Synthesis of New Pyrrolo [2,3-D] Pyrimidinehydrazine Derivatives Bearing Pyrrole Moiety for Evaluation as Antimicrobial Agents

Mrs. Rubeena Mansuri[1*], Dr. Raj Badekar[2], Dr. Priyanka Jhalora[3] and Dr. Kalpana Patankar-Jain[4]

[1*,3]Dept of Chemistry, Pacific University of Higher Education and Research, Udaipur-India, rubeena2711@gmail.com.
[2]R and D, RIVA Industries, Ambernath MIDC, Ambernath West, Thane-Maharashtra.
[4]Chemistry Dept, Royal College of Arts, Science and Commerce, Mira Road, Maharashtra 401107.

***Corresponding Author:** Mrs. Rubeena Mansuri
Dept of Chemistry, Pacific University of Higher Education and Research, Udaipur-India, rubeena2711@gmail.com

Abstract
The pyrrolopyrimidinehydrazone was used as a precursor in the synthesis of pyrrolo [2, 3-*d*] pyrimidinehydrazide compounds. Elemental analysis, infrared spectroscopy, $^{1}H/^{13}C$ NMR, and UV spectral data were used to confirm the structures of these substances. All of the newly synthesized compounds were evaluated for cytotoxicity against *artemia salina* in vitro. All novel compounds were also tested for antimicrobial activity in vivo. When compared to streptomycin and fluconazole, several derivatives demonstrated potential antimicrobial activity. We investigate and discuss the structure-activity relationships and antimicrobial activities of these compounds in this article.

Keywords: Hydroxy benzaldehydes, pyrrolo [2,3-*d*] pyrimidinehydrazide, dihydroxy benzaldehydes, 4-hydroxy-3-methoxybenzaldehyde

1. Introduction:

Purines and pyrimidines are useful leads for drug discovery due to their important roles in cellular processes. 2-thiopyrimidine (2-TP) and its derivatives, also known as 2-mercaptopyrimidine compounds, are an important family of pyrimidines [1]. The sulfur atom in the 2-TP ring is an intriguing replacement for the current oxygen atom bonded to C-2 in the uridine base [2]. Given this assumption, 2-TPs have piqued the attention of synthetic-biochemists [3]. The European patent [4] disclosed the use of 2-TP derivatives in the production of cardiotonic drugs. Pathak et al. investigated the main antimicrobial activity of 2-TP derivatives against Mycobacterium tuberculosis (Mtb) [5]. 6-Thiopurine (6 TP) [6] is a thioanalog of hypoxanthine, a naturally occurring result of purine metabolism.

Several thousand 6-TP derivatives have been synthesized and characterized in biological studies since the discovery of this antimetabolite more than half a century ago. Which has been shown to be very successful in the treatment of leukemias [7], autoimmune and rheumatic disorders [7–10], and immunosuppression during organ transplantation [7, 8]. Because of their similarity to pyrimidines and purines, pyrrolo[3,2-d]pyrimidines, a class of 7-deazapurine analogues, show interesting biological activity. Tolmetin (Rumatol) and ketorolac (Ketolac), two well-known nonsteroidal anti-inflammatory drugs (NSAIDs) [11], act by inhibiting prostaglandin synthesis, which is the main mechanism of these drugs' anti-inflammatory action. PNU-142731A [12] is a pyrrolopyrimidine anti-inflammatory suppressing cytokine production in vivo. Three pyrrolo [2,3-d]pyrimidine nucleoside antibiotics identified in nature are tubercidin, toyocamycin, and sangivamycin [13, 14]. Some microorganisms' development has been seen to be inhibited by these compounds.

In previous research, many scientists discovered [15–17] that nitrogen and/or hetero atoms in rings increased the activity in the direction of anti-microbial and anti-inflammatory effects. In response to the results above and as part of ongoing research in this area [15–17], we set out to create new pyrrolopyrimidines hydrazone derivatives and related hydroxybenzaldehyde derivatives in order to examine the relationships between structure and activity. The heterocyclic system pyrrole was chosen considering previous results [15–17].

2. Experimental

2.1. General methods

The Lab Junction Melting Point/Boiling Point Apparatus determines the uncorrected melting points of all substances. On a BRUKER FT-IR spectrophotometer, potassium bromide pellet IR bands were captured. Chemical shifts were reported as ppm against TMS as an internal reference, and 1H NMR spectra were determined on a Bruker 400 MHz. The VCarlo Erba 1108 was used to conduct microanalyses, and the findings fell within the calculated values' acceptable range (0.40). A JASCO V650 spectrophotometer was used to record UV bands at room temperature. Silica gel plates that had been precoated were used for thin-layer chromatography. As the developing solvent system, a 9:1 mixture of pet ether and ethyl acetate was used, and UV light was used to see the spots.

2.2. Preparation of 4-hydrazinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine:

In dry ethanol (50 mL), 4-chloropyrrolo[2,3-d]pyrimidine (0.10 mol) and hydrazine hydrate (0.15 mol) were refluxed for three hours. The residues were then recrystallised from methanol and evaporated under decreased pressure to yield 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine.

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

In the presence of a catalytic quantity of concentrated hydrochloric acid, a mixture of substituted hydroxy benzaldehydes (**a–j**) and 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine (1.49 g, 0.01 mol.) was refluxed in ethanol for 3–7 hours. The acquired solid was separated by filtering and then recrystallised from ethanol to produce **a-j**.

2.3.1. 2-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazinylidene]methyl}phenol (a).

Yield % 78.66, m.p. 187 ºC, IR: υ_{max}./cm^{-1} 3412 (-NH- aromatic), 3190 (-NH- aliphatic), 3054 (-OH), 2841 (-CH=), 1579/1435 (>C=C<), 1487 (>C=NN-), 1031 (-N-N-), 735 (di sub benzene ring). ^{1}H-NMR (DMSO-d$_6$) δ: 12.421 (s, 1H, -NH- aliphatic), 10.421 (s, 1H, NH, aromatic), 8.634 (s, 1H, -OH), 8.316 (s, 1H, -CH=), 7.865 (s, 1H, pyrimidine-H), 6.942-7.378 (m, 6H, aromatic-H). ^{13}C-NMR (DMSO-d$_6$): 143.28 (-CH=), 151.38 (C2), 101.71 (C3), 148.90 (C4), 116.82 (C5), 124.03 (C6), 102.04 (C2), 119.96 (C3), 127.31 (C4). Anal. Calcd. for $C_{13}H_{11}N_5O$ (253.26): C, 61.65; H, 4.38; N, 27.65; O, 6.32. Found: C, 61.12; H, 4.37; N, 27.59; O, 6.21.

2.3.2. 3-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazinylidene]methyl}phenol (b).

Yield % 80.24, m.p. 189 ºC, IR: υ_{max}./cm^{-1} 3468 (-NH- aromatic), 3134 (-NH- aliphatic), 3050 (-OH), 2827 (-CH=), 1590/1481 (>C=C<), 1481 (>C=NN-), 1019 (-N-N-), 735 (di sub benzene ring). ^{1}H-NMR (DMSO-d$_6$) δ: 14.318 (s, 1H, -NH- aliphatic), 13.023 (s, 1H, NH, aromatic), 9.921 (s, 1H, -OH), 8.468 (s, 1H, -CH=), 8.741 (s, 1H, pyrimidine-H), 6.993-7.552 (m, 6H, aromatic-H). ^{13}C-NMR (DMSO-d$_6$): 143.27 (-CH=), 150.38 (C2), 101.78 (C3), 148.70 (C4), 116.82 (C5), 124.03 (C6), 102.04 (C2), 119.96 (C3), 127.31 (C4). Anal. Calcd. for $C_{13}H_{11}N_5O$ (253.26): C, 61.65; H, 4.38; N, 27.65; O, 6.32. Found: C, 61.19; H, 4.34; N, 27.62; O, 6.29.

2.3.3. 4-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazinylidene]methyl}phenol (c).

Yield % 83.79, m.p. 191 ºC, IR: υ_{max}./cm^{-1} 3512 (-NH- aromatic), 3388 (-NH- aliphatic), 3138 (-OH), 2824 (-CH=), 1662/1470 (>C=C<), 1570 (>C=NN-), 1025 (-N-N-), 723 (di sub benzene ring). ^{1}H-NMR (DMSO-d$_6$) δ: 14.078 (s, 1H, -NH- aliphatic), 12.983 (s, 1H, NH, aromatic), 12.676 (s, 1H, -OH), 10.339 (s, 1H, -CH=), 8.473 (s, 1H, pyrimidine-H), 6.645-7.896 (m, 6H, aromatic-H). ^{13}C-NMR (DMSO-d$_6$): 142.42 (-CH=), 150.92 (C2), 99.93 (C3), 148.69 (C4), 116.25 (C5), 124.55 (C6), 102.89 (C2), 116.99 (C3), 130.53 (C4). Anal. Calcd. for $C_{13}H_{11}N_5O$ (253.26): C, 61.65; H, 4.38; N, 27.65; O, 6.32. Found: C, 61.44; H, 4.36; N, 27.60; O, 6.31.

2.3.4. 3-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazinylidene]methyl}benzene-1,2-diol (d).

Yield % 77.70, m.p. 193 ºC, IR: υ_{max}./cm^{-1} 3355 (-NH- aromatic), 3183 (-NH- aliphatic), 3115/3125 (-OH), 2873 (-CH=), 1587/1472 (>C=C<), 1528 (>C=NN-), 1022 (-N-N-), 739 (di sub benzene ring). ^{1}H-NMR (DMSO-d$_6$) δ: 13.978 (s, 1H, -NH- aliphatic), 12.963 (s, 1H, NH, aromatic), 9.660 (s, 1H, -OH), 9.029 (s, 1H, -CH=), 8.431 (s, 1H, pyrimidine-H), 6.741-7.652 (m, 6H, aromatic-H). ^{13}C-NMR (DMSO-d$_6$): 146.62 (-CH=), 120.71 (C2), 146.11 (C3), 146.34 (C4), 125.97 (C5), 124.55 (C6), 100.08 (C2), 103.05 (C3), 146.54 (C4). Anal. Calcd. for $C_{13}H_{11}N_5O_2$ (269.26): C, 57.99; H, 4.12; N, 26.01; O, 11.88. Found: C, 57.49; H, 4.03; N, 26.00; O, 11.31.

2.3.5. 4-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazinylidene]methyl}benzene-1,3-diol (e).

Yield % 81.04, m.p. 195 ºC, IR: υ_{max}./cm^{-1} 3310 (-NH- aromatic), 3190 (-NH- aliphatic), 3513/3123 (-OH), 2880 (-CH=), 1595/1443 (>C=C<), 1529 (>C=NN-), 974 (-N-N-), 718 (di sub benzene ring). ^{1}H-NMR (DMSO-d$_6$) δ: 13.851 (s, 1H, -NH- aliphatic), 12.931 (s, 1H, NH, aromatic), 10.288/10.175 (s, 2H, -OH), 8.920 (s, 1H, -CH=), 8.417 (s, 1H, pyrimidine-H), 6.414-8.060 (m, 6H, aromatic-H). ^{13}C-NMR (DMSO-d$_6$): 146.62 (-CH=), 120.71 (C2), 146.11 (C3), 146.34 (C4), 125.97 (C5), 124.55 (C6), 100.08 (C2), 103.05 (C3), 146.54 (C4). Anal. Calcd. for $C_{13}H_{11}N_5O_2$ (269.26): C, 57.99; H, 4.12; N, 26.01; O, 11.88. Found: C, 57.88; H, 4.10; N, 27.56; O, 11.79.

2.3.6. 2-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazinylidene]methyl}benzene-1,4-diol (f).

Yield % 84.39, m.p. 194 ºC, IR: υ_{max}./cm^{-1} 3188 (-NH- aromatic), 3130 (-NH- aliphatic), 3082 (-OH), 2837 (-CH=), 1594/1458 (>C=C<), 1529 (>C=NN-), 1022 (-N-N-), 736 (di sub benzene ring). ^{1}H-NMR (DMSO-d$_6$) δ: 14.050 (s, 1H, -NH- aliphatic), 12.995 (s, 1H, NH, aromatic), 9.722/9.004 (s, 2H, -OH), 8.441 (s, 1H, -CH=), 7.553 (s, 1H, pyrimidine-H), 6.872-7.258 (m, 6H, aromatic-H). ^{13}C-NMR (DMSO-d$_6$): 142.60 (-CH=), 119.99 (C2), 143.93 (C3), 145.34 (C4), 125.90 (C5), 124.50 (C6), 102.17 (C2), 102.55 (C3), 145.93 (C4). Anal. Calcd. for $C_{13}H_{11}N_5O_2$ (269.26): C, 57.99; H, 4.12; N, 26.01; O, 11.88. Found: C, 57.91; H, 4.07; N, 27.91; O, 11.81.

2.3.7. 4-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazinylidene]methyl}benzene-1,2-diol (g).

Yield % 87.36, m.p. 198 ºC, IR: υ_{max}./cm^{-1} 3178 (-NH- aromatic), 3118 (-NH- aliphatic), 3057/2996 (-OH), 2870 (-CH=), 1583/1472 (>C=C<), 1519 (>C=NN-), 1020 (-N-N-), 756 (di sub benzene ring). ^{1}H-NMR (DMSO-d$_6$) δ: 14.161 (s, 1H, -NH- aliphatic), 13.569 (s, 1H, NH, aromatic), 12.973/9.873 (s, 2H, -OH), 9.206 (s, 1H, -CH=), 8.652 (s, 1H, pyrimidine-H), 6.937-7.542 (m, 6H, aromatic-H). ^{13}C-NMR (DMSO-d$_6$): 142.58 (-CH=), 119.95 (C2), 143.90 (C3), 145.32 (C4), 125.91 (C5), 124.52 (C6), 102.19 (C2), 102.57 (C3), 145.90 (C4). Anal. Calcd. for $C_{13}H_{11}N_5O_2$ (269.26): C, 57.99; H, 4.12; N, 26.01; O, 11.88. Found: C, 57.89; H, 4.11; N, 25.99; O, 11.77.

2.3.8. 2-methoxy-4-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4yl)hydrazinylidene]methyl} phenol (h).

Yield % 86.88, m.p. 196 ºC, IR: υ_{max}./cm^{-1} 3380 (-NH- aromatic), 3232 (-NH- aliphatic), 3135 (-OCH$_3$), 2977 (-OH), 2924 (-CH=), 1584/1441 (>C=C<), 1523 (>C=NN-), 1024 (-N-N-), 755 (di sub benzene ring). ^{1}H-NMR (DMSO-d$_6$) δ: 13.918 (s, 1H, -NH- aliphatic), 12.901 (s, 1H, NH, aromatic), 8.581 (s, 2H, -OH), 9.801 (s, 1H, -CH=), 8.427 (s, 1H, pyrimidine-H), 6.896-7.683 (m, 6H, aromatic-H), 3.897 (s, 3H, -OCH$_3$). ^{13}C-NMR (DMSO-d$_6$): 56.58 (OCH$_3$), 142.57 (-

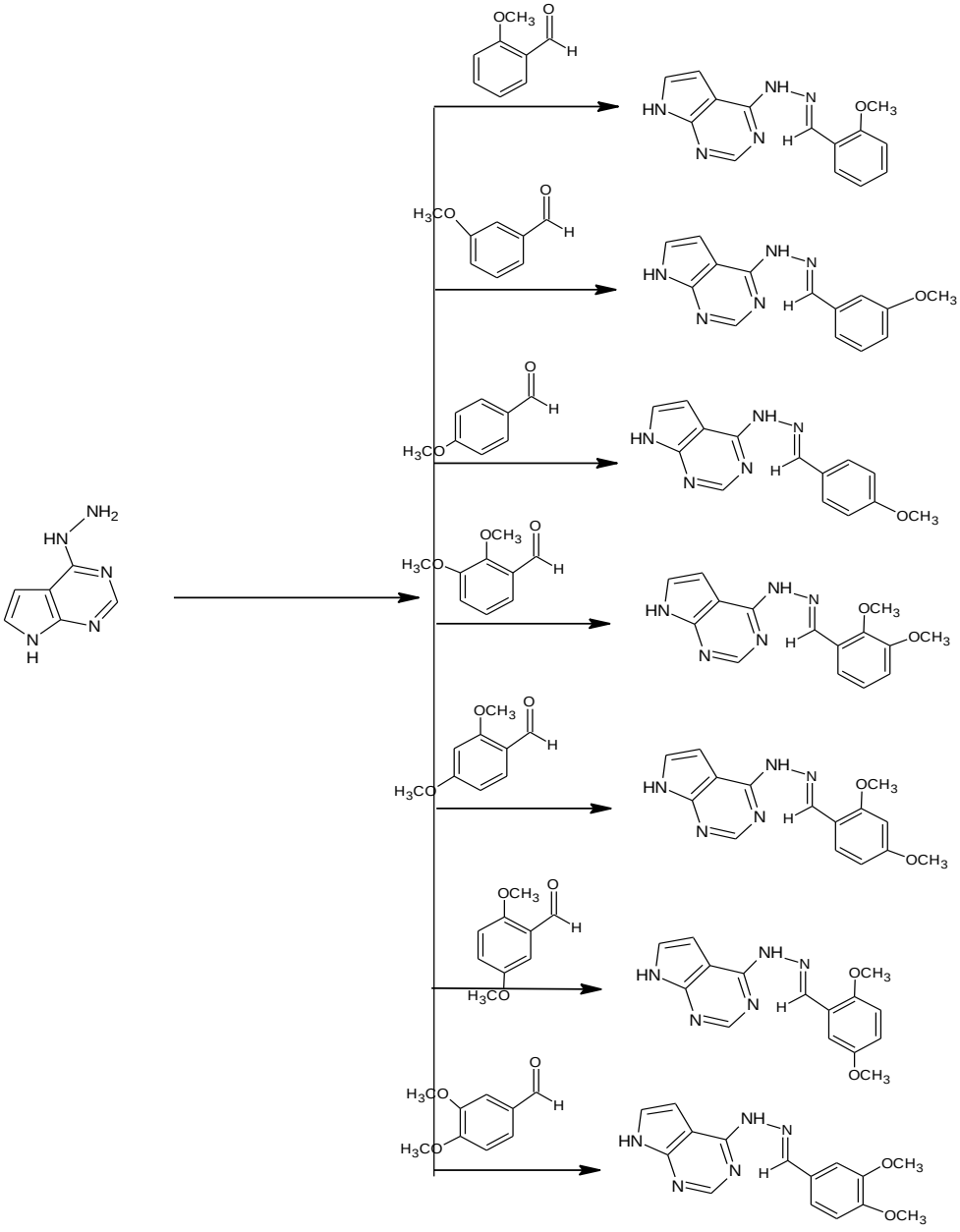
CH=), 150.57 (C2), 102.97 (C3), 148.67 (C4), 115.90 (C5), 123.96 (C6), 99.98 (C2), 119.50 (C3), 125.90 (C4). Anal. Calcd. for $C_{14}H_{13}N_5O_2$ (283.28): C, 59.36; H, 4.63; N, 24.72; O, 11.30. Found: C, 56.31; H, 4.59; N, 24.69; O, 11.21.

2.3.9. 5-nitro-2-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazinylidene]methyl} phenol (i).

Yield % 83.89, m.p. 199 ºC, IR:υ_{max}./cm^{-1} 3382 (-NH- aromatic), 3230 (-NH- aliphatic), 3068 (-OH), 2615 (-CH=), 1586/1450 (>C=C<), 1524 (>C=NN-), 1024 (-N-N-), 741 (di sub benzene ring). ^{1}H-NMR (DMSO-d_6) δ: 14.277 (s, 1H, -NH- aliphatic), 12.997 (s, 1H, NH, aromatic), 12.172 (s, 2H, -OH), 9.001 (s, 1H, -CH=), 8.507 (s, 1H, pyrimidine-H), 7.243-7.542 (m, 6H, aromatic-H). ^{13}C-NMR (DMSO-d_6): 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. for $C_{14}H_{13}N_5O_2$ (283.28): C, 59.36; H, 4.63; N, 24.72; O, 11.30. Found: C, 56.31; H, 4.59; N, 24.69; O, 11.21.

2.3.10. 2,4-dichloro-6-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazinylidene]methyl} phenol (j).

Yield % 88.82, m.p. 195 ºC, IR:υ_{max}./cm^{-1} 3190 (-NH- aromatic), 3065 (-NH- aliphatic), 3594 (-OH), 2805 (-CH=), 1590/1451 (>C=C<), 1529 (>C=NN-), 1029 (-N-N-), 856/893 (Ar-Cl), 732 (tetra sub benzene ring). ^{1}H-NMR (DMSO-d_6) δ: 12.518 (s, 1H, -NH- aliphatic), 8.406 (s, 1H, NH, aromatic), 7.990 (s, 2H, -OH), 8.702 (s, 1H, -CH=), 7.624 (s, 1H, pyrimidine-H), 6.952-7.479 (m, 6H, aromatic-H). ^{13}C-NMR (DMSO-d_6): 143.84 (-CH=), 151.03 (C2), 122.96 (C3), 130.25 (C4), 115.90 (C5), 123.96 (C6), 101.15 (C2), 101.84 (C3), 150.23 (C4). Anal. Calcd. for $C_{14}H_9N_5OCl_2$ (283.28): C, 48.47; H, 2.82; N, 21.74; O, 4.97; Cl, 22.01. Found: C, 48.41; H, 2.79; N, 21.72; O, 4.92; Cl, 21.93.



Scheme 1: Synthetic pathways for compounds (**a** to **j**)

2.4. Biological assay:

2.4.1. Anti-microbial activity:

2.4.1.1. Materials and methods:

The disc-diffusion and MIC methods were used to test antibacterial activity under National Committee for Clinical Laboratory Standards conditions [18]. The anti-microbial activity of the synthesised compounds was tested in vitro against several pathogenic Gram-positive bacteria (*Staphylococcus aureus* MCC 2010, and *Bacillus subtilis* MCC 2010) and Gram-negative bacteria (*Pseudomonas aeruginosa* (MCC 2080, and *Escherichia coli* MCC 2412), as well as *Candida albicans* MCC1439). All microorganisms used in this study originated from the Konkan Gyanpeeth Rahul Dharkar College of Pharmacy and Research Institute, Karjat, Dist-Raigad, Maharashtra-India.)
Hi-Media supplied the nutrient agar and Muller Hinton agar (MHA) media for disc sensitivity studies. (India). The tested compound was dissolved in sterile DMSO to produce 20 and 30 mg/mL and then passed through 0.2 mm membrane filters. Filtrates were dispensed as 2 mL samples into sterile, small screw-capped containers, frozen, and stored at 15 degrees Celsius. After thawing, the containers were refrozen. The disc diffusion sensitivity test was performed in the same way as Bauer et al. [19]. DMSO demonstrated no blocking zones. As control drugs, streptomycin and fluconazole were used.
The NCCLS recommendations from 1997 were followed in determining the MIC (minimum inhibitory concentration) for each compound tested on Mueller-Hinton agar [20]. All bacterial isolates were subculture in MHA plates and left in the incubator at 37 °C overnight, while all fungicide isolates were subculture in SDA plates and left in the incubator at 35 °C for 24-48 hours. To make sure they were pure and viable, the microorganisms underwent at least two passages. Each concentration was combined with sterile nutrient agar in sterile plates, and bacteria inoculum was added to each well of the micro dilution trays. The solutions of the newly synthesized compounds and standard drugs were prepared at 1000, 500, 250, 125, 65, 30, and 15 mg/ml concentrations using serial twofold dilutions in DMSO. After 24 hours of incubation, the trays were read for MIC end points while being incubated at 37 °C in a humid room. Minimum inhibitory concentrations (MICs)-the lowest concentration of a substance required to fully prevent macroscopic growth-were reported. As reference wells, DMSO, pure microorganisms, and pure media were used.

3. Results and discussion:

The synthetic methods used to obtain the target molecules are depicted in **Figure 1**. This research looked into the interaction between substituted hydroxy benzaldehydes and 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine. **Table 1** lists the physicochemical details for compounds **a** to **j**. Prepared substances have been identified by the IUPAC names and initials 2-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazinylidene]methyl}phenol **(a)**, 3-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazinylidene]methyl}phenol **(b)**, 4-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazinylidene]methyl}phenol **(c)**, 3-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazinylidene]methyl}benzene-1,2-diol **(d)**, 4-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazinylidene]methyl}benzene-1,3-diol **(e)**, 2-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazinylidene]methyl}benzene-1,4-diol **(f)**, 4-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazinylidene]methyl}benzene-1,2-diol **(g)**, 2-methoxy-4-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4yl)hydrazinylidene]methyl} phenol **(h)**, 5-nitro-2-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazinylidene]methyl} phenol **(i)**, and 2,4-dichloro-6-{(*Z*)-[2-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)hydrazinylidene]methyl} phenol **(j)**. The synthetic methods used to obtain the desired molecules are depicted in **Scheme 1**. This research looked into the interaction between substituted hydroxy benzaldehydes and 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine.

3.1. FT(IR) spectra:

It was possible to analyse the bonding of the 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine to substituted hydroxy benzaldehydes by comparing the FT(IR) spectra of the synthesized compounds with those of the free 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine. Several prominent bands were used to examine the impact of 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine vibration on modified hydroxybenzaldehyde's. The absence of stretching vibrations brought on by the aldehyde (CHO) and amino (NH_2) moieties is evidence that all prepared molecules have developed as expected. Instead, a powerful new band that is associated with the azomethine (HC=NN) group [21] formed in the range 1487–1570 cm^{-1}. A broad band that has been designated as aromatic in the 3310–3512 cm^{-1} region suggests the existence of the prepared compounds [22–23] (NH). All substances have been connected to the (-CH=) aldehydic bands between 2828 and 2873 cm^{-1}. Sharp lines can be seen in the IR spectra of **a-j** compounds at 1580–1595 and 1421–1462 cm^{-1}, which are connected to an aromatic ring's >C=C group. Strong bands can be found in the 1316–1335 cm^{-1}, 722–739 cm^{-1}, and the 650–691 cm^{-1}. The aromatic (C-N) ring, the di- or trisubstituted benzene ring, and the mono substituted benzene ring can all be found in the FT(IR) bands of the **a-j** compounds. The FT-IR spectroscopy of the **a-j** compound showed a band at 3054–3138 cm^{-1} that is associated with the aromatic -OH group.

3.2. ^{1}H NMR spectra:

The broad singlet signals seen at 12.421–14.318 ppm in the ^{1}H NMR spectra of all synthesized compounds are caused by the presence of the aromatic NH moiety in the pyrrolyl ring. The singlet aliphatic -NH- peak appears in the 10.421-34.023 ppm range, and the singlet peak associated with the aldehydic -CH= group of all prepared molecules appears in the 8.316–8.768 ppm range. The fact that none of the synthesised derivatives exhibit a wide singlet signature at 9.84 ppm (2H), which corresponds to the -NH_2 of 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine, indicates that the amino group was successfully substituted by Schiff base [24]. The 1H-NMR spectra of compounds **a** through **j** show a singlet for the pyrimidine proton at 7.865–7.768 ppm. All chemicals produced have singlet signals ranging from 8.634 to 9.210 ppm.

1H NMR results support the aromatic ring's OH structure. The ^{1}H NMR bands match those identified in previously published studies [24–25].

3.3. Antimicrobial evaluation:

The new compounds were tested in vitro for antibacterial and antifungal activity using the broth microdilution method against *Candida albican*s (MCC1439) and *Saccharomyces cerevisiae* (MCC1033) as well as Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus* (MCC 2010), *Bacillus subtilis* (MCC 2010), and *Pseudomonas aeruginosa* (MCC 2080).

After being incubated for 24 hours at 37 °C, all of the bacterial isolates were grown in nutrient broth. The sabouraud dextrose agar was kept in malt broth after being incubated for 24 hours at 25 °C, and tween 80 was used to gather the suspensions of fungal spores from fungi that had been actively growing for 7 days. The final inoculum optical densities (OD) for bacteria and fungus were 0.2–0.3 and 0.5, respectively. The DMSO used to create the stock solutions has no effect on the microbe at the concentrations being investigated. At concentrations of 1000 g/mL, both bacteria and fungus were multiplied by two. *Fluconazole* and *streptomycin* were common medication powders for pathogens and fungi. The antimicrobial activity was evaluated following 24 hours at 37 °C and 48 hours at 25 °C for the antifungal test.

3.3.1. Antibacterial activity:

Streptomycin, a broad-spectrum antibiotic with a MIC of 1mg/mL against the bacterial species, served as the study's reference medication. The suppression zones for Escherichia coli (MCC 2412), Bacillus subtilis (MCC 2010), Pseudomonas aeruginosa (MCC 2080), and Staphylococcus aureus (MCC 2010) were 17–20mm, 19-25 mm, 19–21 mm, and 16–27 mm, respectively. The antibacterial results in **Table 1** clearly showed that all of the bacteria tested were susceptible to the tested compounds, with MICs varying from 15 to 65 ppm.

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

Compound	Antibacterial Activity (zone of inhibition)			
	S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***
(a)	16.00	21.00	19.00	19.00
(b)	17.00	25.00	17.00	19.00
(c)	16.00	20.00	20.00	21.00
(d)	16.00	21.00	20.00	20.00
(e)	18.00	19.00	20.00	20.00
(f)	17.00	23.00	19.00	19.00
(g)	17.00	24.00	17.00	19.00
(h)	18.00	20.00	19.00	19.00
(i)	17.00	21.00	20.00	19.00
Streptomycin	20.00	22.00	20.00	19.00

With respect to each bacterial species, the (**c**) (21mm) was found to be more effective than the reference drug for S. aureus, while the (**b**), (**f**), (**g**), (**h**), and (**i**) were less effective for P. aeruginosa, and the (c) was found to be more effective than the reference drug for P. aeruginosa. The (**c**), (**d**), (**e**), and (**i**) were found to be the most active chemicals for E. coli, whereas the (**c**), (**e**), and (**g**) were found to be the most active for B. subtilis. The antimicrobial effect is most likely a result of microorganisms' less lipophilic cell walls, which make it simpler for them to enter cells. This is most likely because the molecule's lipophilic alkyl chain allows it to travel through the lipid cell membrane of gram-negative bacteria. The findings indicate that the antibacterial activity decreases as the length of the carbon chain increases. This might be because the molecule can't travel through the bacterial cell membrane due to the carbon chain's bulk [26].

3.3.2. Antifungal activity

Fluconazole was the standard drug used in this research; its MIC for the tested fungi was 50 g/ml, and its inhibition zones for Candida albicans (MCC1439) and Saccharomyces cerevisiae (MCC1033), respectively, were 29-33 mm and 19-29 mm, respectively. With a MIC of 50 mg/mL against Candida albicans (MCC1439) and Saccharomyces cerevisiae (MCC1033), all of the compounds evaluated in **Table 2** demonstrated high fungicidal potential and were therefore more effective than the standard treatment.

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

Compound	***Candida albicans***	***Saccharomyces cerevisiae***
(a)	29.00	19.00
(b)	30.00	25.00
(c)	33.00	29.00
(d)	34.00	23.00
(e)	29.00	25.00
(f)	20.00	22.00
(g)	29.00	23.00
(h)	31.00	24.00

(i)	25.00	25.00
Fluconazole	26.00	21.00

4. Conclusion

In this research, we have produced a number of novel substituted 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine derivatives of **a-j**, hydroxy benzaldehydes. The formation of the proposed compounds is confirmed by analytical data, FT-IR, UV-vis, NMR spectral investigations, and electrochemical data. The ^{1}H NMR, UV-vis, elemental analysis (C, H, N, and O), and FT-IR spectra of the synthesized hydroxy benzaldehydes-based substances were recorded and investigated. The results advise mixing 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine and modified hydroxy benzaldehydes in a 1:1 mixture. All synthetic materials exhibited outstanding antibacterial activity.

5. References:

1. S.M. Sondhi, R.N. Goyal, A.M. Lahoti, *Bioorg. Med. Chem.* 13 (2005) 3185.
2. E. Sochacka, I. Fratczak, *Tetrahedron Lett*. 45 (2004) 6729.
3. J.C. Hazelton, B. Iddon, H. Suschitzky, L.H.J. Woolley, *Chem. Soc. Perkin Trans*. 1 6 (1992) 685.
4. Z.G. Hajos, R.M. Kanojia, *Chem. Abstr*. 116 (1991) 83701.
5. A.K. Pathak, V. Pathak, L.E. Seit, W.J. Sulng, R.C.J. Reynolds, *Med. Chem*. 41 (2004) 273.
6. E.V. Aleksandrova1, *Pharm. Chem. J*. 37 (2003) 645.
7. M.D. Mashkovskii, *Drugs. Moscow* 1e2 (2000) (in Russian).
8. G.N. Pershin, Z.h. Vses, o-vaim, *Mendeleev Communications* 15 (2) (1970) 216.
9. E. Berger, L. Rumbach, *La Revue de médecine interne* 20 (1999) 3465.
10. T. Munster, D.E. Furst, *Clin. Exp. Rheumatol.* 17 (1999) 529;
11. J.L.S. Banitt, G.L. Bundy, I.M. Richards, *J. Pharmacol. Exp. Ther*. 290 (1999) 188.
12. B. Howard Cottam, Z. Kazimierczuk, S. Geary, P. McKernan, *J. Med. Chem*. 28 (1985) 1461.
13. M.J. Motsuoka, *Antibiotics (Tokyo)* 13A (1960) 121.
14. K. Anzai, S.J. Marumo, *Antibiotics* (Tokyo) 10A (1957) 20.
15. M.A. Mohamed, A.E. Rashad, M.E.A. Zaki, S.S. Fatahala, *Acta Pharm*. 55 (2005) 237.
16. M.A. Mohamed, A.E. Rashad, M.E.A. Zaki, S.S. Fatahala, *Arch. Pharm. Chem. Life Sci.* (2006) 339.
17. M.A. Mohamed, A.E. Rashad, M.E.A. Zaki, S.S. Fatahala, M.Z. Monem, *Naturforsch C* (2007) 62
18. M.T. Cocco, C. Congiu, A. Maccioni, V. Onnis, *J. Heterocycl*. 30 (1993) 253.
19. A.W. Bauer, M.M. Kirby, J.C. Sherris, *Am. J. Clin. Pathol*. 45 (1996) 493.
20. A.C. Gales, H.S. Sader, R.N. Jones. *Diagn. Microbial. Infect. Dis*. 44 (1997e2000) 289.
21. Almáši, Miroslav, Mária Vilková, and Jozef Bednarčík. *Inorganica Chimica Acta* 515 (2021): 120064.
22. Karthikeyan, S., V. K. Gupta, R. Boopathy, A. Titus, and G. Sekaran. *Journal of Molecular Liquids* 173 (2012): 153-163.
23. Carolin, C. Femina, P. Senthil Kumar, B. Chitra, C. Fetcia Jackulin, and Racchana Ramamurthy. *Journal of Hazardous Materials* 415 (2021): 125716.
24. Oregioni, Alain, Benjamin Stieglitz, Geoffrey Kelly, Katrin Rittinger, and Tom Frenkiel. *Scientific reports* 7, no. 1 (2017): 1-8.
25. Ghorab, Mostafa M., Mariangela Ceruso, Mansour S. Alsaid, Yassin M. Nissan, Reem K. Arafa, and Claudiu T. Supuran. *European Journal of Medicinal Chemistry* 87 (2014): 186-196.
26. Sharma, Pradyumn, Rakesh Vaiwala, Srividhya Parthasarathi, Nivedita Patil, Anant Verma, Morris Waskar, Janhavi S. Raut, Jaydeep Kumar Basu, and K. Ganapathy Ayappa. *Langmuir* 38, no. 50 (2022): 15714-15728.