to N-H stretching [34,35]. Compounds 2a, 2b and 2j each exhibited distinctive absorption bands in their individual IR spectra at 578, 523, and 485 cm⁻¹ due to ortho, meta and para-iodo groups on the aromatic ring, respectively [34]. The presence of characteristic FTIR band at 1592-1587 cm⁻¹ for C=N stretching. The 1H NMR signal at δ 8.55-8.57 ppm confirmed structure of new synthesized compounds 2a-j. The results of characterization data of compounds 2a-j synthesized in this study were also found to be in agreement with the results of the other studies especially for the hydrazide and imino groups [27,28,30,36].

Antimicrobial activity: The new synthesized compounds 2a-j were subjected to evaluation of their antibacterial and antifungal potential against Gram-positive/negative bacterial strains and fungal strains using cup plate method (Table-1). Study revealed that compounds 2c, 2f, 2h and 2j exhibited highest activity, however the antibacterial activity against E. coli was observed for compounds 2a, 2b, 2c and 2d but not for compound 2e. The E. coli inhibition activity of new synthesized compounds was significantly increased when the iodo group was changed.
Compounds 2c, 2e, 2f and 2j were all quite active against \( P. \) aeruginosa; however, compounds 2h and 2i were extremely active against this particular strain of bacteria [37]. Compounds 2a, 2b and 2j exhibited substantial antimicrobial activity when tested against \( S. \) aureus. Compounds 2d and 2f showed promising action against \( C. \) albicans during the experiment. After switching out the iodo group for a bromo group, the antifungal activity against \( C. \) albicans improved significantly. Compounds 2c, 2e and 2j were among the effective chemicals against \( S. \) cerevisiae. The results of the present study were also found to be in agreement with the results of other studies [37,38].

**Conclusion**

A straightforward multicomponent, one-pot synthesis for novel pyrrolopyrimidine analogues (2a-j) has been synthesized using starting materials that are easily accessible. As part of our research on halo-substituted benzaldehyde ring systems and attempts to identify novel lead compounds by compiling pyrazole and pyrimidine heterocycles, compounds 2c, 2f, 2h, and 2g show potential activity against bacterial strains, and compounds 2e, 2g and 2j show potential activity against fungal strains when compared with commercially standard. The structure activity association demonstrates that adding electron-donating iodo or bromo groups as a substituent improves an agent’s ability to fight bacteria and fungi. The outcomes of the current research endeavors aimed at optimizing the efficacy of the leadership framework will establish the basis for our continuous investigation. Present study concludes that novel pyrrolopyrimidine analogues (2a-j) possess high antibacterial and antifungal potential, however, it is necessary to conduct further preclinical and clinical evaluations in order to determine their therapeutic importance.

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interests regarding the publication of this article.

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