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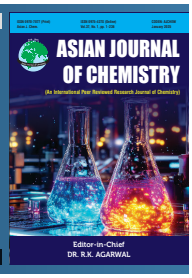

Synthesis, Characterization and Antimicrobial Evaluation of Aromatic Aldehyde Derivatives of 4-Hydrazinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine

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A novel series of pyrrolo[2,3-*d*]pyrimidine derivatives (**2a-j**), incorporating a biologically active hydrazone moiety, was synthesized and subjected to antibacterial evaluation. Structural elucidation of the synthesized compounds was achieved using various spectroscopic methods, including IR, ^{1}H and ^{13}C NMR and mass spectrometry. The compounds demonstrated antibacterial and antifungal activities, with MIC values ranging between 50 and 250 µg/mL. The most resistant strains tested were *Escherichia coli* (MCC 2412), *Staphylococcus aureus* (MCC 2408), *Bacillus subtilis* (MCC 2010), *Pseudomonas aeruginosa* (MCC 2080), *Saccharomyces cerevisiae* (MCC 1033) and *Candida albicans* (MCC 1439). All of the compounds showed significant efficacy, according to the antimicrobial screening, with several of them surpassing the common drugs fluconazole and ciprofloxacin.

Keywords: 4-Aminopyrrolo[2,3-*d*]pyrimidine, Hydrazine moiety, Antibacterial activity, Antifungal activity.

INTRODUCTION

Nitrogen-containing heterocycles are of significant importance, representing a key class of both naturally occurring and synthetic compounds, many of which possess notable biological activities. Many biological activities, including antibacterial [1], anti-inflammatory [2] and anticancer [3] qualities are exhibited by pyrazoles and their derivatives. Because of their increased importance in synthesis and bioactivity, pyrazole derivatives have attracted the attention of chemists and biologists in recent years. Pyrimidines, similarly, have garnered attention for their chemical and pharmacological potential, showing antibacterial [4], antifungal [5], antimalarial [6], anticonvulsant [7], anticancer [8] activities, *etc*. Moreover, it has been observed that molecules with pyrrole and pyrrolopyrimidine frameworks exhibit a variety of biological characteristics, particularly antibacterial effects [9-13]. Hydrazides and their derivatives, owing to their pharmacological properties, especially antibacterial effects, are widely utilized in medicinal research [14-16].

In medicinal chemistry, pyrrolo[2,3-*d*]pyrimidine derivatives have attracted a lot of interest because of their wide range of biological activities, which include antiviral, anticancer and antibacterial qualities. This heterocyclic framework, comprising fused pyrrole and pyrimidine rings, has been explored extensively for drug development, as the incorporation of various substituents onto the core structure often leads to enhanced pharmacological potential [17].

The ability of the hydrazinyl functional group to react with aldehydes and ketones to prepare hydrazones is one of its most well-known uses in medicinal chemistry. Antibacterial, antifungal and anticancer properties are only a few of the biological activities that these hydrazone derivatives have shown [18,19]. Moreover, aromatic aldehydes, with their conjugated systems and electron-withdrawing or donating substituents, offer a versatile platform for the modification of bioactive scaffolds, often resulting in compounds with improved pharmacokinetic and pharmacodynamic profiles [20].

One such promising modification involves the attachment of hydrazinyl groups at specific positions on the pyrrolo[2,3-*d*]-pyrimidine nucleus, yielding compounds with potent bioactivity. The synthesis of novel aromatic aldehyde derivatives of 4-hydrazinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine is the main focus of this study due to the biological significance of both pyrrolo[2,3-*d*]-pyrimidines and hydrazone derivatives. Emphasizing the potential antibacterial properties of the compounds derived from modifying this framework with various aromatic aldehydes.

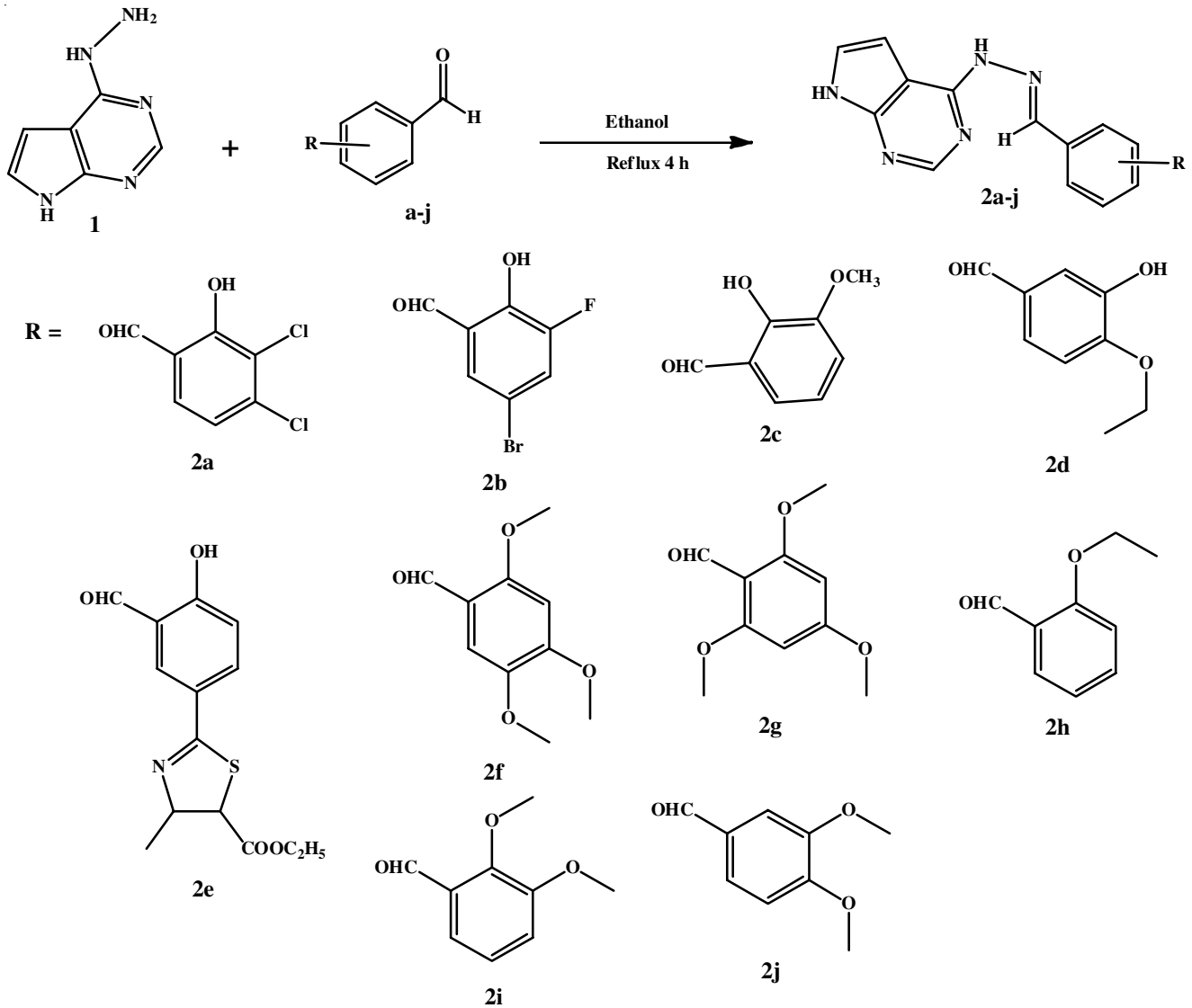
Moreover, the range of structures resulting from the choice of aldehydes could influence the effectiveness of the compounds in addressing various microbial diseases, including fungi and both Gram-positive and Gram-negative bacteria. This work aims to contribute to the interest of research on pyrrolo[2,3-d]pyrimidine derivatives by offering insights into the structure-activity relationships and their potential as novel antibacterial agents.

EXPERIMENTAL

All chemicals including aromatic aldehydes, 4-hydrazinyl-7*H*-pyrrolo[2,3-*d*]-pyrimidine, solvents and reagents were all purchased commercially and utilized without additional purification. The melting points were determined using a capillary method and are uncorrected. FTIR spectra were recorded on the Perkin-Elmer FTIR spectrophotometer using KBr method. ^{1}H NMR spectra were recorded on a Bruker Avance II 400F (400 MHz) NMR spectrophotometer in $CDCl_3$ or DMSO-d_6 solvent using TMS as an internal reference. Mass spectra were acquired using an ESI-MS.

General procedure of synthesis of 4-hydrazinyl-7H-pyrrolo[2,3-*d*]pyrimidines (2a-j): A series of aromatic aldehyde derivatives of 4-hydrazinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine were synthesized through condensation reactions. The standard procedure involved addition of 1.0 mmol of 4-hydrazinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine in 10 mL of ethanol with 1.1 mmol of the appropriate aromatic aldehyde. The reaction mixture was refluxed with stirring for 4 h. Upon completion, the resulting solid was cooled, filtered, washed with cold ethanol and then recrystallized from ethanol to yield the desired products (**2a-j**) (**Scheme-I**).

6-((2-(7*H*-Pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazono)-methyl)-2,3-dichlorophenol (2a): Yield: 74.11%, m.p.: 173-175 ºC, IR (KBr, ν_{max}, cm^{-1}): 3197 (-NH- arom.), 3079 (-NH- aliph.), 2978 (-CH=), 1582,1479 (>C=C<), 1479 (>C=NN-), 1647 (N-H), 1084 (-N-N-), 704 (benzene ring); ^{1}H NMR (DMSO-d_6) δ ppm: 11.96 (s, 1H, -NH- aliphatic), 8.35 (s, 1H, NH, aromatic), 8.74 (s, 1H, -CH=), 7.354 (s, 1H, pyrimidine-H), 6.90-7.00 (m, 5H, aromatic-H); ^{13}C NMR (DMSO-d_6) δ ppm: 151.10, 123.63, 102.63, 102.13. Anal. calcd. (found) % for



Scheme-I: Preparation of pyrrolo[2,3-*d*]pyrimidine derivatives (**2a-j**)

$C_{13}H_9N_5OCl_2$ (*m.w.* 321.02): C, 48.47 (48.39); H, 2.82 (2.81); N, 21.74 (21.69); O, 4.97 (4.96); Cl, 22.01 (22.00).

(*E*)-2-((2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazono)methyl)-4-bromo-6-fluorophenol (2b): Yield: 73.58%, m.p.: 195-197 ºC, IR (KBr, ν_{max}, cm^{-1}): 3190 (-NH- arom.), 3106 (-NH- aliph.), 2953 (-CH=), 1590, 1478 (>C=C<), 1589 (>C=NN-), 1625 (N-H), 1291 (C-F), 1084 (-N-N-), 729 (benzene ring); ^{1}H NMR (DMSO-d_6) δ ppm: 11.85 (s, 1H, -NH- aliphatic), 8.44 (-OH), 8.27 (s, 1H, NH, aromatic), 7.70 (s, 1H, -CH=), 6.79-7.52 (m, 6H, aromatic-H); ^{13}C NMR (DMSO-d_6) δ ppm: 151.14, 129.78, 123.38, 102.48, 102.44. Anal. calcd. (found) % for $C_{13}H_9N_5OBrF$ (*m.w.* 349.00): C, 44.59 (44.53); H, 2.59 (2.58); N, 20.00 (19.96); O, 4.57 (4.55); Br, 22.82 (22.80); F, 5.43 (5.42).

3-Methoxy-2-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4yl)hydrazinylidene]methyl}phenol (2c): Yield: 86.88%, m.p.: 196 ºC, IR (KBr, ν_{max}, cm^{-1}): 3380 (-NH- arom.), 3232 (-NH- aliph.), 3135 (-OCH_3), 2977 (-OH), 2924 (-CH=), 1584, 1441 (>C=C<), 1523 (>C=NN-), 1024 (-N-N-), 755 (disubst. benzene ring); ^{1}H NMR (DMSO-d_6) δ ppm: 13.92 (s, 1H, -NH- aliphatic), 12.90 (s, 1H, NH, aromatic), 8.58 (s, 2H, -OH), 9.80 (s, 1H, -CH=), 8.43 (s, 1H, pyrimidine-H), 6.90-7.68 (m, 6H, aromatic-H), 3.90 (s, 3H, -OCH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 56.58 (OCH_3), 142.57 (-CH=), 150.57 (C2), 102.97 (C3), 148.67 (C4), 115.90 (C5), 123.96 (C6), 99.98 (C2), 119.50 (C3), 125.90 (C4). Anal. calcd. (found) % for $C_{14}H_{13}N_5O_2$ (*m.w.* 283.28): C, 59.36 (56.31); H, 4.63 (4.59); N, 24.72 (24.69); O, 11.30 (11.21).

5-((2-(7*H*-Pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazono)-methyl)-2-ethoxyphenol (2d): Yield: 78.46%, m.p.: 194-195 ºC, IR (KBr, ν_{max}, cm^{-1}): 3268 (-NH- arom.), 3137 (-NH- aliph.), 2994 (-CH=), 1581,1475 (>C=C<), 1574 (N-H), 1535 (>C=NN-), 1043 (-N-N-), 763 (benzene ring). ^{1}H NMR (DMSO-d_6) δ ppm: 11.29 (s, 1H, -NH- aliphatic), 11.29 (-OH), 8.39 (s, 1H, NH, aromatic), 8.21 (s, 1H, -CH=), 6.48-7.59 (m, 6H, aromatic-H); ^{13}C NMR (DMSO-d_6) δ ppm: 149.47, 124.36, 116.98, 116.97, 110.54, 99.25. 98.33, 62.01, 14.94. Anal. calcd. (found) % for $C_{15}H_{15}N_5O_2$ (*m.w.* 297.12): C, 60.60 (60.49); H, 5.09 (5.07); N, 23.56 (23.51); O, 10.56 (10.53).

Ethyl (*E*)-2-(3-((2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)-hydrazono)methyl)-4-hydroxyphenyl)-4-methyl-4,5-dihydrothiazole-5-carboxylate (2e): Yield: 74.56%, m.p.: 199-201 ºC, IR (KBr, ν_{max}, cm^{-1}): 3442 (-NH- arom.), 3192 (-NH- aliph.), 2986 (-CH=), 1655 (N-H), 1585,1476 (>C=C<), 1515 (>C=NN-), 1049 (-N-N-), 728 (benzene ring); ^{1}H NMR (DMSO-d_6) δ ppm: 14.088 (s, 1H, -NH- aliphatic), 12.976 (s, 1H, -OH), 11.284 (s, 1H, NH, arom.), 9.046 (s, 1H, -CH=), 7.167-8.631 (m, 5H, aromatic-H), 4.299 (s, 2H, -OCH_2-), 2.697 (s, 3H, -CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 142.64, 126.44, 103.28, 100.50. Anal. calcd. (found) % for $C_{20}H_{20}N_6O_3S$ (*m.w.* 424.13): C, 56.59 (59.52); H, 4.75 (4.70); N, 19.80 (19.73); O, 11.31 (11.79); S, 7.55 (7.54).

4-(2-(2,4,5-Trimethoxybenzylidene)hydrazinyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (2f): Yield: 82.24%, m.p.: 186-189 ºC, IR (KBr, ν_{max}, cm^{-1}): 3376 (-NH- arom.), 3235 (-NH- aliph.), 3054, 3114, 3001 (-OCH_3) 2969 (-CH=), 1657 (N-H), 1585 (>C=NN-), 1575-1506 (>C=C<), 1068 (-N-N-), 736 (benzene ring). ^{1}H NMR (DMSO-d_6) δ ppm: 13.66 (s, 1H, -NH- aliphatic), 12.90 (s, 1H, NH, aromatic), 8.88 (s, 1H, -CH=), 6.77-8.43 (m, 3H, aromatic-H, 3.89 (s, 9H, -OCH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 142.58, 125.94, 112.77, 110.02, 102.67, 99.95, 98.07, 57.67, 56.36. Anal. calcd. (found) % for $C_{16}H_{17}N_5O_3$ (*m.w.* 327.34): C, 58.71 (58.68); H, 5.23 (5.22); N, 21.39 (21.39); O, 14.66 (14.64).

4-(2-(2,4,6-Trimethoxybenzylidene)hydrazinyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (2g): Yield: 78.15%, m.p.: 189-192 ºC, IR (KBr, ν_{max}, cm^{-1}): 3176 (-NH- arom.), 3085 (-NH- aliph.), 3469, 3398, 3305 (-OCH_3), 2969 (-CH=), 1661 (N-H), 1575, 1506 (>C=C<), 1591 (>C=NN-), 1055 (-N-N-), 791 (benzene ring); ^{1}H NMR (DMSO-d_6) δ ppm: 13.90 (s, 1H, -NH- aliph.), 12.82 (s, 1H, NH, aromatic), 8.90 (s, 1H, -CH=), 6.32-8.63 (m, 3H, aromatic-H, 3.88 (s, 9H, -OCH_3); ^{13}C NMR (DMSO-d_6) δ: 142.63, 124.69, 106.27, 103.21, 101.21, 99.83, 91.02, 56.61, 56.07. Anal. calcd. (found) % for $C_{16}H_{17}N_5O_3$ (*m.w.* 327.34): C, 58.71 (58.62); H, 5.23 (5.20); N, 21.39 (21.35); O, 14.66 (14.63).

4-(2-(2-Ethoxybenzylidene)hydrazinyl)-7*H*-pyrrolo-[2,3-*d*]pyrimidine (2h): Yield: 84.53%, m.p.: 180-182 ºC, IR (KBr, ν_{max}, cm^{-1}): 3204 (-NH- arom.), 3134 (-NH- aliph.), 3074 (-OCH_2-), 2978 (-CH=), 1664 (N-H), 1491,1441 (>C=C<), 1488 (>C=NN-), 1059 (-N-N-), 748 (benzene ring); ^{1}H NMR (DMSO-d_6) δ ppm: 12.37 (s, 1H, -NH- aliphatic), 8.66 (s, 1H, -NH-aromatic), 8.28 (s, 1H, -CH=), 6.84-7.35 (m, 6H, aromatic-H (7.77), 4.14 (s, 2H, -OCH_2-), 1.15 (s, 3H, -CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 150.72, 121.26, 113.42, 102.54, 64.38, 15.16. Anal. calcd. (found) % for $C_{15}H_{15}N_5O$ (*m.w.* 281.32): C, 64.04 (64.04); H, 5.37 (5.36); N, 24.90 (24.88); O, 5.69.

4-[(2*Z*)-2-(2,3-dimethoxybenzylidene)hydrazinyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (2i): Yield: 80.53%, m.p. 196-198 ºC, IR (KBr, ν_{max}, cm^{-1}): 3274 (-NH- arom.), 3138 (-NH- aliph.), 3083 (-OCH_3), 2835 (-CH=), 1589,1428 (>C=C<), 1538 (>C=NN-), 1378,1270 (-C-O), 1025 (-N-N-), 738 (trisub. benzene ring); ^{1}H NMR (DMSO-d_6) δ ppm: 14.25 (s, 1H, -NH- aliphatic), 12.97 (s, 1H, NH, aromatic), 8.73 (s, 1H, -CH=), 8.46 (s, 1H, pyrimidine-H), 7.09-8.00 (m, 6H, arom.-H), 3.87 (s, 3H, -OCH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 55.91 (-OCH_3), 142.57 (-CH=), 150.18 (C2), 100.10 (C3), 148.84 (C4), 114.83 (C5), 125.90 (C6), 103.22 (C2), 114.98 (C3), 126.26 (C4). Anal. calcd. (found) % for $C_{15}H_{15}N_5O_2$ (*m.w.* 297.31): C, 60.60 (59.99); H, 5.09 (4.99); N, 23.56 (23.51); O, 10.76 (10.71).

4-[(2*Z*)-2-(3,4-dimethoxybenzylidene)hydrazinyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (2j): Yield: 81.14%, m.p. 196 ºC, IR (KBr, ν_{max}, cm^{-1}): 3381 (-NH- arom.), 3182 (-NH- aliph.), 3112 (-OCH_3), 2832 (-CH=), 1582/1462 (>C=C<), 1513 (>C=NN-), 1348/1264 (-C-O), 1023 (-N-N-), 767 (trisub. benzene ring); ^{1}H NMR (DMSO-d_6) δ ppm: 14.136 (s, 1H, -NH- aliphatic), 12.919 (s, 1H, NH, aromatic), 8.653 (s, 1H, -CH=), 8.444 (s, 1H, pyrimidine-H), 7.642-7.752 (m, 5H, aromatic-H), 3.899 (s, 6H, -OCH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 56.09, 56.56 (-OCH_3), 142.61 (-CH=), 100.06 (C2), 149.66 (C3), 152.07 (C4), 111.74 (C5), 123.61 (C6), 103.19 (C2), 110.01 (C3), 152.25 (C4). Anal. calcd. (found) % for $C_{15}H_{15}N_5O_2$ (*m.w.* 297.31): C, 60.60 (60.49); H, 5.09 (5.06); N, 23.56 (23.51); O, 10.76 (10.72).

Antibacterial screening: The antibacterial activity of the synthesized compounds was assessed using the disc diffusion method [21]. To replicate the typical conditions used in antibacterial experiments, the Muller Hinton agar medium was autoclaved for 15 min at 15 lbs/in^2 of pressure. By suspending cultures in sterile distilled water, the bacterial inoculum was created, with the concentration adjusted to roughly 10^8 cfu/mL. After swabbing the corresponding microbial cultures onto Petri dishes with 20 mL of Muller Hinton agar, the cultures were incubated for 15 min to allow for absorption. A 100 µL of compound (4 mg/mL dissolved in DMSO) was added to wells, measuring 6 mm in diameter, using a sterile borer. After that, the plates were incubated for 24 h at 37 ºC. By measuring the zone of inhibition surrounding each well, antibacterial activity was evaluated. The positive control was streptomycin, while the negative control was dimethyl sulfoxide (DMSO) [22].

Antifungal activity: Two different fungal species *viz. Candida albicans* (MCC1439) and *Saccharomyces cerevisiae* (MCC1033) were employed in cup and plate assay to evaluate the antifungal activity of the synthesized compounds [23,24]. Using a micropipette, test solutions were carefully poured into discs that were 1 mm thick and 5 mm in diameter. The plates were subsequently incubated for one week at 37 ºC, during which the diffusion of the test solution inhibited the formation of the fungal cultures. The diameter of the inhibitory zones was evaluated following a 36 h incubation period at 37 ºC. Moreover, the compounds suspected of possessing antifungal action were subjected to minimum inhibitory concentration (MIC) testing.

***In vitro* cytotoxicity:** A brine shrimp mortality assay was employed to evaluate the cytotoxic potential of the synthesized compounds [25]. Shrimp eggs were placed in one compartment of the tank, while the opposite side was filled with a simulated brine solution comprising 38 g of NaCl per 1000 mL of tap water. After 2 days, the shrimp hatched and developed into nauplii, which were then harvested for the bioassay. Dried compounds were prepared in varying concentrations (2.5, 5.5, 7.5, 10 and 12.5 mg/mL) by dissolving in DMSO. A Pasteur pipette was used to introduce ten live prawns into each test vial. To confirm the experimental protocol and cytotoxicity results, a control group was included. Following a 24 h incubation period, the vials were inspected under a microscope in order to note any observations and tally the number of surviving nauplii. Every experiment was conducted three times with five replicates. The acquired data were utilized to compute chi-square values, LC_{50}, LC_{90} and 95% CI. When the control group experienced mortality, the data were adjusted using Abbott's formula, which is described in reference [26]:

$$\text{Deaths (\%)} = \frac{\text{Test} - \text{Control}}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION

The synthetic procedures illustrated in **Scheme-I** were employed to synthesize the desired compounds. It was postulated that 4-hydrazinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine could potentially undergo reactions with substituted aromatic benzaldehydes, prompting further investigation by researchers. Assuming that 4-hydrazinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine would interact with substituted aromatic benzaldehydes to prepare the expected products, the target compounds were successfully synthesized using the pathways shown in **Scheme-I**.

The FTIR spectra of the synthesized compounds were compared to the free 4-hydrazinyl-7H-pyrrolo[2,3-*d*]pyrimidine in order to determine the degree of interaction with the methoxy group of benzaldehyde. Within a limited band range, the impact of 4-hydrazinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine vibrations on substituted aromatic benzaldehydes was investigated. The lack of stretching vibrations connected to the amino (-NH_2) and aldehyde (-CHO) groups comfirmed that all synthesized molecules were successfully synthesized. The azomethine (HC=NN-) group has been identified as the source of a significant new band observed between 1479 and 1591 cm^{-1} [27]. It is suggested that the synthesized compounds in 3176-3442 cm^{-1} region corresponds to the aromatic (-NH) group [28]. All of the compounds could be categorized as the aldehydic (-CH=) bands were found between 2986 and 2926 cm^{-1}. The >C=C group of the aromatic ring is linked to two prominent peaks located at 1590-1493 and 1483-1441 cm^{-1}. The strongest band, observed at 1330-1318 cm^{-1}, is followed by bands at 738-728 cm^{-1} and 791-704 cm^{-1}. Compounds **2a-j** also show the characteristics bands of aromatic (C–N), di- or trisubstituted and monosubstituted benzene rings in their FTIR spectra.

The aromatic -NH- moiety in the pyrrolyl ring is responsible for the broad singlet signals observed in the ^{1}H NMR spectra of all synthesized molecules between 7.724 and 12.905 ppm. Furthermore, in all the synthesized compounds, the aldehydic -CH= group is between 7.702 and 9.046 ppm, whereas a singlet peak corresponding to the aliphatic -NH- group is detected in at δ 11.29-14.09 ppm. In the ^{1}H NMR spectra, the broad singlet signal at δ 9.84 ppm (2H), which would correspond to the -NH_2 of 4-hydrazinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine, is absent, indicating that the amino group has been successfully substituted by Schiff base [29,30]. The ^{1}H NMR spectra show a singlet for the pyrimidine proton in the 6.98-8.28 ppm region.

The ^{13}C NMR spectra of compounds **2a-j** were thoroughly analyzed to confirm the structural integrity and electronic environments of key carbon atoms within the heterocyclic framework. The observed chemical shifts (δ) are consistent with the expected structural motifs of pyrrole and pyrimidine rings, as well as conjugated systems. The signals in the range of 142.58-151.14 ppm were assigned to the alkenic carbon atoms (-CH=), indicative of sp^2 hybridized carbons in a conjugated environment. These downfield shifts are typical for carbons involved in double bonds, where deshielding arises from the π-electron cloud.

The chemical shifts between δ 121.26-129.78 ppm correspond to the carbon atoms in the pyrrole ring directly bonded to the nitrogen atom (C-NH). This range is characteristic of pyrrolic carbons and aligns well with literature values, demonstrating the electron-rich nature of the pyrrole ring, which influences the deshielding of adjacent carbons. Signals at 106.27-123.38 ppm were attributed to the C4 carbon of pyrrole ring, whereas shifts observed at 100.36-116.98 ppm were assigned

to the C3 position. These values are typical for pyrrole rings, reflecting the influence of the electronic environment due to the nitrogen atom and the conjugation within the ring. The chemical shifts between 102.13-110.54 ppm was assigned to the C2 position of the pyrrole ring. This slightly downfield shift, compared to other positions, could be attributed to the subtle electronic interactions from adjacent functional groups or atoms. In pyrimidine ring, the C4 carbon exhibited chemical shifts in the range of 99.25-100.50 ppm and the C3 carbon was observed between 91.02-98.33 ppm. These shifts are consistent with the electron-deficient nature of the pyrimidine ring, where the nitrogen atoms exert a strong deshielding effect on the adjacent carbons.

In **2d** and **2h** compounds, the observed shifts δ 62.01-64.80 ppm for the methylene carbons are consistent with ether-linked systems commonly found in heterocyclic frameworks. These findings confirm the successful incorporation of the -OCH_2- group into the molecular structures of compounds **2d** and **2h.** The shifts observed are characteristic of carbons attached to electronegative oxygen atoms in such linkages, supporting the proposed structural assignments. The characteristic signals for the methyl carbons were observed in the range of 14.94-15.16 ppm, which is typical for aliphatic methyl groups. According to this range of chemical shifts, methyl carbons bound to sp^3 hybridized carbon atoms are compatible, reflecting the relatively shielded nature of the methyl group due to the absence of strongly electronegative atoms nearby. Thus, the ^{13}C NMR spectral data for compounds **2a-j** are in good agreement with the proposed structures, confirming the integrity of the pyrrole and pyrimidine rings and the presence of conjugated systems.

The mass spectral data for compounds **2a-j** revealed molecular ion peaks ranging from *m/z* 282.0941 to 425.1338, consistent with their expected molecular weights based on the structural variations. The increasing molecular masses across the series reflect the introduction of additional or more complex substituents such as alkyl, methoxy, halogens or aryl groups. The mass spectra confirm the successful synthesis of the target compounds, with each molecular ion peak closely matched to the calculated molecular weights for the proposed structures.

Antibacterial activity: Table-1 illustrates that all tested microorganisms demonstrated susceptibility to the investigated compounds, with MICs varying from 0.5 to 64 µg/mL. A broad spectrum antibiotic ciprofloxacin, the reference drug, demonstrated a minimum inhibitory concentration (MIC) of 10 µg/mL against the bacterial species. The inhibition zones measured between 8 and 17 mm for *S. aureus* (MCC 2010) and between 8 and 29 mm for *P. aeruginosa* (MCC 2080).

The most effective compounds against *S. aureus* across all tested bacteria were identified as **2a**, **2b**, **2c**, **2d**, **2e** and **2f**. Compounds **2a-i** demonstrated greater efficacy than the standard ciprofloxacin. In contrast, compound **2g** exhibited lower antibacterial activity against *P. aeruginosa*. Notably, compound **2f** was found to be more effective than the recommended treatment. Compounds **2a**, **2b** and **2d** were efficient against *B. subtilis*, whereas compounds **2a-d** was the most effective against *E. coli*. The observed antibacterial activity may be attributed to the enhanced penetration of the cell walls of less lipophilic microorganisms. This might be because the molecules' lipophilic alkyl chain can pass through Gram-negative bacteria's lipid membranes. The results indicate that antibacterial efficacy decreases as carbon chain length increases, likely due to the carbon chain gets excessively long to efficiently penetrate the bacterial cell membrane [31].

TABLE-1
ANTIBACTERIAL STUDIES OF COMPOUNDS **2a-j**

Compound	Zone of inhibition (mm)			
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
2a	15	15	19	15
2b	17	18	23	16
2c	12	10	19	13
2d	13	0	23	16
2e	12	17	15	25
2f	13	12	16	29
2g	8	7	6	8
2h	7	7	0	19
2i	9	10	9	8
2j	0	0	0	0
Ciprofloxacin	10	10	12	11

Antifungal activity: The reference drug fluconazole (MIC 50 µg/mL) was used to treat *C. albicans* (MCC1439) and *S. cerevisiae* (MCC1033) and the corresponding inhibition zones were measured at 6-17 mm and 7-20 mm. Table-2 shows that all of the compounds under investigation have significiant higher fungicidal activity than the reference medication. For all compounds under investigation, the MIC against *S. cerevisiae* (MCC1033) and *C. albicans* (MCC1439) was found to be 54 µg/mL.

TABLE-2
ANTIFUNGAL STUDIES OF COMPOUNDS **2a-j**

Compound	Zone of inhibition (mm)	
	Candida albicans	*Saccharomyces cerevisiae*
2a	12	14
2b	14	11
2c	11	13
2d	6	20
2e	0	16
2f	13	11
2g	17	9
2h	9	7
2i	10	9
2j	0	0
Fluconazole	9	12

***In vitro* cytotoxicity:** The cytotoxic activity of the synthesized compounds on *Artemia salina* were also assessed, with the results summarized in Table-3. The LD_{50} values ranged from 3.99 to 9.67 × 10^{-4} µM/mL, representing the concentration at which 50% of the organisms exhibited adverse effects [32-34].

Conclusion

In this study, a series of aromatic aldehyde derivatives of 4-hydrazinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine were synthesized and characterized. The antimicrobial evaluation revealed that several derivatives exhibited significant biological activities, with electron-withdrawing substituents on the aromatic ring

TABLE-3
BRINE SHRIMP BIOASSAY OF COMPOUNDS **2a-j**

Compd.	LD_{50} (M)	Compd.	LD_{50} (M)
2a	$> 6.45 \times 10^{-4}$	**2f**	$> 4.66 \times 10^{-4}$
2b	$> 4.22 \times 10^{-4}$	**2g**	$> 3.99 \times 10^{-4}$
2c	$> 5.25 \times 10^{-4}$	**2h**	$> 7.67 \times 10^{-4}$
2d	$> 4.49 \times 10^{-4}$	**2i**	$> 4.49 \times 10^{-4}$
2e	$> 7.31 \times 10^{-4}$	**2j**	$> 4.98 \times 10^{-4}$

enhancing efficacy. These results highlight the potential of these novel hydrazone derivatives as promising candidates for developing new antimicrobial agents.

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CONFLICT OF INTEREST

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

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