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CHARACTERIZATION, MULTICOMPONENT SYNTHESIS, AND ANTIMICROBIAL EVALUATION OF NEW PYRROLO [2, 3-d]-PYRIMIDINE DERIVATIVES WITH HYDRAZONE MOIETY

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Abstract:
The novel compound pyrrolo [2, 3-d], a series of pyrimidine derivatives (**2a-i**) containing a physiologically active hydrazone moiety was produced and subsequently evaluated for their antibacterial efficacy. The assignment of structures for the synthesized compounds is determined through the utilization of various analytical techniques, including infrared spectroscopy (IR), proton nuclear magnetic resonance spectroscopy (^{1}H NMR), and mass spectrometry (MS). The synthesized compounds exhibited antibacterial and antifungal properties, as evidenced by their minimum inhibitory concentration (MIC) values ranging from 50–250 µg/mL. The species that exhibited the highest level of resistance 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). The antimicrobial screening results indicate that all the compounds examined had substantial activity, with several compounds demonstrating greater efficacy compared to the reference drugs employed (*ciprofloxacin* and *fluconazole*).
Keywords: Fluorobenzaldehydes, 4-aminopyrrolo[2,3-*d*]pyrimidine, Difluoroaldehydes, Hydrazine moiety.

1. Introduction:

Nitrogen heterocycles hold significant significance as they represent a crucial category of both naturally occurring and synthetic compounds, a substantial number of which exhibit valuable biological properties. Pyrazoles and their derivatives reveal a wide range of biological behaviour, particularly antibacterial [1], anti-inflammatory [2], and anticancer [3] properties. In recent years, there has been a significant focus from chemists and biologists on the investigation of pyrazole derivatives due to their increasing application in synthesis and bioactivity.

Pyrimidines are of chemical and pharmacological interest and molecules containing the pyrimidine ring system have been suggested to possess antibacterial [4], antifungal [5], antimalarial [6], anticonvulsant [7], and anticancer [8] properties. In addition, it has been

discovered that compounds comprising pyrrole and pyrrolopyrimidines exhibit a range of biological activities, such as antibacterial effects [9-13]. Conversely, hydrazides and their various derivatives are commonly used in the field of medical research as a result of their pharmacological characteristics, particularly their antibacterial effects [14, 15]. Paminobenzoic acid (PABA) is impeded from being used in the biosynthesis of tetrahydrofolic acid, a crucial enzyme cofactor in both human and bacterial cells. This cofactor is vital in providing a one-carbon unit for synthesizing pyrimidine nucleic acid bases, which are essential for DNA synthesis [16]. This paper focuses on the synthesis of new pyrrole, pyrazole, pyrimidine, and pyrrolo [2, 3-d] pyrimidine derivatives that contain a hydrazide group. Specifically, the nitrogen atom of the hydrazide group is substituted with thiazole, pyrimidine, or quinoxaline moieties. This substitution is based on the established antimicrobial properties of these biologically active moieties, as documented in previous studies [17-20]. The recently synthesized compounds were assessed for their antibacterial properties against both gram-positive and gram-negative bacteria, as well as fungi.

2. Experimental

2.1. General methods

The chemicals needed for the study could only be obtained from the s. d. Fine Chem Company. The melting points were measured using an open capillary method with a Lab Junction Melting Point/Boiling Point Apparatus. The temperature at which the ice melted was recorded. Thin-layer chromatography was performed on plates consisting of precoated silica gel. Pet ether and ethyl acetate, in a 9:1 ratio, formed the developing solvent solution, and ultraviolet light was used to examine the spots. Spectra of KBr discs were taken in the infrared using a BRUKER FT-IR spectrophotometer. The material was dissolved in DMSO-d_6, and NMR spectra were taken using a Bruker 400 MHz spectrometer, with TMS as the internal standard. The elements were the subject of experiments conducted at Pune University. UV spectra were obtained at room temperature using a JASCO V650 spectrophotometer with methanol as the solvent. Element analysis was performed using a Carlo Erba 1108 Elemental Analyzer.

2.2. Fluorobenzaldehyde derivatives of 4-hydrazinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (2a-j)

In the conditions of this experiment, a catalytic amount of concentrated hydrochloric acid was used to facilitate the reaction. Specifically, 1 mmol of 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine (**1**) and 1 mmole of substituted fluorobenzaldehydes (**a-j**) were subjected to reflux in a 20 mL ethanol solution for 3-7 hours. Following the separation process, the solid substance denoted as **2a-j** was subjected to recrystallization using ethanol as the solvent after filtration.

2.2.1. 4-{(2Z)-2-[(2, 3-difluorophenyl)methylidene]hydrazinyl}-7H-pyrrolo[2, 3-d]pyrimidine (2a).

Yield % 84.67, m. p. 184 ^{o}C, IR: υ_{max}/cm^{-1} 3110 (-NH- aromatic), 3178 (-NH- aliphatic), 2815 (-CH=), 1582/1479 (>C=C<), 1655 (>C=NN-), 1233 (C-F), 1070 (-N-N-), 741 (benzene ring). ^{1}H-NMR (DMSO-d_6) δ: 14.247 (s, 1H, -NH- aliphatic), 12.970 (s, 1H, NH, aromatic), 8.726 (s, 1H, -CH=), 8.464 (s, 1H, pyrimidine-H), 7.087-8.009 (m, 6H, aromatic-H)). Anal. Calcd. for $C_{13}H_9N_5F_2$ (273.24): C, 57.14; H, 3.32; N, 25.63; F, 13.91. Found: C, 56.78; H, 3.27; N, 25.09; F, 13.77.

2.2.2. 4-{(2Z)-2-[(2, 4-difluorophenyl)methylidene]hydrazinyl}-7H-pyrrolo[2, 3-d]pyrimidine (2b).

Yield % 72.77, m. p. 193 ^{o}C, IR: υ_{max}/cm^{-1} 3139 (-NH- aromatic), 3292 (-NH- aliphatic), 2884 (-CH=), 1590/1478 (>C=C<), 1661 (>C=NN-), 1291 (C-F), 1029 (-N-N-), 687 (benzene ring). ^{1}H-NMR (DMSO-d_6) δ: 14.41 (s, 1H, -NH- aliphatic), 12.94 (s, 1H, NH, aromatic),

8.49 (s, 1H, -CH=), 7.20-7.56 (m, 6H, aromatic-H (7.20 (1H, *d*, (*J* = 3.88Hz), 7.34 (1H, *dd*, (*J* = 7.62, 1.62Hz), 7.45 (1H, *dd*, (*J* = 8.00, 1.75Hz), 7.48 (1H, *dd*, (*J* = 8.03, 1.62Hz), 7.56 (2H, *d*, (*J* = 3.88Hz)). Anal. Calcd. for $C_{13}H_9N_5F_2$ (273.24): C, 57.14; H, 3.32; N, 25.63; F, 13.91. Found: C, 56.93; H, 3.20; N, 25.55; F, 13.88.

2.2.3. 4-{(2Z)-2-[(2, 6-difluorophenyl)methylidene]hydrazinyl}-7H-pyrrolo[2, 3-d]pyrimidine (2c).

Yield % 74.85, m. p. 188 ^oC, IR: υ_{max}/cm^{-1} 3096 (-NH- aromatic), 3195 (-NH- aliphatic), 2982 (-CH=), 1587/1477 (>C=C<), 1624 (>C=NN-), 1224 (C-F), 1001 (-N-N-), 731 (benzene ring). ^{1}H-NMR (DMSO-d_6) δ: 11.82 (s, 1H, -NH- aliphatic), 11.69 (s, 1H, NH, aromatic), 7.51 (s, 1H, -CH=), 7.05-8.28 (m, 6H, aromatic-H (7.05 (1H, *d*, (*J* = 3.86Hz), 7.27 (2H, *dd*, (*J* = 8.40, 1.08Hz), 8.28 (2H, *s*), 7.48 (1H, *dd*, (*J* = 8.03, 1.62Hz), 7.56 (2H, *d*, (*J* = 3.88Hz)). Anal. Calcd. for $C_{13}H_9N_5F_2$ (273.24): C, 57.14; H, 3.32; N, 25.63; F, 13.91. Found: C, 57.01; H, 3.29; N, 25.58; F, 13.90.

2.2.4. 4-{(2Z)-2-[(3, 5-difluorophenyl)methylidene]hydrazinyl}-7H-pyrrolo[2, 3-d]pyrimidine (2d).

Yield % 80.05, m. p. 196 ^oC, IR: υ_{max}/cm^{-1} 3127 (-NH- aromatic), 3289 (-NH- aliphatic), 2948 (-CH=), 1581/1475 (>C=C<), 1662 (>C=NN-), 1224 (C-F), 1023 (-N-N-), 682 (benzene ring). ^{1}H-NMR (DMSO-d_6) δ: 14.42 (s, 1H, -NH- aliphatic), 12.94 (s, 1H, NH, aromatic), 8.66 (s, 1H, pyrimidine H), 8.11 (s, 1H, -CH=), 6.81-7.52 (m, 6H, aromatic-H (7.52 (1H, *d*, (*J* = 3.89Hz), 7.04 (1H, *dd*, (*J* = 1.64, 1.43Hz), 6.87 (2H, *dd*, (*J* = 1.68, 1.53Hz), 6.81 (1H, *d*, (*J* = 3.89Hz)). Anal. Calcd. for $C_{13}H_9N_5F_2$ (273.24): C, 57.14; H, 3.32; N, 25.63; F, 13.91. Found: C, 56.99; H, 3.30; N, 25.61; F, 13.89.

2.2.5. 4-{(2Z)-2-[(2, 3, 5-Trifluorophenyl)methylidene]hydrazinyl}-7H-pyrrolo[2, 3-d]pyrimidine (2e).

Yield % 73.33, m. p. 169 ^oC, IR: υ_{max}/cm^{-1} 3046 (-NH- aromatic), 3290 (-NH- aliphatic), 2684 (-CH=), 1585/1476 (>C=C<), 1660 (>C=NN-), 1223 (C-F), 1023 (-N-N-), 682 (benzene ring). ^{1}H-NMR (DMSO-d_6) δ: 14.52 (s, 1H, -NH- aliphatic), 12.48 (s, 1H, NH, aromatic), 8.66 (s, 1H, pyrimidine H), 8.09 (s, 1H, -CH=), 6.81-7.53 (m, 5H, aromatic-H (6.81 (1H, *d*, (*J* = 3.90Hz), 6.89 (1H, *d*, (*J* = 1.68Hz), 7.14 (1H, *d*, (*J* = 1.68Hz), 7.53 (1H, *d*, (*J* = 3.90Hz). Anal. Calcd. for $C_{13}H_8N_5F_3$ (291): C, 53.61; H, 2.72; N, 19.57; F, 24.05. Found: C, 53.22; H, 2.70; N, 19.53; F, 23.83.

2.2.6. 4-{(2Z)-2-[(2, 4, 5-Trifluorophenyl)methylidene]hydrazinyl}-7H-pyrrolo[2, 3-d]pyrimidine (2f).

Yield % 77.77, m. p. 183 ^oC, IR: υ_{max}/cm^{-1} 3109 (-NH- aromatic), 3194 (-NH- aliphatic), 2985 (-CH=), 1575/1506 (>C=C<), 1629 (>C=NN-), 1206 (C-F), 1046 (-N-N-), 695 (benzene ring). ^{1}H-NMR (DMSO-d_6) δ: 11.87 (s, 1H, -NH- aliphatic), 11.74 (s, 1H, NH, aromatic), 8.28 (s, 2H, Ar H), 7.56 (s, 1H, -CH=), 6.93-7.70 (m, 3H, aromatic-H (6.93 (1H, *d*, (*J* = 3.86Hz), 7.34 (1H, *d*, (*J* = 0.54Hz). Anal. Calcd. for $C_{13}H_8N_5F_3$ (291): C, 53.61; H, 2.72; N, 19.57; F, 24.05. Found: C, 53.18; H, 2.66; N, 19.49; F, 23.97.

2.2.7. 4-{(2Z)-2-[(3, 4, 5-Trifluorophenyl)methylidene]hydrazinyl}-7H-pyrrolo[2, 3-d]pyrimidine (2g).

Yield % 79.04, m. p. 186 ^oC, IR: υ_{max}/cm^{-1} 3021 (-NH- aromatic), 3118 (-NH- aliphatic), 2973 (-CH=), 1582/1480 (>C=C<), 1648 (>C=NN-), 1251 (C-F), 1026 (-N-N-), 731 (benzene ring). ^{1}H-NMR (DMSO-d_6) δ: 11.93 (s, 1H, -NH- aliphatic), 11.66 (s, 1H, NH, aromatic), 8.66 (s, 1H, pyrimidine H), 8.12 (s, 1H, -CH=), 6.81-7.53 (m, 4H, aromatic-H (6.81 (1H, *d*, (*J* = 3.89Hz), 7.12 (2H, *d*, (*J* = 1.95Hz), 7.53 (1H, *d*, (*J* = 3.89Hz). Anal. Calcd. for $C_{13}H_8N_5F_3$ (291): C, 53.61; H, 2.72; N, 19.57; F, 24.05. Found: C, 53.52; H, 2.71; N, 19.55; F, 24.00.

2.2.8. 4-[(2Z)-2-{[2-(trifluoromethyl)phenyl]methylidene}hydrazinyl]-7H-pyrrolo[2,3-d]pyrimidine (2h).

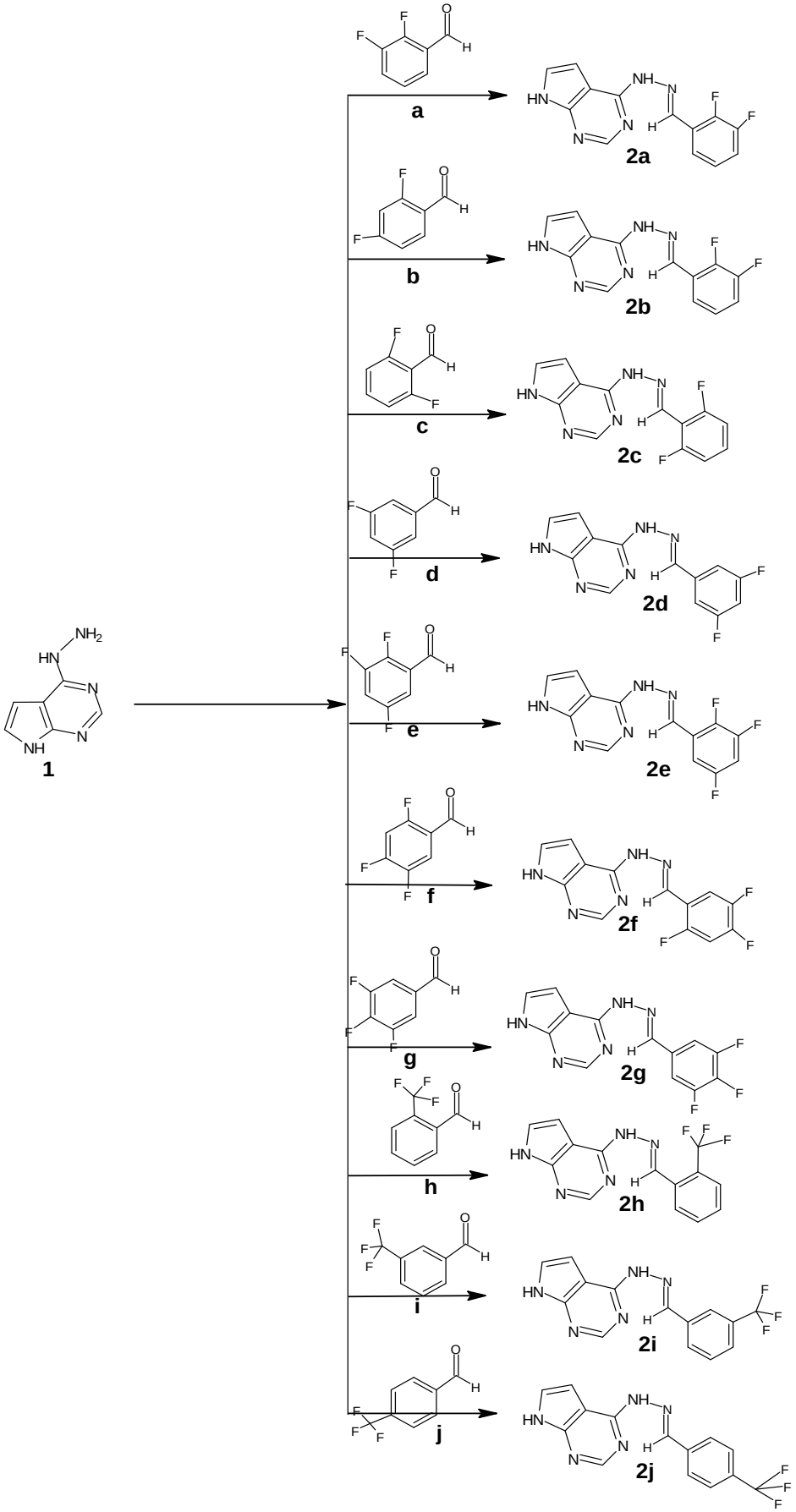
Yield % 73.53, m. p. 180 ^{o}C, IR: υ_{max}/cm^{-1} 3070 (-NH- aromatic), 3196 (-NH- aliphatic), 2982 (-CH=), 1491/1441 (>C=C<), 1664 (>C=NN-), 1219 (C-F), 1066 (-N-N-), 680 (benzene ring). ^{1}H-NMR (DMSO-d$_6$) δ: 11.93 (s, 2H, -NH- aliphatic and aromatic), 6.93 (s, 1H, pyrimidine H), 7.37 (s, 1H, -CH=), 7.77-8.29 (m, 6H, aromatic-H (7.77 (2H, *d*, (*J* = 3.88Hz), 8.07 (2H, *ddd*, (*J* = 8.32, 7.34, 1.88Hz), 8.29 (2H, *ddd*, (*J* = 7.70, 7.34, 1.33Hz). Anal. Calcd. for $C_{14}H_{10}N_5F_3$ (305): C, 56.08; H, 3.30; N, 22.94; F, 18.67. Found: C, 55.59; H, 3.26; N, 22.88; F, 18.59.

2.2.9. 4-[(2Z)-2-{[3-(trifluoromethyl)phenyl]methylidene}hydrazinyl]-7H-pyrrolo[2,3-d]pyrimidine (2i).

Yield % 82.59, m. p. 192 ^{o}C, IR: υ_{max}/cm^{-1} 3075 (-NH- aromatic), 3196 (-NH- aliphatic), 2962 (-CH=), 1595/1483 (>C=C<), 1573 (>C=NN-), 1219 (C-F), 1031 (-N-N-), 672 (benzene ring). ^{1}H-NMR (DMSO-d$_6$) δ: 11.84-11.90 (s, 2H, -NH- aliphatic and aromatic), 8.58 (s, 1H, pyrimidine H), 6.95 (s, 1H, -CH=), 7.36-8.30 (m, 6H, aromatic-H (7.36 (1H, *ddd*, (*J* = 7.60, 1.29, 1.10Hz), 7.61 (1H, *dt*, (*J* = 7.64, 1.31Hz), 7.86 (2H, *td*, (*J* = 7.62, 0.44Hz), 8.30 (2H, *ddd*, (*J* = 1.34, 1.10, 0.44Hz). Anal. Calcd. for $C_{14}H_{10}N_5F_3$ (305): C, 56.08; H, 3.30; N, 22.94; F, 18.67. Found: C, 55.90; H, 3.26; N, 22.88; F, 18.49.

2.2.10. 4-[(2Z)-2-{[4-(trifluoromethyl)phenyl]methylidene}hydrazinyl]-7H-pyrrolo[2,3-d]pyrimidine (2j).

Yield % 77.25, m. p. 196 ^{o}C, IR: υ_{max}/cm^{-1} 3054 (-NH- aromatic), 3193 (-NH- aliphatic), 2981 (-CH=), 1493/1440 (>C=C<), 1658 (>C=NN-), 1225 (C-F), 1011 (-N-N-), 715 (benzene ring). ^{1}H-NMR (DMSO-d$_6$) δ: 12.43-12.45 (s, 2H, -NH- aliphatic and aromatic), 6.98 (s, 1H, pyrimidine H), 7.48 (s, 1H, -CH=), 7.88-8.41 (m, 6H, aromatic-H (7.88 (2H, *ddd*, (*J* = 8.31, 1.80, 0.53Hz), 8.10 (2H, *d*, (*J* = 3.88Hz), 8.41 (2H, *ddd*, (*J* = 8.29, 1.66, 0.45Hz). Anal. Calcd. for $C_{14}H_{10}N_5F_3$ (305): C, 56.08; H, 3.30; N, 22.94; F, 18.67. Found: C, 56.97; H, 3.22; N, 22.91; F, 18.43.



Scheme 1. Synthetic pathways for compounds (**2a-j**)

2.3. Biological screening

2.3.1. Antibacterial screening

In this study, *Staphylococcus aureus* MCC 2010 and *Bacillus subtilis* MCC 2010 were employed to evaluate the antimicrobial efficacy of the synthesized compounds against gram-positive and gram-negative bacteria, correspondingly. The bacterial strains used in this study were *Escherichia coli* (MCC 2412), *Staphylococcus aureus* (MCC 2408), *Bacillus subtilis* (MCC 2010), *Pseudomonas aeruginosa* (MCC 2080). The Muller Hilton agar medium underwent autoclaving at a pressure of 15 lbs/in^2 for a duration of 15 minutes, to replicate the conditions typically employed in an antibacterial assay. The researchers employed the disc diffusion technique [21] to evaluate the antibacterial activity of the recently synthesized compounds. To assess the antibiotic action, the inoculum was modified by suspending the culture in sterile distilled water, resulting in a reduction in the organism count to around 108 colony-forming units per milliliter (cfu/mL). Petri plates containing 20 mL of Muller Hilton agar medium were subjected to swabbing using the corresponding microbial strain cultures. Subsequently, the Petri dishes were incubated for a duration of 15 minutes to facilitate the absorption of the cultures. A sterile borer was utilized to generate wells with a diameter of 6 mm. Subsequently, 100 L of a solution containing each chemical at a concentration of 4.0 mg/mL, reconstituted in DMSO, was introduced into the contaminated plates. Every plate was subjected to incubation for a complete 24-hour period at a temperature of 37 °C. Every plate was subjected to incubation for a complete 24-hour period at a temperature of 37 °C. The antibacterial activity of all produced compounds was assessed by quantifying the area of inhibition surrounding the test wells. The negative control utilized dimethyl sulfoxide (DMSO), whereas the positive control involved the use of streptomycin [22].

Antifungal Activity

Two distinct fungal species were used in cup and plate assays involving the chemicals [23, 24]. The two strains under investigation are *Candida albicans* (MCC1439) and *Saccharomyces cerevisiae* (MCC1033). The test solution was introduced into the discs, which had a thickness of 1 mm and a diameter of 5 mm, via a micropipette. Subsequently, the plates were incubated at a temperature of 37 °C for a duration of one week. During this period, the growth of the injected fungus was impeded by the diffusion of the test solution. The diameter of the inhibitory zone was determined following a 36-hour incubation period at 37°C. The investigation involved conducting minimum inhibitory concentration (MIC) experiments on substances hypothesized to possess antifungal properties. The minimum inhibitory concentration (MIC) of an antifungal agent refers to the concentration at which all detectable microbial growth is effectively suppressed after a complete incubation period of 24 hours. Diagnostic laboratories utilize the minimum inhibitory concentration (MIC) method to validate the resistance of microorganisms to antimicrobial agents, as well as to assess the effectiveness of newly developed antimicrobial agents.

2.3.2. *In vitro* cytotoxicity

The cytotoxicity of the generated compounds was assessed by a bioassay involving brine shrimp [25]. The shrimp eggs were allocated to one section of the tank. In contrast, the other section was filled with a simulated seawater solution of 38 g of NaCl/1000 ml of tap water. The shrimp require two days for hatching and subsequent development into *nauplii*. The just-emerged crustaceans were extracted to conduct a bioassay. The sample vials were filled with dried complexes of different strengths, specifically 2.5, 5.5, 7.5, 10, and 12.5 mg/mL. The cytotoxicity of the complexes was assessed by dissolving them in dimethyl sulfoxide (DMSO). Ten live shrimp were introduced into each test container using a Pasteur pipette. To ensure the validity of the test protocol and the results derived from the cytotoxic activity of the drug, a control group was incorporated. Following 24 hours, the tubes were subjected to

microscopic examination to document any observations made and quantify the surviving nauplii. The experiment was conducted with a total of five replications, each being repeated three times. The acquired data was subjected to calculations for LC_{50}, 95% confidence interval (CI), LC_{90}, and chi-square. When fatalities occurred within the control group, the numerical values were modified utilizing Abbott's formula as outlined in reference [26].

% deaths = [(test-control)/control] x 100.

3. Results and Discussion:

Synthetic methods indicated in **Scheme 1** were applied to obtain the necessary compounds. It was hypothesized that 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine might react with substituted fluoro benzaldehydes. Thus, researchers investigated this possibility. **Table 1** provides a synopsis of the physicochemical data for molecules **2a-j**. The target compounds were obtained by the synthetic routes shown in **Scheme 1**. It was hypothesized that 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine might react with substituted fluoro benzaldehydes, thus scientists investigated this possibility.

3.1. FT(IR) spectra:

The FT(IR) spectra of the obtained compounds were compared to the free 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine to determine the extent to which the molecule bonded to the methoxy group of the benzaldehyde. The effect of 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine vibration on substituted fluorobenzaldehydes was investigated using a narrow range of bands. All synthesized compounds have been verified as mature by the lack of stretching vibrations from aldehyde (CHO) and amino (NH_2) moieties. Instead, a potent new band at 1573–1662 cm^{-1} was produced by the azomethine (HC=NN-) group [27]. It has been hypothesized that the prepared compounds exist because of a broadband identified as aromatic (NH) in the 3054-3110 cm^{-1} area [28, 29]. The aldehydic (-CH=) bands at 2815-2882 cm^{-1} have been analyzed, and all of the compounds have been categorized. The infrared spectra of **2a-j** compounds exhibit two prominent lines at 1493-1590 and 1441-1483 cm^{-1}, which can be attributed to the >C=C group of an aromatic ring. The 1318-1330 cm^{-1} band is the strongest, followed by the 728-738 cm^{-1} and the 654-687 cm^{-1} bands. Aromatic (C-N), di/trisubstituted benzene ring, and monosubstituted benzene ring FT(IR) spectra are all observed in compounds **2a-j**. A band at 1206-1291 cm^{-1} was observed in the FT(IR) spectra of the **2a-j** molecule, corresponding to the aromatic C-F group.

3.2. ^{1}H NMR spectra:

The broad singlet signals at 11.82-14.41 ppm in the ^{1}H NMR spectra of all prepared compounds can be attributed to the presence of the aromatic -NH- moiety in the pyrrolyl ring. In the 11.69-12.94 ppm range, the aliphatic -NH- singlet peak is seen, and in the 7.48-8.49 ppm range, the aldehydic -CH= group is ascribed to all of the produced compounds. The ^{1}H NMR spectra of all the synthesized derivatives demonstrate that the amino group was successfully replaced by Schiff base [30], as there is no broad singlet signal at 9.84 ppm (2H) corresponding to the -NH_2 of 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine. The ^{1}H-NMR spectra of compounds **2a-j** show a singlet for the pyrimidine proton between 6.98 and 8.28 ppm. Identical bands can be seen in ^{1}H NMR spectra when comparing published publications [31].

3.3. Antimicrobial evaluation:

Microdilution in vitro testing was performed to determine the antibacterial and antifungal efficacy of the novel compounds against Gram-positive bacteria like *Staphylococcus aureus* and *Bacillus subtilis* and Gram-negative bacteria like *Pseudomonas aeruginosa* and *Escherichia coli*.

After 24 hours of incubation at 37 °C, all of the bacterial isolates were effectively cultivated in nutritious broth. After 24 hours of incubation at 25 °C, the sabouraud dextrose agar was

transferred to malt broth, and spore suspensions from quickly growing fungi were collected using tween 80 over the course of 7 days. The final bacterial inoculum OD was between 0.20 and 0.30, while the fungal inoculum OD was 0.50. At the concentrations employed here, the DMSO used to make the stock solutions is safe for the microorganisms. The bacterial and fungal populations were concentrated to 1000 g/mL and beyond. Anti-fungal and antibacterial medications in powder form, such as fluconazole and ciprofloxacin, were widely utilized. After incubation at 37 °C for 24 hours and at 25 °C for 48 hours, the antibacterial activity was determined.

3.3.1. Antibacterial activity:

Table 1 shows that all of the microorganisms tested were susceptible to the chemicals investigated, with MICs ranging from 0.5 to 64 g/mL, with the reference drug being *ciprofloxacin*, a broad-spectrum antibiotic with a MIC of 10 g/mL against the bacterial species. Inhibition zones ranged from 8-17 mm for *Staphylococcus aureus* (MCC 2010), and 8-29 mm for *Pseudomonas aeruginosa* (MCC 2080).

Table 1: Antibacterial studies of **2a-j** compounds

Compound	Antibacterial Activity (zone of inhibition)			
	S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***
2a	15	15	19	15
2b	17	18	23	16
2c	16	0	24	19
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	13	8	15	9
2j	19	11	19	12
Ciprofloxacin	10	10	12	11

The most potent bacteria against *S. aureus* when considering all bacteria together are the **2a**, **2b**, and **2j**. It was demonstrated that compounds **2a**-**2f**, **2i**, and **2j** were more efficient than the industry standard *ciprofloxacin*. Against *P. aeruginosa*, 2**g** had less antibacterial activity. The outcomes proved that **2f** outperformed the recommended course of administration. It was revealed that the compounds **2a-d** were the most effective against *E. coli*. For *B. subtilis*, see **2a**, **2b**, and **2d**. The fact that fewer lipophilic microorganisms' cell walls are more readily penetrated is most likely what causes the antibacterial action. This is most likely due to the molecule's ability to pass through the lipid cell membrane of gram-negative bacteria thanks to the lipophilic alkyl chain. The findings show that as the carbon chain length increases, the antibacterial activity decreases. This might occur as a result of the carbon chain's size being too great to pass through the bacterial cell membrane [32].

3.3.2. Antifungal activity

The *Candida albicans* (MCC1439) and *Saccharomyces cerevisiae* (MCC1033) exhibited inhibition zones ranging from 6-17 mm and 7-20 mm, respectively, upon exposure to the reference drug *fluconazole* (MIC 50 µg/ml). According to the data presented in **Table 2**, it is evident that all the compounds examined exhibited significantly greater fungicidal efficacy compared to the reference drug. The minimum inhibitory concentration (MIC) against both *Candida albicans* (MCC1439) and *Saccharomyces cerevisiae* (MCC1033) was measured at 54 µg/mL for all the investigated compounds.

Table 2: Antifungal studies of **2a-j** compounds

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

3.3.3. *In vitro cytotoxicity*:

The cytotoxicity of the caused compounds on Artemia salina was evaluated, and the results are presented in **Table 3**. The LD_{50} values, ranging from 3.99 to 9.67 x 10^{-4} µM/mL, indicate the concentration at which 50% of the organisms were affected [33-35].

Table 3: Brine shrimp bioassay of **2a-j** compounds

Compound	**LD_{50} (M)**
2a	$>6.45 \times 10^{-4}$
2b	$>4.22 \times 10^{-4}$
2c	$>5.25 \times 10^{-4}$
2d	$>4.49 \times 10^{-4}$
2e	$>7.31 \times 10^{-4}$
2f	$>4.66 \times 10^{-4}$
2g	$>3.99 \times 10^{-4}$
2h	$>7.67 \times 10^{-4}$
2i	$>4.44 \times 10^{-4}$
2j	$>9.67 \times 10^{-4}$

4. Conclusion

Several novel derivatives of **2a-j**, 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine have been synthesized using various substituted methoxy benzaldehydes. The production of the proposed compounds is supported by the results obtained from various analytical techniques, including FT-IR, UV-vis, NMR spectrum studies, and electrochemical data. The synthesized compounds were subjected to various analytical techniques, including ^{1}H NMR, UV-vis, elemental analysis (C, H, N), and FT-IR spectroscopy. The obtained spectra were recorded and subsequently analyzed. The findings suggest that a 1:1 ratio of 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine and modified methoxy benzaldehydes should be combined. All the synthesized compounds exhibited significant antibacterial activity. The cytotoxicity of all the synthesized compounds is significantly high towards the susceptible cell types.

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