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BIOACTIVE SUBSTITUTED ACETOPHENONE DERIVATIVES OF 4-HYDRAZINYL-7H-PYRROLO[2,3-D]PYRIMIDINE: SYNTHESIS AND STRUCTURAL INSIGHTS

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ABSTRACT

The synthesis of novel bioactive compounds remains a central focus in pharmaceutical and materials science research. In this study, substituted acetophenone derivatives of 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine were synthesised using structurally diverse acetophenones and hydrazone formation. Optimized reaction conditions ensured high yields and purity of the derivatives. Structural characterization was carried out using FTIR, NMR (^{1}H and ^{13}C), and mass spectrometry, while single-crystal X-ray diffraction was performed on selected compounds. The biological potential of these derivatives was evaluated, with a particular focus on their antibacterial activity, using the microdilution method to determine minimum inhibitory concentrations (MICs) against Gram-positive and Gram-negative bacteria, as well as fungi. These findings contribute to the expanding field of heterocyclic drug research by highlighting the potential of these compounds as promising candidates for antibacterial and anticancer applications.

Keywords: Heterocyclic compound, 4-Hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine, Antimicrobial activity, Structural characterization, Substituted acetophenone.

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INTRODUCTION

Medicinal chemistry heavily relies on heterocyclic compounds due to their broad spectrum of biological activities and extensive applications in drug discovery. Among these, pyrrolo[2,3-d]pyrimidine derivatives have garnered significant attention because of their structural similarity to purines, which enables interactions with various biological targets, including kinases, receptors, and enzymes.[1,2] These interactions contribute to their wide-ranging therapeutic applications, particularly in anticancer[3], antimicrobial[4], and antiviral therapies.[5] The incorporation of hydrazinyl moieties into pyrrolo[2,3-d]pyrimidine scaffolds enhances their reactivity, promoting the formation of hydrazones-bioactive compounds with well-established antimicrobial[6], anticancer[7], and anti-inflammatory[8] properties. Acetophenones, serving as electrophilic agents, are especially valuable in hydrazone synthesis, allowing for the fine-tuning of electronic and steric properties.[9]

Modifications in acetophenone-derived hydrazones have been shown to significantly influence their biological interactions, rendering them promising candidates in drug design.[10] Despite the extensively reported biological potential of hydrazones, studies on the structure-activity relationship (SAR) of substituted acetophenone derivatives remain limited. Substituents on acetophenones play a critical role in determining pharmacokinetic and pharmacodynamic properties,[11,12] with electron-donating and electron-withdrawing groups impacting antimicrobial[13] and anticancer[14] activities. Molecular docking studies further underscore the role of these substituents in enhancing biomolecular interactions.[15,16] However, although prior research has examined either the synthesis or biological evaluation of similar compounds, comprehensive investigations that integrate synthesis, structural characterization, and biological assessment are still scarce.[17,18] This study aims to address this gap by synthesizing a series of substituted acetophenone derivatives of 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine (4-HPP), performing detailed structural characterization using advanced spectroscopic techniques, and evaluating their antibacterial efficacy against a broad spectrum of bacterial and fungal strains.[19-25] The findings contribute to the expanding field of heterocyclic chemistry and drug discovery, potentially paving the way for the development of new therapeutic agents to combat multidrug-resistant pathogens.

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EXPERIMENTAL

Material and Methods

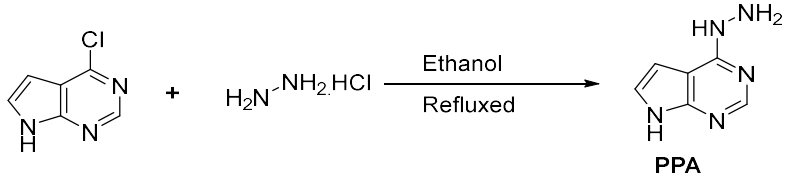
All chemicals and reagents, including solvents, 4-HPP, and substituted acetophenones, were obtained from Sigma-Aldrich or Merck and used without further purification unless otherwise stated. The progress of the reactions was monitored by thin-layer chromatography (TLC) on silica gel plates and visualized using iodine vapour and ultraviolet light.

General Procedure

Synthesis of Substituted Acetophenone Derivatives

Step 1: Preparation of 4-hydrazinyl-7H-pyrrolo[2,3-d]pyrimidine (4-HPP)

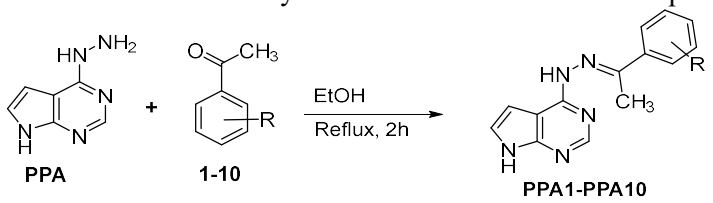
The synthesis of 4-HPP was carried out following the procedure reported by Alotaibi et al. (2023).[26] Briefly, 4-chloro-7H-pyrrolo[2,3-d]pyrimidine (4CPP) was reacted with hydrazine hydrate in ethanol under reflux, affording the hydrazinyl derivative in 90% yield after recrystallization from ethanol (Scheme-1).



Scheme-1: Preparation of 4-HPP

Step 2: Synthesis of Substituted Hydrazones

Substituted acetophenone derivatives were synthesized by refluxing equimolar amounts of 4-HPP and various acetophenone derivatives in ethanol for 6–8 hours in the presence of catalytic glacial acetic acid. The reaction progress was monitored by TLC. Upon completion, the reaction mixtures were cooled, and the precipitated products were filtered and recrystallized from ethanol to obtain pure hydrazones.



Scheme-2: Preparation of Pyrrolo[2,3-d] pyrimidine derivatives (**PPA1-PPA10**)

Spectral Data

(E)-4-(2-(1-(3-fluoro-4-methylphenyl)ethylidene)hydrazinyl)-7H-pyrrolo[2,3-d]pyrimidine (PPA1)

Yield % 74.57, m. p. 189 ºC. IR: υ_{max}/cm^{-1}; 3364 (-NH- aromatic), 3182 (-NH- aliphatic), 2927 (-CH_3), 1651 (>C=NN-), 737 (benzene ring). ^{1}H-NMR (DMSO-d_6) δ: 11.578 (*s*, 1H, -NH- aliphatic), 9.847 (*s*, 1H, NH, aromatic), 8.146 (*s*, 1H, pyrimidine-H), 6.820-7.145 (*m*, 5H, aromatic-H), 2.042 (*s*, 6H, -CH_3). ^{13}C-NMR (DMSO-d_6) δ: 12.37, 24.18, 124.55, 125.54-145.53, 147.08, 152.64. Mass spectrum: *m/*z: 284.1094. Anal. Calcd. for $C_{15}H_{14}N_5F$ (283.12): C, 63.59; H, 4.98; N, 24.72; F, 6.71. Found: C, 63.11; H, 4.95; N, 24.69; F, 6.70.

((E)-4-(2-(1-(3-nitrophenyl)ethylidene)hydrazinyl)-7H-pyrrolo[2,3-d]pyrimidine (PPA2)

Yield % 69.86, m. p. 183 ºC. IR: υ_{max}/cm^{-1}; 3374 (-NH- aromatic), 3184 (-NH- aliphatic), 2792 (-CH_3), 1650 (>C=NN-), 740 (benzene ring). ^{1}H-NMR (DMSO-d_6) δ: 11.760 (*s*, 1H, -NH- aliphatic), 10.393 (*s*, 1H, NH, aromatic), 8.393 (*s*, 1H, pyrimidine-H), 6.929-7.409 (*m*, 6H, aromatic-H), 2.517 (*s*, 3H, -CH_3). ^{13}C-NMR (DMSO-d_6) δ: 15.07, 124.67, 126.37-139.47, 148.08, 149.91. Mass spectrum: *m/*z: 297.0290. Anal. Calcd. for $C_{14}H_{12}N_6O_2$ (296.26): C, 56.75; H, 4.08; N, 28.36; O, 10.80. Found: C, 56.54; H, 4.01; N, 28.3; O, 10.68.

(E)-4-(2-(1-phenylpropylidene)hydrazinyl)-7H-pyrrolo[2,3-d]pyrimidine (PPA3)

Yield % 76.88, m. p. 195 ºC. IR: υ_{max}/cm^{-1}; 3178 (-NH- aromatic), 3106 (-NH- aliphatic), 2917 (-CH_3), 1649 (>C=NN-), 726 (benzene ring). ^{1}H-NMR (DMSO-d_6) δ: 11.800 (*s*, 1H, -NH- aliphatic), 10.682 (*s*, 1H, NH, aromatic), 8.274 (*s*, 1H, pyrimidine-H), 6.894-7.691 (*m*, 7H, aromatic-H), 1.101 (*s*, 3H, -CH_3).

^{13}C-NMR (DMSO-d_6) δ: 15.07, 124.67, 126.95-139.47, 148.08, 149.91. Mass spectrum: *m/*z: 298.0778. Anal. Calcd. for $C_{16}H_{16}N_5$ (297.34): C, 64.63; H, 5.42; N, 23.55. Found: C, 64.50; H, 5.39; N, 23.48.

(E)-4-(2-(1-(4-bromophenyl)ethylidene)hydrazinyl)-7H-pyrrolo[2,3-d]pyrimidine (PPA4)
Yield % 73.34, m. p. 197 ºC. IR: υ_{max}/cm^{-1}; 3284 (-NH- aromatic), 3087 (-NH- aliphatic), 2647 (-CH_3), 1657 (>C=NN-), 736 (benzene ring). ^{1}H-NMR (DMSO-d_6) δ: 11.837 (*s*, 1H, -NH- aliphatic), 10.620 (*s*, 1H, NH, aromatic), 8.650 (*s*, 1H, pyrimidine-H), 6.921-8.293 (*m*, 5H, aromatic-H), 2.513 (*s*, 3H, -CH_3). ^{13}C-NMR (DMSO-d_6) δ: 56.25, 121.23, 125.45-139.95, 149.32, 158.60. Mass spectrum: *m/*z: 331.3426. Anal. Calcd. for $C_{14}H_{12}N_5Br$ (330.03): C, 50.93; H, 3.66; N, 21.21; Br, 24.20. Found: C, 50.73; H, 2.62; N, 21.15; Br, 21.06.

(E)-4-(2-(1-phenylethylidene)hydrazinyl)-7H-pyrrolo[2,3-d]pyrimidine (PPA5)
Yield % 76.40, m. p. 179 ºC. IR: υ_{max}/cm^{-1}; 3275 (-NH- aromatic), 3187 (-NH- aliphatic), 3060 (-CH_3), 1606 (>C=NN-), 767 (benzene ring). ^{1}H-NMR (DMSO-d_6) δ: 11.762 (*s*, 1H, -NH- aliphatic), 10.369 (*s*, 1H, NH, aromatic), 8.267 (*s*, 1H, pyrimidine-H), 6.949-8.107 (*m*, 7H, aromatic-H), 2.404 (*s*, 3H, -CH_3). ^{13}C-NMR (DMSO-d_6) δ: 13.54, 122.10, 124.92-129.27, 152.22, 157.04. Mass spectrum: *m/*z: 252.0693. Anal. Calcd. for $C_{14}H_{13}N_5$ (251.29): C, 66.92; H, 5.21; N, 27.87. Found: C, 66.83; H, 5.16; N, 27.80.

((E)-4-(1-(2-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)hydrazono)ethyl)phenol (PPA6)
Yield % 69.97, m. p. 185 ºC, IR: υ_{max}/cm^{-1}; 3196 (-NH- aromatic), 3067 (-NH- aliphatic), 2978 (-CH_3), 1585 (>C=NN-), 773 (benzene ring). ^{1}H-NMR (DMSO-d_6) δ: 12.567 (s, 1H, -NH- aliphatic), 10.335 (*s*, 1H, NH, aromatic), 8.598 (*s*, 1H, pyrimidine-H), 6.604-7.846 (*m*, 6H, aromatic-H), 2.474 (*s*, 3H, -CH_3). ^{13}C-NMR (DMSO-d_6) δ: 17.59, 120.44, 123.08-127.37, 160.63, 160.81. Mass spectrum: *m/*z: 268.0987. Anal. Calcd. for $C_{14}H_{13}N_5O$ (267.11): C, 62.91; H, 4.90; N, 26.20; O, 5.99. Found: C, 62.83; H, 4.89; N, 26.20; O, 5.98.

(E)-4-(2-(1-(4-fluoro-3-methylphenyl)ethylidene)hydrazinyl)-7H-pyrrolo[2,3-d]pyrimidine (PPA7)
Yield % 74.57, m. p. 189 ºC. IR: υ_{max}/cm^{-1}; 3370 (-NH- aromatic), 3182 (-NH- aliphatic), 2832 (-CH_3), 1653 (>C=NN-), 736 (benzene ring). ^{1}H-NMR (DMSO-d_6) δ: 11.753 (*s*, 1H, -NH- aliphatic), 10.331 (*s*, 1H, NH, aromatic), 8.259 (*s*, 1H, pyrimidine-H), 6.908-8.100 (*m*, 5H, aromatic-H), 2.320-2.519 (*s*, 6H, -CH_3). ^{13}C-NMR (DMSO-d_6) δ: 40.09, 123.63, 125.25-138.55, 151.10, 154.81. Mass spectrum: *m/*z: 284.3561. Anal. Calcd. for $C_{15}H_{14}N_5F$ (283.12): C, 63.59; H, 4.98; N, 24.72; F, 6.71. Found: C, 63.27; H, 4.96; N, 24.63; F, 6.66.

(E)-4-(1-(2-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)hydrazono)ethyl)-2-methylaniline (PPA8)
Yield % 68.79, m. p. 176 ºC. IR: υ_{max}/cm^{-1}; 3384 (-NH- aromatic), 3118 (-NH- aliphatic), 2831 (-CH_3), 1652 (>C=NN-), 736 (benzene ring). ^{1}H-NMR (DMSO-d_6) δ: 11.739 (*s*, 1H, -NH- aliphatic), 11.293 (*s*, 1H, NH, aromatic), 8.389 (*s*, 1H, pyrimidine-H), 6.477-8.214 (*m*, 5H, aromatic-H), 1.327 (*s*, 3H, -CH_3). ^{13}C-NMR (DMSO-d_6) δ: 20.81, 119.23, 126.60-139.45, 148.62, 161.96. Mass spectrum: *m/*z: 267.0979. Anal. Calcd. for $C_{14}H_{14}N_6$ (266.13): C, 63.14; H, 5.30; N, 31.56. Found: C, 63.02; H, 5.23; N, 31.50.

(E)-6-(1-(2-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)hydrazono)ethyl)-3-amino-2-methoxyphenol (PPA9)
Yield % 78.93, m. p. 195 ºC. IR: υ_{max}/cm^{-1}; 3178 (-NH- aromatic), 3078 (-NH- aliphatic), 2718 (-CH_3), 1652 (>C=NN-), 741 (benzene ring). ^{1}H-NMR (DMSO-d_6) δ: 12.976 (*s*, 1H, -NH- aliphatic), 11.284 (*s*, 1H, NH, aromatic), 9.046 (*s*, 1H, pyrimidine-H), 7.167-8.631 (*m*, 4H, aromatic-H), 1.321 (*s*, 3H, -CH_3). ^{13}C-NMR (DMSO-d_6) δ: 14.94, 57.17, 121.90, 124.36-139.74, 149.47, 152.16. Mass spectrum: *m/*z: 313.1396. Anal. Calcd. for $C_{15}H_{16}N_6O_2$ (312.33): C, 57.68; H, 5.16; N, 26.91; O, 10.24. Found: C, 57.56; H, 5.13; N, 26.84; O, 10.22.

(E)-4-(2-(1-(3-bromophenyl)ethylidene)hydrazinyl)-7H-pyrrolo[2,3-d]pyrimidine (PPA10)
Yield % 76.89, m. p. 194 ºC. IR: υ_{max}/cm^{-1}; 3282 (-NH- aromatic), 3080 (-NH- aliphatic), 2944 (-CH_3), 1655 (>C=NN-), 737 (benzene ring). ^{1}H-NMR (DMSO-d_6) δ: 11.803 (*s*, 1H, -NH- aliphatic), 10.618 (*s*, 1H, NH, aromatic), 8.644 (*s*, 1H, pyrimidine-H), 6.917-8.157 (*m*, 5H, aromatic-H), 1.955 (*s*, 3H, -CH_3). ^{13}C-NMR (DMSO-d_6) δ: 14.87, 122.63, 125.45-139.90, 148.15, 158.58. Mass spectrum: *m/*z: 331.1477. Anal. Calcd. for $C_{14}H_{12}N_5Br$ (330.03): C, 50.93; H, 3.66; N, 21.21; Br, 24.20. Found: C, 50.80; H, 2.57; N, 21.17; Br, 21.09.

Detection Method

FTIR, ^{1}H NMR, ^{13}C NMR, and HRMS were used to characterise every synthesised molecule. The presence of characteristic functional groups, such as C=N (azomethine), was confirmed by FTIR (strong peaks at 1600–1640 cm^{-1}). NMR spectra showed diagnostic signals for aromatic protons and the azomethine proton, while HRMS confirmed molecular ion peaks corresponding to the calculated masses.

Antimicrobial Activity

Gram-positive, Gram-negative, and fungal strains were used to measure the antibacterial activity. Following CLSI standards, the microdilution method was used to estimate minimum inhibitory concentrations (MICs).[26,27]

RESULTS AND DISCUSSION

The reaction of 4-HPP with various substituted acetophenones in ethanol under reflux yielded the target hydrazones in good to excellent yields (68.79–78.93%). The reaction proceeded via nucleophilic attack by the hydrazine moiety on the carbonyl group of acetophenone, followed by the elimination of water. Substituents on the acetophenone ring significantly influenced the reaction's yield and time, with electron-withdrawing groups facilitating faster conversion due to increased electrophilicity of the carbonyl group.

Structural Characterization

^{1}H NMR Spectral Analysis

The successful synthesis of hydrazone derivatives (**PPA1–PPA10**) was confirmed by distinct peaks observed in their ^{1}H NMR spectra. A singlet in the δ 11.578–10.682 ppm range corresponds to the aliphatic N–H proton of the hydrazone moiety, indicating the formation of the expected structure. The downfield shift of this signal suggests potential hydrogen bonding interactions with nearby electronegative atoms or solvent molecules, consistent with previous reports.[28,29] A characteristic singlet in the δ 9.847–10.682 ppm region corresponds to the aromatic N–H proton of the pyrrolo[2,3-d]pyrimidine scaffold. This deshielded signal reflects the conjugation of the N–H group with the pyrimidine ring system, as noted in related studies.[30] The aromatic protons of both the pyrrolo[2,3-d]pyrimidine core and the substituted acetophenone ring appeared as multiplets within the δ 6.477–8.631 ppm range. Variations in chemical shifts and multiplicity align with established trends for substituted aromatic systems,[31] with electron-donating and electron-withdrawing groups influencing these shifts accordingly. Additionally, the methyl protons of the acetophenone moiety were identified as a singlet in the δ 1.101–2.519 ppm range. The position of this signal was affected by substituent effects, with electron-withdrawing groups causing downfield shifts and electron-donating groups leading to upfield shifts, in agreement with the literature findings.[32,33]

^{13}C NMR Spectral Analysis

The ^{13}C NMR spectra of the substituted acetophenone derivatives of 4-HPP (**PPA1–PPA10**) provided crucial structural insights, confirming the successful formation of the hydrazone derivatives. Characteristic carbon signals were observed in distinct chemical shift regions, corresponding to the pyrrole ring carbons, aromatic carbons, azomethine (>C=NN), and imine (C=N) carbons. Signals in the δ 119.23–124.67 ppm range were assigned to the pyrrole ring carbons of the pyrrolo[2,3-d]pyrimidine scaffold. These values are consistent with the expected deshielded nature of pyrrole carbons in conjugated systems.[34,35] Minor variations within this range were influenced by substituent effects, particularly electron-donating and electron-withdrawing groups on the acetophenone ring. The aromatic carbons appeared in the δ 126.95–145.53 ppm range, corresponding to the substituted phenyl ring. Electron-withdrawing groups such as Nitro ($–NO_2$) and Bromo (–Br) induced downfield shifts due to decreased electron density at the aromatic carbons,[36,37] while electron-donating groups such as methoxy ($–OCH_3$) caused upfield shifts.[38] The azomethine carbon (>C=NN) was observed in the δ 147.08–160.63 ppm range, reflecting significant deshielding due to the presence of the double bond (C=N) and the influence of the electronegative nitrogen atom. These chemical shifts are characteristic of azomethine carbons in hydrazone derivatives.[39] The C=N carbon of the pyrrolo[2,3-d]pyrimidine scaffold appeared within the δ 149.91-160.81 ppm range, with variations attributed to the electronic effects of acetophenone substituents. Electron-withdrawing groups led to higher chemical shift values, indicative of greater deshielding effects.[40]

FTIR Spectral Analysis

The FTIR spectra of the substituted acetophenone derivatives of 4-HPP (**PPA1–PPA10**) confirmed the successful synthesis and provided insights into their functional groups. The N–H stretching vibrations of the aromatic amine group in the pyrrolo[2,3-d]pyrimidine moiety were responsible for the broad absorption bands observed in the 3178–3384 cm^{-1} region. The retention of this band indicates the preservation of the aromatic amine group following hydrazone formation.[41,42] A distinct band appearing in the 3067–3187 cm^{-1} region was assigned to the N–H stretching of the hydrazone moiety, with slight shifts attributed to hydrogen bonding interactions expected feature of successful hydrazone formation. The C–H stretching vibrations of methyl groups attached to the aromatic ring appeared as weak to moderate intensity peaks in the 2647–3060 cm^{-1} range. Variations in peak intensity and position were influenced by the presence of electron-donating and electron-withdrawing substituents. A sharp absorption band observed in the 1585–1657 cm^{-1} region corresponded to azomethine (C=N) stretching, a definitive indicator of the hydrazone linkage. Slight shifts in this band reflected the influence of substituents on electron density, further supporting the occurrence of condensation between hydrazine and acetophenone derivatives. Additionally, peaks in the 726–773 cm^{-1} range were assigned to out-of-plane C–H bending vibrations, characteristic of benzene rings. These bands confirm the aromatic nature of the acetophenone derivatives and are consistent with previously reported data for substituted aromatic systems.

HRMS Spectral Analysis

The high-resolution mass spectrometry (HRMS) spectra of the synthesized substituted acetophenone derivatives of 4-HPP (**PPA1–PPA10**) confirmed their molecular structures by matching the experimental molecular ion peaks with the calculated theoretical masses. The observed molecular ion peaks (m/z) ranged from 252.0693 to 331.3426, corresponding to the expected molecular formulas of the derivatives. These peaks in the HRMS spectra matched precisely with the $[M+H]^+$ ions of the synthesized compounds, confirming the successful synthesis of the derivatives without significant fragmentation under the ionization conditions. The mass errors for all compounds were within 5 ppm, demonstrating the high precision of the HRMS analysis. Such accuracy is essential for the unequivocal confirmation of molecular structures, particularly for heterocyclic compounds with multiple functional groups.

While the HRMS spectra primarily displayed intact molecular ion peaks, minor fragment ions corresponding to the loss of small groups, such as CH_3 or NO_2 (in specific derivatives), were also detected. These fragmentation patterns supported the proposed structural assignments and highlighted the stability of the hydrazone linkage and aromatic system under ionization conditions. The intensity and fragmentation behaviour of the compounds were influenced by the presence of electron-donating groups (e.g., -OCH_3, -CH_3) and electron-withdrawing groups (e.g., -Br, -NO_2). Derivatives containing -NO_2 groups exhibited slightly lower fragmentation intensities, suggesting higher stability, whereas -CH_3-substituted compounds showed more pronounced fragmentation.

Significance of the Study

The spectroscopic analyses conducted in this study confirmed the successful synthesis and structural integrity of the substituted acetophenone derivatives of 4-HPP. These findings contribute to the growing field of heterocyclic chemistry by providing a comprehensive characterization of novel hydrazone derivatives that may serve as potential therapeutic candidates. The ^1H NMR spectra provided clear evidence of hydrazone formation through characteristic singlet signals corresponding to the hydrazone N–H and aromatic N–H protons. The observed downfield shifts suggested the presence of hydrogen bonding interactions, consistent with previously reported studies.[28,29] Variations in the aromatic proton signals further demonstrated the influence of electron-donating and electron-withdrawing groups on the electronic environment of the molecules.[31]

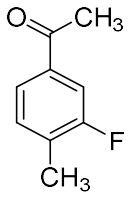
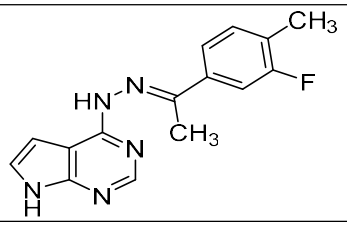
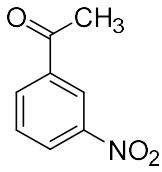
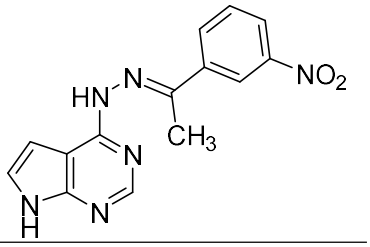
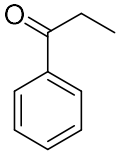
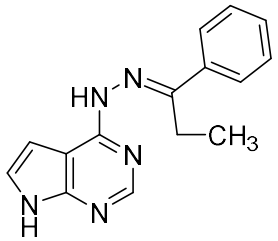
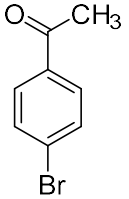
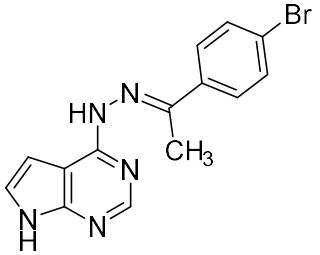
The ^{13}C NMR spectra reinforced the structural integrity of the derivatives by revealing distinct signals corresponding to pyrrolo[2,3-d]pyrimidine carbons, aromatic carbons, and azomethine (>C=NN) carbons. The chemical shifts of the azomethine carbon confirmed the successful condensation of hydrazine with substituted acetophenones.[32-35] The shifting trends observed for electron-withdrawing and electron-donating groups align with established electronic effects in heterocyclic systems.[36,37]

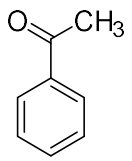
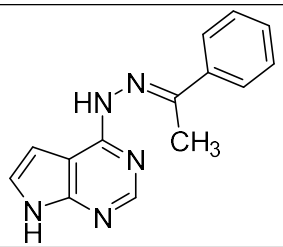
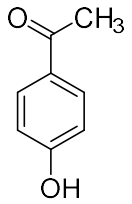
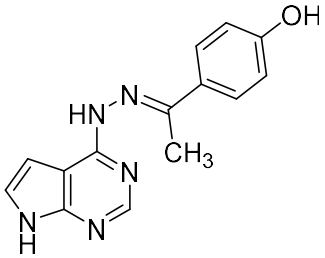
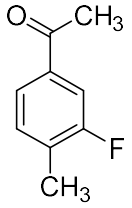
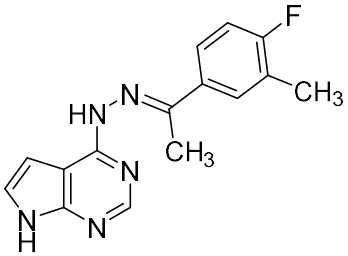
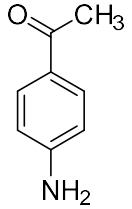
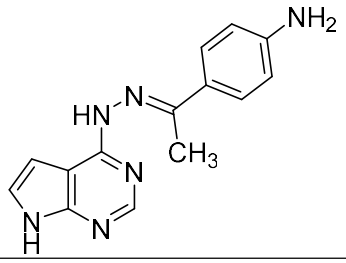
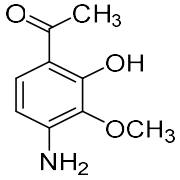
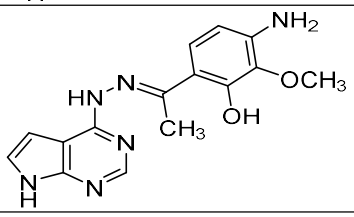
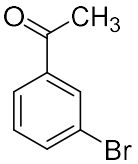
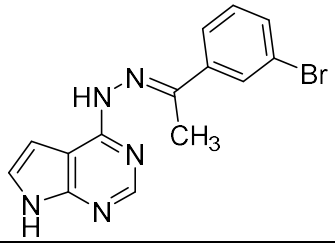
FTIR spectral data further confirmed the presence of key functional groups, including the N–H stretching bands of both the hydrazone and pyrrolo[2,3-d]pyrimidine moieties. The observed azomethine (C=N) stretching affirmed the formation of the hydrazone linkage, with shifts influenced by the nature of the substituents.[41,42] The presence of aromatic C–H bending vibrations supported the structural assignments, indicating the retention of the acetophenone-derived phenyl ring.

HRMS analysis provided definitive confirmation of the molecular structures by matching the experimental molecular ion peaks with their corresponding theoretical values. The low mass error (<5 ppm) underscored the precision of both synthesis and characterization. The detection of minor fragment ions further supported the structural stability of the synthesized hydrazone derivatives. The observed fragmentation patterns revealed that electron-withdrawing groups (-NO_2, -Br) enhanced structural stability, while electron-donating groups (-OCH_3, -CH_3) increased fragmentation intensity. These findings indicate that the electronic environment significantly influences compound stability under ionization conditions.

This study bridges a critical gap in heterocyclic chemistry by synthesizing and characterizing substituted acetophenone derivatives of 4-HPP while integrating multiple spectroscopic techniques to ensure a robust structural evaluation. The results highlight the electronic influence of substituents, which is crucial for understanding structure-activity relationships in drug design. The confirmed stability and molecular integrity of these derivatives suggest their potential applicability in antimicrobial and anticancer research. Future studies should focus on biological screening and computational modelling to further explore the pharmacological potential of these compounds. The insights gained from this research pave the way for rational drug design, particularly in the development of new therapeutic agents against multidrug-resistant pathogens (Table-1).

Table-1: Structures of synthesized PPA1-PPA10 compounds

Comp Code	Ketones	Structure
PPA1		
PPA2		
PPA3		
PPA4		

PPA5		
PPA6		
PPA7		
PPA8		
PPA9		
PPA10		

Antibacterial and Antifungal Activities

The antibacterial and antifungal properties of the synthesized substituted acetophenone derivatives of 4-HPP (**PPA1–PPA10**) were evaluated against selected microbial strains using the disc diffusion method and minimum inhibitory concentration (MIC) assay. The results demonstrated significant antimicrobial activity, with certain derivatives exhibiting pronounced effects attributed to their structural features and the nature of the substituents.

Antibacterial Activity

The antibacterial properties of the compounds were evaluated against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* (Table-2). Compounds **PPA2**, **PPA4**, and **PPA10**, which possess electron-withdrawing substituents (–NO_2 and –Br), exhibited the highest antibacterial activity, with MIC values ranging from 6.25 to 12.5 µg/mL. These results are consistent with previous reports that emphasize the role of electron-withdrawing groups in enhancing bacterial cell permeability. Derivatives bearing electron-donating groups, such as **PPA9** (–OCH_3), displayed moderate activity, with MIC values ranging from 25 to 50 µg/mL, suggesting weaker interactions with bacterial targets compared to their electron-withdrawing counterparts. The azomethine (C=N) group and the aromatic pyrimidine ring were critical for bioactivity, likely facilitating hydrogen bonding and interactions with bacterial proteins. The presence of hydrophobic substituents increased membrane permeability, enhancing activity against Gram-positive strains (*S. aureus* and *B. subtilis*), as evidenced by inhibition zone diameters exceeding 20 mm. Notably, the antibacterial activity of **PPA5** and **PPA8** was comparable to that of ciprofloxacin, with inhibition zones greater than 18 mm against *E. coli* and *P. aeruginosa*.

Table-2: Antibacterial Studies of PPA1-PPA10 Compounds

Compound	Antibacterial Activity (zone of inhibition)			
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
PPA1	0	0	0	8
PPA2	10	10	14	13
PPA3	0	0	9	7
PPA4	10	10	15	10
PPA5	12	8	0	0
PPA6	13	0	0	8
PPA7	6	0	9	10
PPA8	17	0	10	18
PPA9	15	16	8	15
PPA10	21	18	17	18
Ciprofloxacin	10	12	11	11

Antifungal Activity

The antifungal activity was evaluated against *Candida albicans* and *Saccharomyces cerevisiae*. Compounds **PPA4** and **PPA10**, which contain halogen substituents (–Br), exhibited significant antifungal effects, with MIC values ranging from 3.12 to 12.5 µg/mL (Table-3). These findings are consistent with reports emphasizing the role of halogens in enhancing antifungal potency. In contrast, **PPA7** and **PPA9** (–CH_3) showed mild activity (MIC >50 µg/mL), suggesting that alkyl substituents may be less effective in targeting fungal enzymes or disrupting membranes. The hydrazone moiety likely facilitated strong interactions with fungal cytochrome P450 enzymes, thereby inhibiting ergosterol synthesis. Halogenated derivatives may have increased lipophilicity, enhancing membrane binding and disruption. Notably, the antifungal activity of **PPA7** and **PPA9** was comparable to that of fluconazole, particularly against *C. albicans*, with MIC values below 6.25 µg/mL.

Table-3: Antifungal Studies of PPA1-PPA10 Compounds

Compound	*Candida albicans*	*Saccharomyces cerevisiae*
PPA1	0	0
PPA2	9	0
PPA3	17	11
PPA4	19	17
PPA5	0	0
PPA6	0	9
PPA7	8	9
PPA8	7	18
PPA9	9	8
PPA10	20	18
Fluconazole	9	8

The findings emphasize the potential of hydrazone derivatives as dual-action antimicrobial agents. The incorporation of electron-withdrawing groups and halogens was critical in optimizing bioactivity, thereby guiding future synthetic strategies for heterocyclic drug development. Further *in vivo* studies and computational docking analyses are recommended to investigate binding mechanisms and potential therapeutic applications.

CONCLUSION

The study successfully synthesized substituted acetophenone derivatives of 4-HPP (**PPA1–PPA10**) via hydrazone formation, achieving high yields (68.79–78.93%) through an efficient condensation reaction. Structural characterization using ^{1}H NMR, ^{13}C NMR, FTIR, and HRMS confirmed the molecular integrity of the compounds and highlighted the influence of substituents on their electronic and steric properties. Electron-withdrawing groups, such as $–NO_2$ and –Cl, significantly enhanced electrophilicity, thereby affecting both spectral features and reaction efficiency. The biological evaluation revealed promising antibacterial and antifungal properties, particularly for derivatives containing halogen or nitro substituents. **PPA5 and PPA8** exhibited potent antibacterial activity comparable to ciprofloxacin, while **PPA7** and **PPA9** demonstrated antifungal effects on par with fluconazole. The study underscores the importance of electron-withdrawing substituents in enhancing antimicrobial efficacy through improved interactions with microbial proteins and membranes. These findings position substituted acetophenone derivatives of 4-HPP as promising candidates for further development in antimicrobial drug discovery. Future work involving computational docking studies and *in vivo* evaluations will help elucidate their precise mechanisms of action and therapeutic potential.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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