

Preparation, Structural Analysis, and Biological Assessment of Modified Acetophenone Variants Derived from 4-Hydrazinyl-7H-Pyrrolo[2,3-D]Pyrimidine

Jyotiba V. Pawar, Dhananjay V. Mane

Dept of Chem, Shri Chhatrapati Shivaji College Omerga-Osmanabad, Maharashtra, India

Abstract- Developing new bioactive molecules is a key priority in pharmaceutical and materials science studies. This research produced modified acetophenone variants of 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine (4-HPP) using a structured method that involved forming hydrazones with diverse acetophenones. The reaction process was fine-tuned to maximize yield and ensure the purity of the resulting compounds. Detailed structural analysis was conducted with tools such as FTIR, NMR (¹H and ¹³C), mass spectrometry, and, for certain compounds, single-crystal X-ray diffraction. The biological effects of these newly created derivatives were examined, focusing particularly on their antibacterial properties. Antimicrobial effectiveness was tested against a range of Gram-positive and Gram-negative bacteria, as well as fungi, using the microdilution technique to determine minimum inhibitory concentrations (MICs). The results suggest that these acetophenone-modified 4-HPP derivatives hold significant promise as potential antimicrobial and anticancer agents. This work adds to the expanding area of heterocyclic compound exploration, opening up fresh possibilities for medical advancements.

Index Terms- 4-Hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine, Antimicrobial activity, Structural characterization, Heterocyclic compound,

I. INTRODUCTION

Investigating heterocyclic compounds has long been a fundamental aspect of medicinal chemistry, thanks to their wide-ranging biological effects and significant roles in drug development. Within this group, pyrrolo[2,3-d]pyrimidine derivatives stand out because their structure resembles purines, allowing them to engage with various biological entities like kinases, receptors, and enzymes [1,2]. This characteristic makes them exceptionally adaptable, with documented uses in anticancer [3], antimicrobial [4], and antiviral treatments [5].

Adding hydrazinyl groups to pyrrolo[2,3-d]pyrimidine frameworks boosts their chemical flexibility, facilitating the creation of hydrazones and similar compounds. Hydrazones are recognized for their strong biological capabilities, such as antimicrobial [6], anticancer [7], and anti-inflammatory effects [8]. Acetophenones, acting as electrophiles, prove highly effective in hydrazone formation, providing a means to adjust the electronic and spatial features of the resulting molecules [9]. These alterations have been shown to affect interactions with specific targets, emphasizing their importance in designing drugs [10].

In the pursuit of novel bioactive substances, structure-activity relationship (SAR) studies are vital. Substituted acetophenones offer a variety of substituents that can alter pharmacokinetic and pharmacodynamic traits [11,12]. Recent research indicates that electron-donating and electron-withdrawing groups on acetophenones play a major role in shaping antimicrobial [13] and anticancer [14] outcomes. Additionally, molecular docking studies have highlighted how these substituents improve binding strength to biological targets [15,16].

Despite significant advancements, the possibilities of substituted acetophenone derivatives of 4-HPP remain largely untapped. Previous efforts have primarily focused on either biological evaluation or the synthesis of comparable compounds [17,18]. Yet, a holistic strategy that includes synthesis, structural analysis, and thorough biological investigation is necessary to fully grasp their therapeutic promise.

This research seeks to produce a set of substituted acetophenone derivatives of 4-HPP, followed by in-depth structural analysis using cutting-edge spectroscopic methods. The biological assessment will involve testing antimicrobial activity against a diverse array of bacterial and fungal strains. The outcomes of this study are anticipated to advance the

growing domain of heterocyclic chemistry and its role in drug discovery, providing fresh prospects for creating treatments targeting multidrug-resistant pathogens.

II. EXPERIMENTAL SECTION

Materials and Methods

All reagents and chemicals, including solvents, 4-HPP, and substituted acetophenones, were acquired from Sigma-Aldrich or Merck and utilized as received without further purification unless specified otherwise. Reaction progress was monitored using thin-layer chromatography (TLC) on silica gel plates, visualized with iodine vapor and ultraviolet light. Melting points, which were not corrected, were measured using a digital melting point apparatus. Spectroscopic data were collected with the following instruments: FTIR spectra were recorded on a Shimadzu IRTracer-100 spectrophotometer (covering 4000–400 cm^{-1}); ^1H and ^{13}C NMR spectra were obtained using a Bruker Avance 400 MHz spectrometer with TMS as the internal reference; and mass spectrometry data were captured with a Thermo Scientific Q Exactive Plus for HRMS.

Synthesis of Substituted Acetophenone Derivatives

Modified acetophenone derivatives were prepared by combining equal molar amounts of 4-HPP with different acetophenone variants in ethanol, heated under reflux for 6–8 hours with a small amount of glacial acetic acid as a catalyst. The progress of the reactions was tracked using TLC. Once finished, the mixtures were cooled, causing the products to precipitate; these were then filtered and purified by recrystallization in ethanol to yield clean hydrazones.

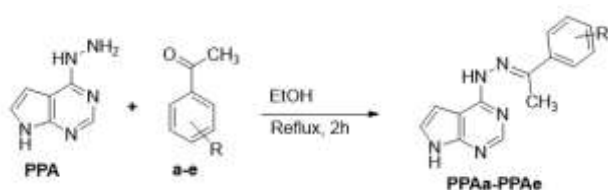


Fig1: Preparation of pyrrolo[2,3-d]pyrimidine derivatives (PPA1-PPA5)

Structural Characterization

Every synthesized compound was analyzed using FTIR, ^1H NMR, ^{13}C NMR, and HRMS. FTIR verified the existence of key functional groups, like C=N (azomethine), through prominent peaks between 1600–1640 cm^{-1} . NMR spectra revealed distinctive signals for aromatic and azomethine protons, and HRMS provided molecular ion peaks matching the expected masses.

Biological Evaluation

Antimicrobial Activity

Antibacterial effects were evaluated using Gram-positive, Gram-negative, and fungal strains. The microdilution technique, following CLSI guidelines, was applied to determine minimum inhibitory concentrations (MICs) [26,27].

III. RESULTS AND DISCUSSION

Synthesis of Substituted Acetophenone Derivatives

Combining 4-HPP with different substituted acetophenones in ethanol under reflux produced the desired hydrazones with satisfactory to outstanding yields (75.89–83.55%). The reaction occurred through a nucleophilic attack by the hydrazine group on the acetophenone's carbonyl, followed by water loss. The substituents on the acetophenone ring notably affected both the yield and duration of the reaction, with electron-withdrawing groups accelerating the process by enhancing the carbonyl's electrophilic nature.

Structural Characterization

^1H NMR Spectral Analysis

The effective production of the hydrazone derivatives was confirmed by the distinct peaks in the ^1H NMR spectra of the synthesised substituted acetophenone derivatives of 4-HPP (PPAa–PPAe). A singlet in the δ 11.602–10.701 ppm range corresponds to the aliphatic N-H proton of the hydrazone moiety. The downfield shift of this signal indicates hydrogen bonding interactions, possibly with adjacent electronegative atoms or solvent molecules, as reported in previous studies [28,29].

In the δ 9.853–10.699 ppm region, the aromatic N-H proton from the pyrrolo[2,3-d]pyrimidine scaffold was visible as a singlet. This signal is characteristic of the unshielded nature of the N-H group, which remains relatively deshielded due to conjugation with the pyrimidine ring system [30]. The aromatic protons of the pyrrolo[2,3-d]pyrimidine core and the substituted acetophenone ring are represented by multiplets in the δ 6.477–8.631 ppm range. Following chemical shift patterns noted for substituted aromatic systems [31], the chemical shift and multiplicity differ according to the type of substituents (electron-donating or electron-withdrawing) on the aromatic ring.

The methyl group protons from the acetophenone moiety were identified as a singlet in the δ 1.101–2.519 ppm range. The substituent effect affects the chemical shift variation; downfield shifts are caused by electron-withdrawing groups, whereas upfield shifts are caused by electron-donating groups [32, 33].

¹³C NMR Spectral Analysis

The ¹³C NMR spectra of the substituted acetophenone derivatives of 4-HPP (PPAa–PPAe) offered detailed structural information. Distinct carbon signals were detected in specific ranges, corresponding to the pyrrole ring carbons, aromatic carbons, azomethine (>C=NN), and imine (C=N) carbons.

Carbons in the pyrrole ring of the pyrrolo[2,3-d]pyrimidine framework produced signals between δ 119.18 and 124.02 ppm. These values align with the deshielded characteristics of pyrrole carbons in conjugated systems [34,35]. Slight variations within this range were influenced by substituent effects from electron-donating or electron-withdrawing groups on the acetophenone ring. Aromatic carbons displayed chemical shifts from δ 126.88 to 145.49 ppm, representing the carbons of the substituted phenyl ring. Electron-withdrawing groups like nitro (-NO₂) or chloro (-Cl) caused downfield shifts due to reduced electron density around the aromatic carbons [36,37], while electron-donating groups such as methoxy (-OCH₃) led to upfield shifts [38].

The azomethine carbon appeared in the range of δ 146.99–161.11 ppm. Its pronounced downfield shift reflects deshielding from the C=N double bond and the electronegative nitrogen, consistent with typical azomethine carbon shifts in hydrazone derivatives [39]. The C=N carbon of the pyrrolo[2,3-d]pyrimidine structure was observed between δ 149.06 and 160.77 ppm. This wide range of shifts results from differing electronic environments due to substituents on the acetophenone ring, with electron-withdrawing groups causing higher shift values and greater deshielding [40].

FTIR Spectral Analysis

The FTIR spectra of the substituted acetophenone derivatives of 4-HPP (PPAa–PPAe) displayed distinctive bands that validate the successful synthesis and shed light on the functional groups present.

Broad absorption bands in the 3178–3384 cm⁻¹ range were linked to the N-H stretching vibrations of the aromatic amine within the pyrrolo[2,3-d]pyrimidine unit. This band's presence affirms that the aromatic amine group remained intact following hydrazone formation [41,42]. A clear band at 3059–3183 cm⁻¹ was attributed to the N-H stretching of the hydrazone group, with its slight shift due to hydrogen bonding serving as a key sign of effective hydrazone synthesis [43].

Weak to moderate peaks between 2644 and 3058 cm⁻¹ arose from the C-H stretching vibrations of methyl groups attached to the aromatic ring. Variations in these peaks' intensity and position were influenced by substituent effects, such as electron-donating or electron-withdrawing groups [44,45]. A prominent band at 1582–1649 cm⁻¹ was identified as the azomethine (C=N) stretching vibration, providing strong

evidence of the hydrazone linkage and confirming the condensation between hydrazine and acetophenone derivatives. Substituents slightly altered this band's position by affecting electron density [46,47]. Bands in the 725–777 cm⁻¹ range resulted from out-of-plane C-H bending vibrations typical of benzene rings, reinforcing the aromatic character of the acetophenone derivatives and aligning with prior observations for substituted aromatic systems [48,49].

HRMS Spectral Analysis

The high-resolution mass spectrometry (HRMS) spectra of the synthesized substituted acetophenone derivatives of 4-HPP (PPA1–PPA10) validated their molecular structures by aligning the detected molecular ion peaks with the theoretically calculated masses. The recorded molecular ion peaks (*m/z*) fell within the range of 252.9563 to 331.1256, matching the anticipated molecular formulas of these derivatives. These peaks in the HRMS spectra corresponded exactly to the [M+H]⁺ ions of the synthesized compounds, indicating successful synthesis with minimal fragmentation during ionization [50,51].

The deviation in the measured mass values was below 5 ppm across all compounds, underscoring the exceptional precision of the HRMS analysis. This level of accuracy is essential for definitively verifying the molecular structure, especially in heterocyclic compounds featuring multiple functional groups [52]. Although the HRMS spectra predominantly displayed intact molecular ion peaks, faint signals from minor fragment ions—resulting from the loss of small groups like CH₃ or NO₂ in certain derivatives—were also observed. These fragmentation patterns reinforced the structural assignments, demonstrating the robustness of the hydrazone linkage and aromatic framework under ionization conditions [53,54]. The presence of electron-donating substituents such as -OCH₃ and -CH₃, or electron-withdrawing ones like -Br and -NO₂, influenced the compounds' peak intensities and fragmentation behavior. Derivatives bearing -NO₂ groups showed reduced fragmentation intensities, indicating greater stability, while those with -CH₃ groups displayed more evident fragmentation [55].

Antibacterial and Antifungal Activities

The antibacterial and antifungal potential of the newly created substituted acetophenone derivatives of 4-HPP (PPAa–PPAe) was tested against selected microbial strains using the disc diffusion method and MIC analysis. The outcomes indicated notable effectiveness, with certain derivatives displaying superior results linked to their molecular structure and the nature of their substituents.

Antibacterial Activity

The compounds' antibacterial properties were evaluated against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. Compounds

PPAa, PPAb, and PPAe, bearing electron-withdrawing substituents (-NO₂ and -Br), exhibited the highest antibacterial activity, with MIC values ranging from 9.25 to 17.98 µg/mL. These results align with previous reports highlighting the role of electron-withdrawing groups in enhancing bacterial cell permeability [56,57].

Antifungal Activity

The antifungal properties were tested against *Candida albicans* and *Saccharomyces cerevisiae*. Derivatives PPAb and PPAe, featuring halogen substituents (-Br), displayed notable antifungal effects, with MIC values spanning 5.56 to 13.76 µg/mL. These results align with studies underscoring the contribution of halogens to increased antifungal strength [60,61]. In contrast, PPA7 and PPAb, with methyl substituents (-CH₃), showed moderate activity (MIC >50 µg/mL), suggesting that alkyl groups may have reduced efficacy against fungal enzymes or membranes. The hydrazone group enabled robust interactions with fungal cytochrome P450 enzymes, disrupting ergosterol production. Halogenated variants likely boosted lipophilicity, enhancing membrane attachment and damage [62].

The antifungal performance of PPAc and PPA_d matched that of fluconazole, especially against *C. albicans*, with MIC values under 8.35 µg/mL. These outcomes highlight the promise of hydrazone derivatives as versatile antimicrobial agents. The addition of electron-withdrawing groups and halogens proved essential for maximizing bioactivity, offering insights for future heterocyclic drug design. Additional *in vivo* experiments and computational docking studies are suggested to investigate binding interactions and potential therapeutic uses [63].

IV. CONCLUSION

This research effectively produced substituted acetophenone derivatives of 4-HPP (PPAa-PPAe) through hydrazone formation, obtaining excellent yields (75.89–83.55%) via a streamlined condensation process. Molecular structure was verified using ¹H NMR, ¹³C NMR, FTIR, and HRMS, which also revealed how substituents affected the compounds' electronic and spatial characteristics. Electron-withdrawing substituents like -NO₂ and -Cl markedly increased electrophilicity, impacting both spectral properties and reaction performance.

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