

Design, synthesis, and antimicrobial evaluation of newly developed pyridine and pyrimidine derivatives derived from enaminones

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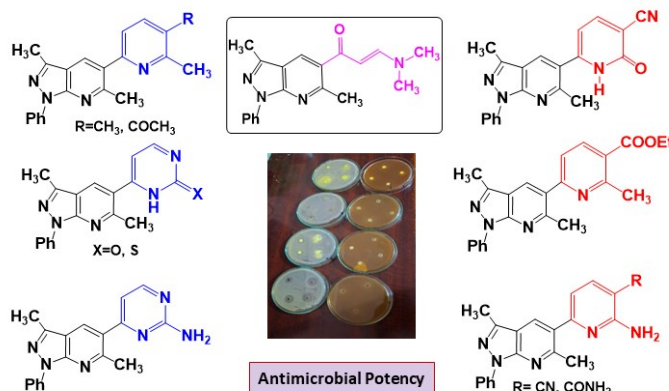
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Abstract

Enaminones have garnered significant attention because of their unique properties and their importance in synthetic chemistry as highly versatile building blocks. In this study, a novel series of heterocyclic compounds based on pyridine and pyrimidine scaffolds was synthesized through the reactions of enaminones **2** with nitriles and various ammonia derivatives. The structures of the newly synthesized compounds were confirmed using multiple spectroscopic techniques, including IR, ¹H-NMR, ¹³C-NMR, and MS, as well as elemental analysis. Evaluation of the antimicrobial activity of these heterocycles demonstrated notable potency, particularly among the pyrimidine analogues.



Keywords: β -enaminone, pyridine, pyrimidine, antimicrobial potency.

Introduction

β -Enaminones are of significant scientific interest due to their dual reactivity as nucleophilic enamines and electrophilic enones, which makes them valuable building blocks in synthetic organic chemistry.¹⁻⁸ Pyridine and its derivatives are polar, ionisable aromatic compounds that enhance solubility and bioavailability.^{9,10} These heterocycles play a critical role in various medical fields.¹¹ Pyridine's solubility, basicity, and hydrogen-bonding ability render it essential in many pharmaceuticals. Researchers are actively investigating pyridine derivatives for drug and insecticide development.¹² The literature underscores the biological effects of pyridines, particularly their anticancer and antibacterial activities.¹³⁻¹⁸ The in vitro antifungal activity of pyridine derivative **A** was assessed against five plant pathogenic fungi-*Helminthosporium maydis*, *Gibberella zeae*, *Botrytis cinerea*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum*-with satisfactory results.¹⁹ (Figure 1). Many pyridines have exhibited antifungal,²⁰⁻²² antiviral,^{23,24} analgesic,²⁵⁻²⁸ anti-inflammatory,^{28,29} antiproliferative,^{30,31} and antidiabetic^{32,33} properties. The primary method for synthesising pyridine involves the reaction of β -enaminones with active methylene reagents.^{34,35}

On the other hand, pyrimidine analogues exhibit a broad spectrum of biological activities, including antioxidant effects and significant anticancer properties.³⁶⁻³⁸ Certain pyrimidines (**B** and **C**) have demonstrated a beneficial impact on normal cells (RPE-1) while showing strong efficacy against cancer cells (MCF-7)³⁹ (Figure 1). Fused pyrimidines, such as pyrazolo[1,5-*a*]pyrimidine-based macrocycles, function as kinase inhibitors that specifically target AAK1.⁴⁰ Pyrimidine derivatives, including azidothymidine, are employed in synthetic strategies for the treatment of HIV infections.⁴¹ Moreover, pyrimidine analogues (5-fluorouracil derivatives) have displayed notable efficacy against strains of *Gram*-positive cocci, regardless of their susceptibility or resistance to approved antibiotics, and they do not exhibit cross-resistance.⁴² Building on prior insights, β -enaminones can be used to synthesize pyrimidine derivatives by heating with ammonia derivatives such as urea, guanidine, or thiourea.^{34,43} Based on the aforementioned information, the current objective is to synthesize innovative heterocycles related to pyridine and pyrimidine structures, utilizing β -enaminone as a precursor compound. This work extends our previous efforts⁴⁴⁻⁴⁸ to advance the development of diverse heterocycles and antimicrobial agents.

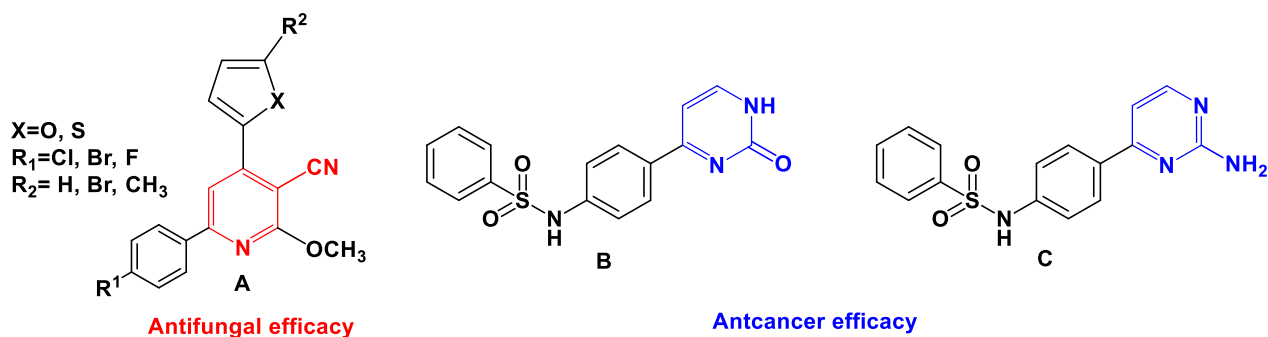


Figure 1. Various biologically active derivatives of pyridine and pyrimidine.

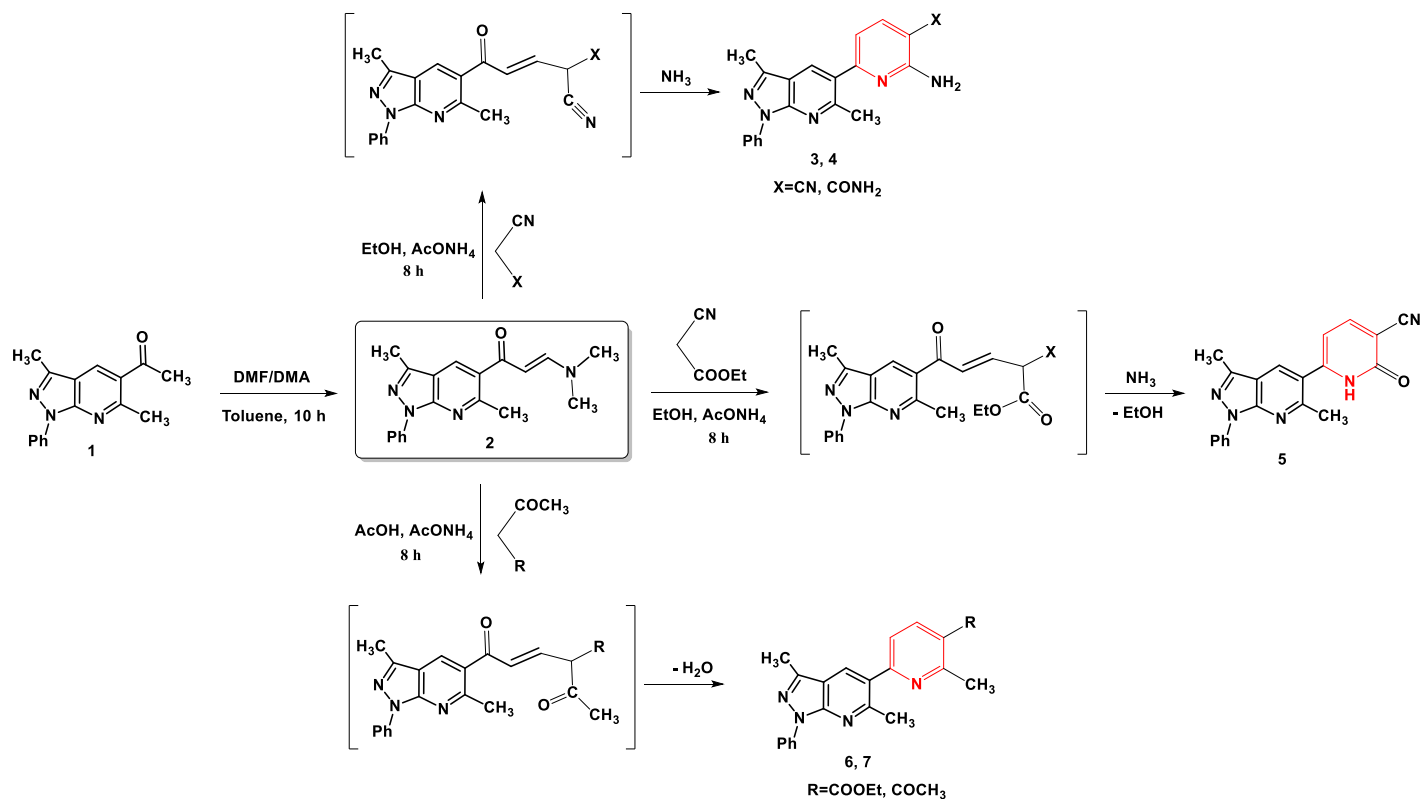
Results and Discussion

1. Chemistry

As part of our efforts to create novel organic heterocyclic compounds with biological activity, we have chosen to develop a new series of pyridine and pyrimidine derivatives based on the pyrazolopyridine structure. The β -

enaminone moiety is significant in synthetic organic chemistry due to its dual nucleophilic and electrophilic sites. The reaction pathways involved the formation of β -enaminone **2**, and its reactivity with active methylene compounds and ammonia derivatives was investigated. The reaction of the acetyl derivative **1**^{49, 50} with the DMFDMA reagent in toluene produced 3-(dimethylamino) prop-2-en-1-one **2**. The structure of compound **2** was confirmed through elemental and spectral analyses. The ¹H-NMR spectrum of **2** presented a signal at $\delta = 1.80$ ppm, indicating the presence of two methyl groups. This conclusion was further supported by the ¹³C-NMR figure, which displayed a signal at $\delta = 29.4$ ppm, corresponding to the two methyl carbons.

The β -enaminone **2** underwent cyclization with various nitrile derivatives, including malononitrile, cyanoacetamide, and ethyl cyanoacetate, in an ethanol and ammonium acetate mixture *via* a Knoevenagel reaction, resulting in the formation of pyridine derivatives **3-6**. Structural confirmation of these products was achieved through analytical and spectral data analysis. The FT-IR spectrum of 2-amino-6-(3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridin-5-yl) pyridine-3-carbonitrile (**3**) showed distinct peaks at $\nu = 2200$ and 3281 cm^{-1} , indicating the presence of a CN group and an NH₂ group, respectively. Additionally, the ¹H-NMR spectrum revealed a signal for the NH₂ group at $\delta = 12.00$ ppm. For the cyanopyridinone derivative **5**, the FT-IR spectrum exhibited three prominent peaks at $\nu = 1672$, 2215 , and 3290 cm^{-1} , which suggest the presence of C=O, CN, and NH groups. The ¹H-NMR figure of **5** showed a signal for the NH group at $\delta = 12.40$ ppm, which means that the two methyl groups were not present. The ¹³C-NMR spectrum of **5** corroborates this conclusion by demonstrating the absence of the methyl groups and indicating a peak at $\delta = 170$ ppm, associated with the CN group (Scheme 1).

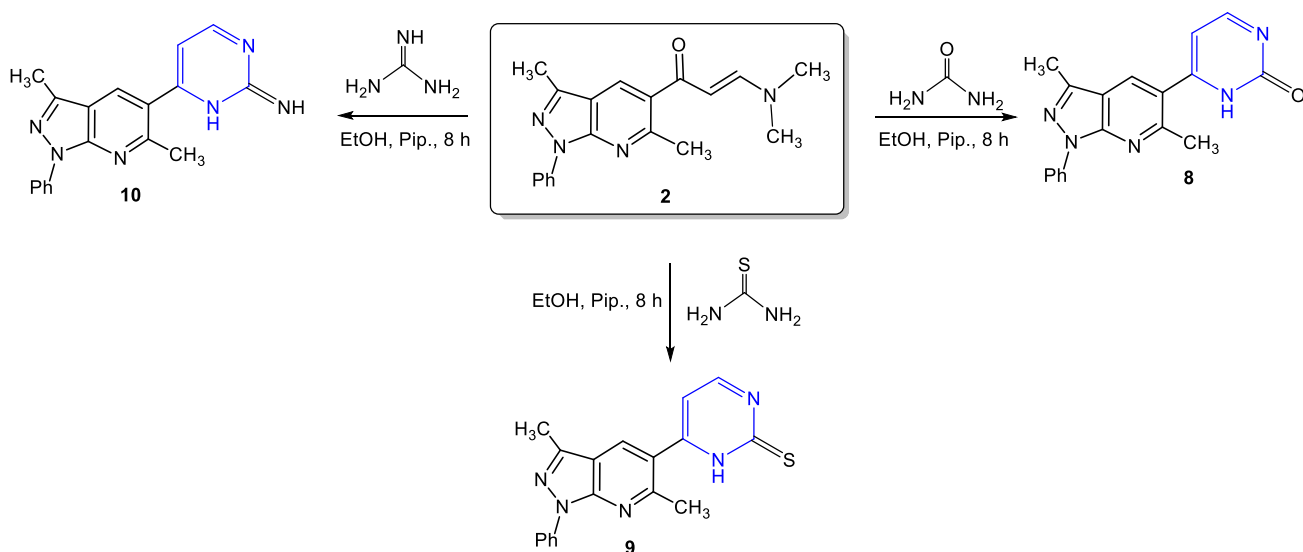


Scheme 1. The formation of pyridine derivatives (**3-7**) *via* the reactions of 3-(dimethylamino)prop-2-en-1-one **2** with active methylene reagents.

Reactions involving β -enaminone **2** with various nucleophiles, including ethyl acetoacetate and acetyl acetone, were conducted in glacial acetic acid supplemented with ammonium acetate. This reaction led to the formation of 2-methylpyridines **6** and **7**. The reaction mechanism proceeds via nucleophilic substitution at the active methylene position of the dimethyl amino group, which is subsequently followed by the elimination of a water molecule from the resulting non-isolable intermediates (Scheme 1). The structures of compounds **6** and **7** were confirmed using FT-IR and NMR spectroscopy, along with elemental analysis. The ^1H -NMR spectrum of **6** exhibited protons from the ethyl group, with a triplet signal at $\delta = 1.30\text{--}1.38$ ppm and a quartet signal at $\delta = 4.22\text{--}4.31$ ppm. The ^{13}C -NMR analysis showed two distinct signals: one at $\delta = 14.6$ ppm, corresponding to the methyl carbon, and another at $\delta = 60.0$ ppm, associated with the methylene carbon.

The β -enaminone derivative **2** was also reacted with active ammonia derivatives, such as urea and thiourea, resulting in the formation of pyrimidines analogues: 6-(3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridin-5-yl)pyrimidin-2(1*H*)-one (**8**) and 6-(3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridin-5-yl)pyrimidine-2(1*H*)-thione (**9**). The spectroscopic analysis of these compounds confirmed the synthesis of pyridine and identified the pyridine-NH signal. The FT-IR spectrum of pyrimidinone **8** displayed a distinct band at $\nu = 3329\text{ cm}^{-1}$, attributed to the (NH) group. Notably, the spectrum does not exhibit the carbonyl group band at $\nu = 1653\text{ cm}^{-1}$, which is typically associated with the enaminone structure. Instead, a new carbonyl group band is observed at $\nu = 1689\text{ cm}^{-1}$, which is attributed to the pyrimidine ring. Additionally, the NH signal was detected in the ^1H -NMR spectrum at $\delta = 60.0$ ppm.

The β -enaminone solution in ethanol was refluxed with guanidine hydrochloride, leading to the formation of 2-amino-4-(3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridin-5-yl)pyrimidine (**10**). The IR spectrum of compound **10** exhibited strong peaks at $\nu = 3419$ and 3294 cm^{-1} , indicating the presence of NH_2 functional group. The ^1H -NMR spectrum revealed that the two protons in the NH_2 group appeared as a broad singlet at $\delta = 6.85$ ppm. The deuterated water (D_2O) exchange spectrum of compound **10** demonstrated the exchange of NH_2 protons with deuterium atoms. The formation of compounds **8**–**10** can be explained by the plausible mechanism depicted in Figure 2. This mechanism involves intramolecular cyclization and subsequent aromatization, occurring through the elimination of dimethylamine and water molecules under the reaction conditions, resulting in compounds **8**–**10**, as depicted in Figure 2.



Scheme 2. Formation of pyrimidine derivatives (**8**–**10**) through the reactions of 3-(dimethylamino)prop-2-en-1-one **2** with ammonia derivatives.

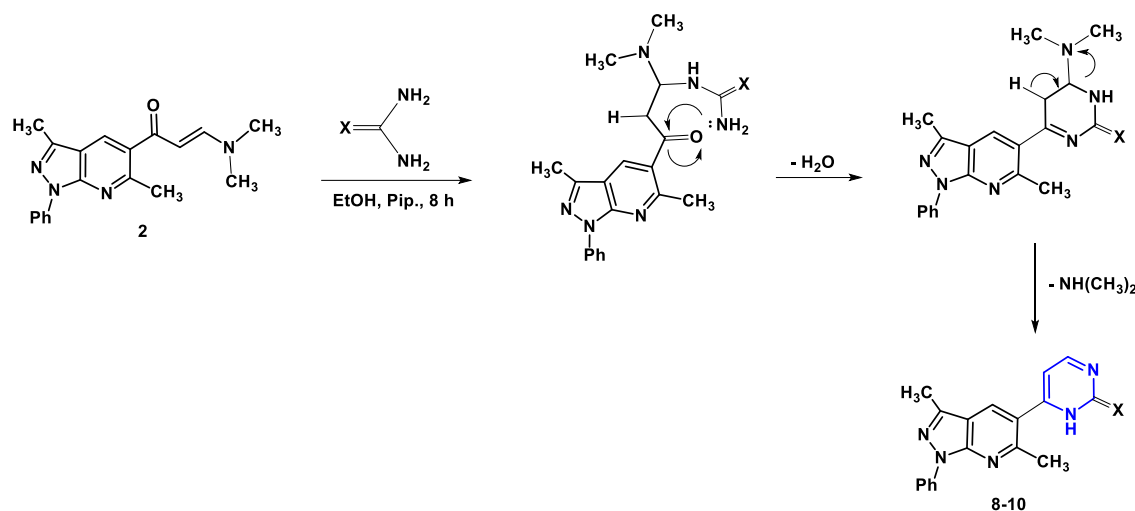


Figure 2. Proposed mechanism for the reactions of enaminone with urea, thiourea, or guanidine.

Antimicrobial evaluation

Antibacterial activity. The compounds **2-10** were assessed for their *in vitro* antimicrobial effects against *Gram*-positive bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, and *Streptococcus pneumoniae*, as well as *Gram*-negative pathogens such as *Haemophilus influenzae* and *Pseudomonas aeruginosa*. The minimal inhibitory concentrations (MICs) were determined, along with the results from standard antibacterial agents, *ciprofloxacin* and *streptomycin*, and their antifungal effectiveness against various fungal species, including *Candida albicans*, *Trichophyton rubrum*, *Aspergillus flavus*, and *Geotrichum candidum*. According to Table 1, all tested compounds exhibited notable antibacterial activity toward *Gram*-positive bacteria. Compound **10** demonstrated the most pronounced antibacterial effects against all tested bacterial strains, showing efficacy comparable to the reference antibiotics *ciprofloxacin* and *streptomycin*.

The β -enaminone **2** showed lower antibacterial activity compared to the other compounds tested. Additionally, compounds **4** and **6** displayed significant effectiveness against *B. cereus* and *S. aureus*. The results presented in Table 1 indicate that compounds **8-10**, which contain a pyrimidine ring, show enhanced activity. In contrast, compound **7**, which lacks this moiety, displays minimal resistance to both types of bacteria.

Antifungal activity. The evaluation of compounds **2-10** for antifungal activity involved testing against *Geotrichum candidum*, *Trichophyton rubrum*, *Candida albicans*, and *Aspergillus flavus*, using *ketoconazole* as the standard drug (Table 2). The pyrimidine derivatives **2-7** demonstrated low antifungal potency, as indicated by minimum inhibitory concentrations (MICs) ranging from 50 to 72 $\mu\text{g/mL}$. In contrast, the aminopyrimidine analogue **10** revealed distinct MIC values of 35, 37, 38, and 37 $\mu\text{g/mL}$ for *G. candidum*, *C. albicans*, *T. rubrum*, and *A. flavus*, respectively.

Table 1. Antibacterial efficacy of the synthesized compounds, presented as MIC ($\mu\text{g/mL}$)

Cpd No.	Gram positive			Gram negative	
	<i>B. cereus</i>	<i>S. aureus</i>	<i>S. pneumoniae</i>	<i>H. influenza</i>	<i>P. Aruginose</i>
2	42	71	74	75	80
3	41	78	70	70	75
4	10	19	20	25	24
5	25	35	31	64	50
6	10	18	24	28	25
7	40	30	32	40	30
8	11	18	15	16	19
9	12	17	14	19	18
10	4	15	12	18	14
Reference*	10	10	10	10	10

*Ciprofloxacin for (Gram +) and Streptomycin for (Gram -)

Table 2. Antifungal efficacy of the synthesized compounds, presented as MIC ($\mu\text{g/mL}$)

Cpd No.	Pathogenic Fungi			
	<i>G. candidum</i>	<i>C. albicans</i>	<i>T. rubrum</i>	<i>A. flavus</i>
2	-	59	-	58
3	61	72	64	-
4	55	68	60	70
5	50	60	57	61
6	50	55	52	57
7	51	50	50	50
8	45	48	49	46
9	41	42	40	40
10	35	37	38	37
Reference*	10	10	10	10

* *Ketoconazole* served as the antifungal standard.

(-) no activity

Conclusions

The aim of this study was to develop new analogues of pyridine and pyrimidine by reacting β -enaminone **2**, nitrile analogues, and ammonia derivatives. We assessed the antimicrobial activity of the synthesized compounds and determined their minimum inhibitory concentration (MIC) values. The findings indicate that heterocycles containing a pyrimidine nucleus exhibit greater antimicrobial potency than those with a pyridine nucleus. The two nitrogen atoms of the pyrimidine ring significantly alter the electron distribution, increasing ring polarity and changing the capacity of the ring to form hydrogen bonds. Increased interactions with the biological target could result in better binding affinity. Additionally, the basicity and solubility of the compound may be affected by the extra nitrogen, which may also account for the observed variations in biological activity.

Experimental Section

General. All melting points were determined on an APP Digital ST 15 apparatus, and the values given are uncorrected. IR spectra (KBr, cm^{-1}) were determined on a Shimadzu-408 infrared analyzer. ^1H -NMR and ^{13}C -NMR spectra were recorded on a JEOL ECA II 500 MHz spectrometer using TMS as the internal standard. Chemical shift values were recorded in ppm on the δ scale. High-resolution ESI mass spectra were obtained on a Varian MAT 312 spectrometer. Elemental analyses were carried out on an Elementar Analysensysteme GmbH VARIO EL V2.3 (July 1998, CHNS mode) device.

1-(3,6-Dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-5-yl)-3-(dimethylamino)prop-2-en-1-one (2). A mixture of acetyl derivative **1** (270 mg, 1 mmol) and DMFDMA (357 mg, 3 mmol) in dioxane (15 mL) was heated for 10 h. Upon cooling, the resulting precipitate was collected by filtration and recrystallized from dioxane. Yield: (68%) white powder, mp 187-189°C. FT-IR (ν_{max}): 2925, 2870 (Aliphatic-H), 1653 (C=O) cm^{-1} ; ^1H -NMR (500 MHz, CDCl_3): δ 2.56 (s, 3H, CH_3 -pyrazole), 2.92 (s, 3H, CH_3 -pyridine), 3.28 (s, 6H, 2 CH_3), 5.81 (d, H, $\text{CH}=\text{CH}$), 7.19-7.40 (m, 4H, 3 Ar-H and $\text{CH}=\text{CH}$), 7.88-7.98 (m, 2H, Ar-H), 8.26 (s, 1H, pyridine-H) ppm; ^{13}C -NMR (100 MHz, CDCl_3): δ 12.4 (CH_3 -pyrazole), 27.8 (CH_3 -pyridine), 42.0 (2 CH_3), 60.0 (CH-pyrazole), 110.8, 119.0, 120.4, 123.8, 125.4, 126.6, 129.4, 131.4, 140.0, 143.8, 149.6, 156.4, 160.1, 176.5 (C=O) ppm. MS (ESI) m/z : 320.0 (M^+ , 90%). Mol. Formula: $\text{C}_{19}\text{H}_{20}\text{N}_4\text{O}$ (320.16): C, 71.23; H, 6.29; N, 17.49. Found: C, 71.12; H, 6.20; N, 17.38%.

General pathway for the synthesis of pyridine derivatives (3-5). A solution of β -enaminone **2** (320 mg, 1 mmol) in ethanol (20 mL) containing ammonium acetate (616 mg, 8 mmol) was prepared, to which active methylene reagents (1 mmol), such as malononitrile, cyanoacetamide, or ethyl cyanoacetate, were added. The resulting mixture was refluxed for 10 h. The product was then separated by filtration and recrystallized from ethanol.

2-Amino-6-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-5-yl) pyridine-3-carbonitrile (3). Yield: (69%) pale orange crystals, mp 203-205°C. FT-IR (ν_{max}): 3430, 3304 (NH_2), 2905 (aliph-H), 2221 (CN) cm^{-1} ; ^1H -NMR (500 MHz, CDCl_3): δ 2.42 (s, 3H, CH_3 -pyrazole), 2.88 (s, 3H, CH_3 -pyridine), 6.80 (s, 2H, NH_2), 7.38-7.80 (m, 7H, 5 Ar-H and 2 pyridine-H), 8.12 (s, 1H, pyridine-H) ppm; ^{13}C -NMR (100 MHz, CDCl_3): δ 14.0 (CH_3 -pyrazole), 19.8 (CH_3 -pyridine), 60.8 (CH-pyrazole), 111.0, 111.7, 118.4, 120.6, 121.8, 125.2, 127.8, 129.0, 130.0, 132.4, 137.4, 141.8, 144.8, 147.4 (CN) ppm. MS (ESI) m/z : 340.1 (M^+ , 48%). Mol. Formula: $\text{C}_{20}\text{H}_{16}\text{N}_6$ (340.39): C, 70.57; H, 4.74; N, 24.69%. Found: C, 70.46; H, 4.63; N, 24.51%.

2-Amino-6-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-5-yl) pyridine-3-carboxamide (4). Yield: (72%) white crystals, mp 230-233°C. FT-IR (ν_{max}): broad 3447, 3312, 3230 (2 NH_2), 1691 (C=O) cm^{-1} ; ^1H -NMR (400 MHz, $\text{DMSO}-d_6$): δ 2.32 (s, 3H, CH_3 -pyrazole), 2.78 (s, 3H, CH_3 -pyridine), 6.39 (s, 2H, NH_2), 7.18-7.89 (m, 7H, 5 Ar-H and

2 pyridine-H), 8.10 (s, 1H, pyridine-H), 8.46 (s, 2H, NH₂) ppm; ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 14.6 (CH₃-pyrazole), 19.6 (CH₃-pyridine), 60.2 (CH-pyrazole), 110.4, 114.8, 119.6, 122.0, 123.6, 125.9, 127.9, 131.0, 132.8, 136.2, 140.6, 142.2, 168.8 (C=O) ppm. MS (ESI) *m/z*: 358.1 (M⁺, 50%). Mol. Formula: C₂₀H₁₈N₆O (358.41): C, 67.02; H, 5.06; N, 23.45%. Found: C, 66.91; H, 4.96; N, 23.30%.

6-(3,6-Dimethyl-1-phenyl-1H-pyrazolo[3,4-*b*]pyridin-5-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (5). Yield: (70%) yellow powder, mp 213-215°C. FT-IR (ν_{max}): 3284 (NH), 2925 (Aliph-H) 2214 (CN), 1678 (C=O) cm⁻¹; ¹H-NMR (400 MHz, CDCl₃): δ 2.52 (s, 3H, CH₃-pyrazole), 2.74 (s, 3H, CH₃-pyridine), 7.28-7.98 (m, 7H, 5 Ar-H and 2 pyridine-H), 8.78 (s, 1H, pyridine-H), 10.18 (s, 1H, NH) ppm; ¹³C-NMR (100 MHz, CDCl₃): 12.8 (CH₃-pyrazole), 28.2 (CH₃-pyridine), 64.4 (CH-pyrazole), 111.4, 119.2, 121.4, 124.4, 126.2, 128.0, 129.8, 132.6, 139.6, 141.8, 148.4 (CN), 158.4 (C=O) ppm. MS (ESI) *m/z*: 341.1 (M⁺, 31%). Mol. Formula: C₂₀H₁₅N₅O (341.37): C, 70.37; H, 4.43; N, 20.52%. Found: C, 70.26; H, 4.31; N, 20.40%.

General pathway for the synthesis of pyrazolo[3,4-*b*]pyridin-5-yl pyridine (6 and 7). β-Enaminone **2** (320 mg, 1 mmol) was added to ethyl acetoacetate or acetylacetone (1 mmol) in the presence of acetic acid (10 mL) and ammonium acetate (1 mmol). The mixture was refluxed for 8 h. Upon completion of the reaction, the solid precipitate was separated by filtration and recrystallized from ethanol.

Ethyl 2-methyl-6-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-*b*]pyridin-5-yl) pyridine-3-carboxylate (6)

Yield: (74%) brown powder, mp 241-243°C. FT-IR (ν_{max}): 2984 (Aliph-H), 1711 (C=O) cm⁻¹; ¹H-NMR (500 MHz, CDCl₃): δ 1.30-1.38 (t, *J*=20.0 Hz, 3H, -CH₂CH₃), 2.42 (s, 3H, CH₃-pyrazole), 2.55 (s, 3H, CH₃), 2.86 (s, 3H, CH₃-pyridine), 4.22-4.31 (q, *J*=27.5, 17.5 Hz, 2H, -CH₂CH₃), 7.29-7.78 (m, 7H, 5 Ar-H and 2 pyridine-H), 8.04 (s, 1H, pyridine-H) ppm; ¹³C-NMR (100 MHz, CDCl₃): δ 14.2 (CH₃-pyrazole), 14.6 (-CH₂CH₃), 18.9 (CH₃-pyridine), 25.8 (CH₃-pyridine), 60.0 (-CH₂CH₃), 62.2 (CH-pyrazole), 115.1, 118.5, 123.5, 126.6, 128.4, 129.1, 129.6, 135.9, 135.0, 140.1, 143.8, 148.4, 167.8 (C=O) ppm. MS (ESI) *m/z*: 386.1 (M⁺, 51%). Mol. Formula: C₂₃H₂₂N₄O₂ (386.46): C, 71.48; H, 5.74; N, 14.50%. Found: C, 71.38; H, 5.67; N, 14.39%.

3-Acetyl-2-methyl-6-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-*b*]pyridin-5-yl) pyridine (7). Yield: (70%) white crystals, mp 221-223°C. FT-IR (ν_{max}): 3001 (Ar-H), 1711 (C=O) cm⁻¹; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 2.45 (s, 3H, CH₃-pyrazole), 2.66 (s, 3H, COCH₃), 2.83 (s, 3H, CH₃), 2.92 (s, 3H, CH₃-pyridine), 7.52-7.93 (m, 7H, 5 Ar-H and 2 pyridine-H), 8.30 (s, 1H, pyridine-H) ppm; ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 14.4 (CH₃-pyrazole), 18.2 (CH₃-pyridine), 25.8 (CH₃-pyridine), 29.4 (CH₃), 117.6, 118.7, 125.3, 127.1, 128.4, 128.7, 128.9, 129.6, 132.0, 132.7, 138.9, 152.6, 187.6 (C=O) ppm. MS (ESI) *m/z*: 356.1 (M⁺, 71%). Mol. Formula: C₂₂H₂₀N₄O (356.43): C, 74.14; H, 5.66; N, 15.72%. Found: C, 74.05; H, 5.60; N, 15.63%.

Formation of pyrimidine derivatives (8-10). β-Enaminone **2** (640 mg, 2 mmol) was dissolved in ethanol (15 mL) with a few drops of piperidine, followed by the addition of urea, thiourea, or guanidine (2 mmol). The mixture was refluxed for 8 h. After cooling, the reaction mixture was poured onto crushed ice and neutralized with dilute hydrochloric acid. The resulting product was filtered, dried, and recrystallized from ethanol.

6-(3,6-Dimethyl-1-phenyl-1H-pyrazolo[3,4-*b*]pyridin-5-yl)pyrimidin-2(1H)-one (8). Yield: (65%) pale yellow crystals, mp 214-216 °C. FT-IR (ν_{max}): 3329 (NH), 3064 (Ar-H), 2993 (Aliph-H), 1689 (C=O) cm⁻¹; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 2.58 (s, 3H, CH₃-pyrazole), 2.91 (s, 3H, CH₃-pyridine), 7.27-8.12 (m, 7H, 5 Ar-H and 2 pyridine-H), 8.30 (s, 1H, pyridine-H), 9.36 (s, 1H, NH exchangeable with D₂O) ppm; ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 14.2 (CH₃-pyrazole), 25.4 (CH₃-pyridine), 118.4, 118.6, 121.8, 126.7, 126.8, 127.3, 127.3, 128.1, 128.8, 129.5, 132.9, 133.2, 138.7, 139.2, 187.8 (C=O) ppm. MS (ESI) *m/z*: 317.0 (M⁺, 77%). Mol. Formula: C₁₈H₁₅N₅O (317.35): C, 68.13; H, 4.76; N, 22.07%. Found: C, 68.13; H, 4.76; N, 22.07%.

6-(3,6-Dimethyl-1-phenyl-1H-pyrazolo[3,4-*b*]pyridin-5-yl)pyrimidine-2(1H)-thione (9). Yield: (63%) yellow crystals, mp 241-243 °C. FT-IR (ν_{max}): 3360 (NH), 3044 (Ar-H) cm⁻¹; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 2.45 (s, 3H, CH₃-pyrazole), 2.79 (s, 3H, CH₃-pyridine), 7.40-8.10 (m, 7H, 5 Ar-H and 2 pyridine-H), 8.40 (s, 1H, pyridine-H),

12.10 (s, 1H, NH exchangeable with D₂O) ppm; ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 14.0 (CH₃-pyrazole), 25.1 (CH₃-pyridine), 118.0, 118.5, 121.8, 126.7, 127.3, 127.5, 128.1, 128.8, 129.5, 129.9, 132.9, 133.3, 138.7, 139.2, 179.8 (C=S) ppm. MS (ESI) *m/z*: 333.0 (M⁺, 31%). Mol. Formula: C₁₈H₁₅N₅S (333.41): C, 64.84; H, 4.53; N, 21.01; S, 9.62%. Found: C, 64.75; H, 4.46; N, 20.01; S, 9.78%.

2(1H)-Imino-6-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-*b*]pyridin-5-yl)pyrimidine (10). Yield: (75%) white plates, mp 233-235°C. FT-IR (ν_{max}): 3419, and 3294 (NH₂), 3087 (Ar-H) cm⁻¹; ¹H-NMR (500 MHz, CDCl₃): δ 2.44 (s, 3H, CH₃-pyrazole), 2.88 (s, 3H, CH₃-pyridine), 6.85 (s, 2H, NH₂), 7.51-8.28 (m, 7H, 5 Ar-H and 2 pyridine-H), 8.38 (s, 1H, pyridine-H) ppm; ¹³C-NMR (100 MHz, CDCl₃): δ 14.9 (CH₃-pyrazole), 24.8 (CH₃-pyridine), 119.6, 120.6, 128.6, 128.7, 128.9, 129.0, 129.5, 139.3, 139.4, 142.9, 147.5, 149.9, 152.0, 157.2, 159.3 ppm. MS (ESI) *m/z*: 317.8 (M⁺, 40%). Mol. Formula: C₁₈H₁₆N₆ (316.37): C, 68.34; H, 5.10; N, 26.56%. Found: C, 68.24; H, 5.00; N, 26.47%.

***In Vitro* Antimicrobial screening method**

A 5% DMSO solution was prepared for the compounds under investigation (**2-10**) as well as for the standard compounds. Three 5 mm Whatman filter paper discs were saturated with this solution and systematically placed on nutrient-enriched hardened agar plates inoculated with bacteria to assess antibacterial activity. Similarly, the discs were arranged on Czapek Dox agar plates inoculated with fungi to evaluate antifungal activity.⁵¹ Fungal cultures were incubated at 28°C for four to seven days, while bacterial cultures were incubated at 37 °C for 24 to 48 hours. Following incubation, the diameters of the inhibition zones were measured in millimetres, providing essential data for analysis. *Ciprofloxacin* and *streptomycin* were used as standards for *Gram*-positive and *Gram*-negative bacteria, respectively, while ketoconazole served as the standard for antifungal controls treated with DMSO, ensuring the reliability of the results. A dilution series incorporating DMSO was established to determine the minimum inhibitory concentration (*MIC*). This approach allowed *MIC* values for both antibacterial and antifungal activities to be expressed in micrograms per millilitre (µg/mL), enhancing the interpretability of the results.

Supplementary Material

All data generated or analyzed during this study are included in this published article and its supplementary information files.

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