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Synthesis of Some 7H-pyrrolo[2,3-d]Pyrimidin-4-Amine Compounds Derived From Fluorobenzaldehyde Using Ultrasonic Waves

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Abstract:

Intermediates were utilized to synthesize 7H-pyrrolo[2,3-d]pyrimidin-4-amine (PPA) derivatives substituted with benzaldehydes. Elemental analysis, infrared spectroscopy, nuclear magnetic resonance, and ultraviolet spectral data were employed to confirm the structures of these compounds. In vitro testing was conducted on all newly synthesized compounds to evaluate their cytotoxicity against *Artemia salina*. Each distinct molecule was assessed for its potential to inhibit microbial growth in living organisms. Several compounds demonstrated promising antibacterial activity when compared to streptomycin and fluconazole. This study focuses on the antibacterial properties and structure-activity relationships of these compounds.

Keywords — ***Artemia salina*, 7H-pyrrolo[2,3-d]pyrimidin-4-amine, difluoro benzaldehydes**

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I. INTRODUCTION

Due to their significance in cellular processes, purines and pyrimidines serve as potential leads for drug discovery. The pyrimidine family includes 2-thiopyrimidine (2-TP) and its related compounds, also referred to as 2-mercaptopyrimidine derivatives [1]. A promising alternative to the oxygen atom currently attached to carbon-2 in the uridine base is the sulfur atom in the 2-TP ring [2]. This hypothesis has made 2-TPs particularly intriguing to synthetic biochemists [3]. The use of 2-TP derivatives in the development of cardiotonic medications is extensively detailed in a European patent [4]. Pathak et al. studied 2-TP derivatives for their potential antibacterial activity against *Mycobacterium tuberculosis* (Mtb) [5]. The thio analogue of hypoxanthine, 6-thiopurine (6-TP) [6], is an organic by-product of purine metabolism.

Since the discovery of this antimetabolite over fifty years ago, extensive biological research has led to the synthesis and characterization of thousands of 6-TP derivatives, which have been shown to be particularly effective in treating leukemias [7], autoimmune and rheumatic disorders [7–10], and for immunosuppression in organ transplantation [7, 8]. Pyrrolo[3,2-d]pyrimidines, structurally similar to purines and pyrimidines, demonstrate intriguing biological activities and belong to the class of 7-deazapurine analogues. Well-known nonsteroidal anti-inflammatory drugs (NSAIDs), such as tolmetin (Rumatol) and ketorolac (Ketolac) [11], achieve their anti-inflammatory effects primarily by inhibiting prostaglandin synthesis. Additionally, the anti-inflammatory pyrrolopyrimidine PNU-142731A [12] blocks cytokine production in living systems. Examples of naturally occurring pyrrolo[2,3-d]pyrimidine nucleoside antibiotics include sangivamycin, tubercidin, and toyocamycin [13, 14], which have been shown to inhibit the growth of certain bacteria.

Previous studies revealed that the incorporation of nitrogen or other heteroatoms in aromatic rings

enhanced antimicrobial and anti-inflammatory activity [15–17]. Building on these findings and as part of ongoing research in this area [15–17], we aimed to develop new PPA derivatives of substituted fluorobenzaldehydes to explore the relationships between structure and activity.

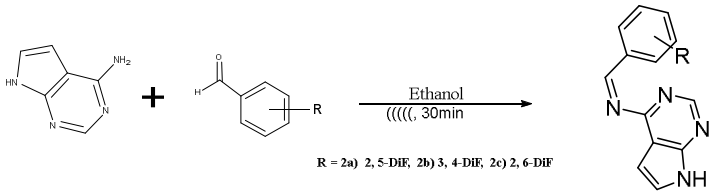
II. EXPERIMENTAL

General methods

The uncorrected melting points of substances were determined using the Lab Junction Melting Point/Boiling Point Apparatus. Infrared light bands from potassium bromide pellets were analyzed with a BRUKER FT-IR spectrophotometer. Chemical shifts were measured on a Bruker 400 MHz spectrometer, with results reported in ppm relative to TMS, which served as the internal reference. Microanalysis conducted using the Carlo Erba 1108 provided results within the permissible range (0.40) of the predicted values. UV bands at room temperature were recorded using a JASCO V650 spectrophotometer. Precoated silica gel plates were employed for thin-layer chromatography (TLC) instead of conventional glass plates. The developing solvent solution consisted of a 9:1 mixture of petroleum ether and ethyl acetate, and the spots were visualized under ultraviolet light.

2.2. Fluorobenzaldehyde derivatives of PPH (2a-2c)

A catalytic amount of concentrated hydrochloric acid was used to facilitate the reaction between substituted fluorobenzaldehydes (a–i) (0.1 mol) and PPA **1** (0.1 mol). After the reaction was completed, as monitored by TLC, the mixture was sonicated at room temperature for 30 minutes. The resulting product was then filtered, washed with ether and water, dried, and recrystallized from ethanol.



Scheme 1: General preparation of compound **2a -2c**

2.2. Biological assay:

2.2.1. Anti-microbial activity:

2.2.1.1. Materials and methods:

The antibacterial efficacy of the synthesized compounds was assessed using disc-diffusion and minimum inhibitory concentration (MIC) techniques, following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) [18]. A range of bacteria was employed for in vitro antimicrobial testing, including Gram-positive *Staphylococcus aureus* MCC 2010 and *Bacillus subtilis* MCC 2010, as well as Gram-negative *Pseudomonas aeruginosa* MCC 2080, *Escherichia coli* MCC 2412, and *Candida albicans* MCC 1439. The microbial strains were obtained from the Konkan Gyanpeeth Rahul Dharkar College of Pharmacy and Research Institute in Karjat, District Raighad, Maharashtra, India.

For the disc diffusion sensitivity tests, nutritional agar and Mueller-Hinton agar (MHA) provided by Hi-Media (India) were used. The test compounds were dissolved in sterile DMSO to achieve concentrations of 20 and 30 mg/mL, filtered through 0.2 µm membrane filters, and stored in sterile, screw-capped containers at -15 °C after flash freezing. The containers were thawed and refrozen as needed. The disc diffusion sensitivity test was conducted following the procedure outlined by Bauer et al. [19], with DMSO showing no inhibitory zones. Streptomycin and fluconazole served as control agents.

To determine the MIC values, testing was carried out on Mueller-Hinton agar by the NCCLS guidelines from 1997 [20]. Subcultures of all bacterial isolates were incubated overnight at 37 °C, while fungal isolates were incubated at 35 °C for 24–48 hours. Each microorganism underwent at least two purification steps to ensure viability and

purity. Bacterial inocula were added to the wells of microdilution trays containing serial two-fold dilutions of the synthesized compounds and reference drugs in DMSO at concentrations of 1000, 500, 250, 125, 65, 30, and 15 mg/mL. The trays were incubated at 37 °C in a humidified environment for 24 hours, after which the MIC endpoints were recorded. The MIC was defined as the lowest concentration of the compound that completely inhibited visible microbial growth. Reference wells included DMSO, sterile microorganisms, and sterile growth medium.

III. RESULTS AND DISCUSSION:

Figure 1 illustrates the synthetic procedures employed to obtain the desired compounds. This study focused on the interactions between substituted hydroxy benzaldehydes and PPA. The physicochemical properties of compounds 2a–c are summarized in Table 1.

3.1. FT(IR) Spectra:

The attachment of PPA to difluoro-substituted benzaldehydes was evaluated by comparing the FT(IR) spectra of the synthesized compounds with those of free PPA. Key bands were analyzed to study the impact of PPA vibrations on modified bromo, cyano, and difluoro-substituted benzaldehydes. The absence of aldehyde (CHO) and amino (NH_2) stretching vibrations confirmed that all prepared compounds were successfully synthesized. In their place, a new characteristic band appeared in the 1512–1523 cm^{-1} region, corresponding to the azomethine (HC=NN) group [21]. Broadband absorption between 3179 and 3280 cm^{-1}, indicative of aromatic υ(NH), was observed in the produced compounds, as reported in references [22, 23].

The aldehydic (-CH=) bands were identified within the 2823–3023 cm^{-1} range for all compounds. The FT(IR) spectra of compounds **2a–c** displayed two prominent bands at 1581–1590 cm^{-1} and 1436–1480 cm^{-1}, which are associated with the aromatic ring's >C=C group. Additional bands were detected in the ranges of 682–739 cm^{-1} and 1315–1333 cm^{-1}. These bands were attributed to various ring structures, including aromatic (C-N), di- or trisubstituted benzene, or mono-substituted benzene.

3.1. ^{1}H NMR spectra:

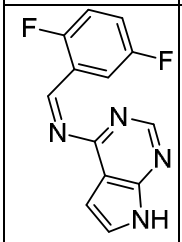
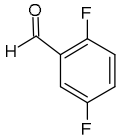
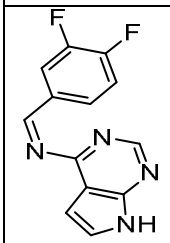
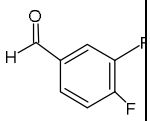
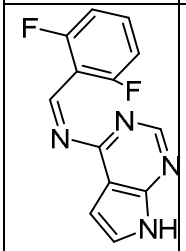
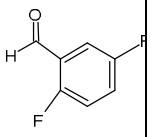
The ^{1}H NMR spectra of all synthesized compounds exhibited singlet signals with chemical shifts between 8.71 and 13.01 ppm, confirming the presence of an aromatic NH group on the pyrrolyl ring. A singlet peak for the aldehydic -CH= group produced a singlet peak in the 8.65–8.69 ppm range across all synthesized compounds. The absence of a broad singlet peak at 9.84 ppm (2H), corresponding to the -NH_2 group of PPA, indicates the successful substitution of the amino group with the Schiff base [24].

Additionally, compounds **2a–c** displayed a single peak in their ^{1}H NMR spectra, corresponding to the pyrimidine proton, within the chemical shift range of 8.44–8.54 ppm. The observed ^{1}H NMR signals are consistent with those reported in previous studies [24–25].

Table 2: NMR spectral data of compounds **2a-2c**

Comp	NH aromatic (s, 1H)	-CH= (s, 1H)	pyrimidine-H (s, 1H)	aromatic-H (m, 6H)
2a	13.01	8.69	8.54	7.24-8.37
2b	8.71	8.66	8.45	7.21-8.28
2c	11.30	8.65	8.44	7.21-8.28

Table 3: Yield, color, reaction time, and physical constants of the product (**2a-c**)

Products	Aldehyde	Color	Yield (%)	Reaction time (min.)	m.p. (°C)
 **2a**	 **a**	Yellow	80.24	30	165
 **2b**	 **b**	Yellow	86.74	30	160
 **2c**	 **c**	Yellow	82.47	30	176

3.2. Antimicrobial evaluation:

The newly synthesized compounds were subjected to in vitro screening to evaluate their antibacterial and antifungal properties. Their activity against *Candida albicans* (MCC1439), *Saccharomyces cerevisiae* (MCC1033), and other Gram-positive and Gram-negative bacteria was tested using the broth microdilution method. The bacterial strains included *Staphylococcus aureus* (MCC 2010), *Bacillus subtilis* (MCC 2010), and *Pseudomonas aeruginosa* (MCC 2080).

Bacterial isolates were cultured in a nutrient-rich broth medium and incubated for 24 hours at 37 °C. For fungi, Sabouraud dextrose agar was transferred to malt broth and incubated at 25 °C for 24 hours. Fungal spore suspensions were prepared using Tween 80 from actively growing fungi over 7 days. The final inoculum optical densities (OD) were 0.2–0.3 for bacteria and 0.5 for fungi. Dimethyl sulfoxide (DMSO), used to prepare stock solutions, showed no noticeable effect on the microorganisms at the concentrations used.

At a concentration of 1000 µg/mL, bacterial and fungal populations demonstrated a twofold increase. Fluconazole and streptomycin were used as standard therapeutic powders to treat infections and fungal growth. Antibacterial activity was evaluated after 24 hours of incubation at 37 °C, while antifungal activity was assessed after 48 hours of incubation at 25 °C.

3.2.1. Antibacterial activity:

The study utilized Streptomycin as the reference drug, an antibiotic known for its broad-spectrum activity. It demonstrated a minimum inhibitory concentration (MIC) of 1 mg/mL against the bacterial species analyzed. The inhibition zones for *Escherichia coli* (MCC 2412), *Bacillus subtilis* (MCC 2010), *Pseudomonas aeruginosa* (MCC 2080), and *Staphylococcus aureus* (MCC 2010) were measured at 19–20 mm, 20–22 mm, 15–19 mm, and 12–15 mm, respectively. **Table 4** reveals that the tested compounds displayed antibacterial activity against all examined microorganisms, with minimum inhibitory concentrations (MICs) ranging from 15 to 65 ppm.

Table 4: Antibacterial studies of **2a-c** compounds

Compound	Antibacterial Activity (zone of inhibition)			
	S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***
(2a)	16.00	22.00	19.00	12.00
(2b)	15.00	20.00	19.00	15.00
(2c)	19.00	21.00	20.00	15.00

Streptomycin	20.00	21.00	20.00	19.00

Antifungal activity

Fluconazole, the reference drug, effectively inhibited the studied fungi, showing a minimum inhibitory concentration (MIC) of 50 µg/mL. The inhibition zones for *Candida albicans* and *Saccharomyces cerevisiae* ranged from 17–21 mm and 24–25 mm, respectively. The compounds listed in **Table 5** demonstrated substantial fungicidal activity, exceeding the efficacy of conventional treatment, as indicated by their MIC of 50 µg/mL against both *Candida albicans* and *Saccharomyces cerevisiae*.

Table 5: Antifungal studies of **2a-c** compounds

Compound	*Candida albicans*	*Saccharomyces cerevisiae*
(2a)	19.00	23.00
(2b)	21.00	24.00
(2c)	17.00	25.00
Fluconazole	22.00	21.00

IV. CONCLUSIONS

The present study focused on synthesizing a series of novel substituted PPA compounds, labeled **2a** to **c**. The successful preparation of the proposed compounds was confirmed through detailed analysis, including FT-IR, UV-vis, NMR spectroscopy, and electrochemical data. Compounds based on bromo, cyano, and difluoro benzaldehydes were synthesized and characterized using various spectroscopic techniques such as ^{1}H NMR, UV-vis, elemental analysis (C, H, N, and X), and FT-IR spectroscopy. The results indicate that a 1:1 ratio of PPA and modified substituted benzaldehydes is optimal. All synthesized compounds exhibited significant antibacterial activity.

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