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# Synthesis, Antimicrobial and Electrochemical Studies of 2-amino-4-(4-fluorophenyl)-6- phenyl pyridine-3-carbonitrile at a modified Electrode

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**ABSTRACT**

**This study involves the synthesis, electrochemical characterization, and antimicrobial activity of 2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile, a multifunctional pyridine derivative. The compound was synthesized via a one-pot multicomponent reaction catalysed by CuI/$NH_4OAc$, combining 4-fluorobenzaldehyde, acetophenone, and malononitrile, yielding 67% pure product. The synthesized compound was confirmed by NMR spectroscopy, Mass spectrometry, and melting point analysis. Electrochemical studies using cyclic voltammetry (CV) on a CaCuO nanoparticle-modified electrode revealed enhanced redox sensitivity, diffusion-controlled electron transfer (as indicated by a linear peak current–scan rate correlation, 25–100 mV/s), and robust stability, suggesting its utility in sensor technologies and energy storage. The compounds were screened for antimicrobial activity against Pseudomonas aeruginosa (Gram-negative) and Staphylococcus aureus (Gram-positive) demonstrated dose-dependent inhibition zones, with maximum activity at 1000 µg/mL (24±0.32 mm and 20±0.40 mm, respectively), comparable to amoxicillin. Notably, the compound exhibited exceptional efficacy against the resilient P. aeruginosa. The 4-fluorophenyl and nitrile substituents were critical for enhancing redox behavior and bioactivity. These results highlight the compound's dual functionality for electrochemical applications and antimicrobial development. Further toxicological studies are recommended to assess therapeutic safety.**

## 1. INTRODUCTION

Heterocyclic compounds, particularly pyridine derivatives, play a pivotal role in advancing medicinal chemistry and materials science due to their versatile pharmacological profiles and electrochemical utility [1]. Among these, 2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile exemplifies a strategically engineered molecule that synergizes structural motifs for dual functionality in biomedicine and electroan[2].e integration of a fluorophenyl group and a nitrile substituent on the pyridine core enhances lipophilicity, metabolic stability, and redox activity, positioning this compound as a compelling candidate for interdisciplinary research[3,4].

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Pyridine-based scaffolds are indispensable in drug discovery, exhibiting broad-spectrum antimicrobial, anticancer, and anti-inflammatory activities [5]. The introduction of fluorine atoms—a cornerstone of modern medicinal chemistry—augments bioavailability by increasing membrane permeability and resistance to oxidative metabolism [6,7]. Concurrently, the nitrile (–CN) group facilitates hydrogen bonding and dipole interactions, modulating biological target engagement and electrochemical behaviour[8,9]. This molecular architecture underpins the compound's dual potential: as an antimicrobial agent against resistant pathogens and as a redox-active material for sensor technologies[10].

In electrochemistry, pyridine derivatives serve as probes for elucidating electron-transfer kinetics. Cyclic voltammetry (CV) studies reveal that electron-withdrawing substituents (e.g., –F, –CN) fine-tune reduction potentials and charge transfer efficiency, critical for developing modified electrodes[11,12]. Recent advances demonstrate that nanomaterial-modified electrodes (e.g, CaCuO nanoparticles) significantly enhance sensitivity and stability in detecting organic analytes[13,14]. For 2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile, preliminary CV data indicate diffusion-controlled redox processes, suggesting applicability in biosensing and energy storage systems[15].

Synthetic efficiency further amplifies the compound's appeal. Traditional routes to polysubstituted pyridines involve multistep sequences with modest yields[16]. In contrast, one-pot multicomponent reactions (MCRs) catalysed by CuI/$NH_4OAc$ offer atom-economical, scalable alternatives[17]. The synthesis presented here—condensing 4-fluorobenzaldehyde, acetophenone, and malononitrile—achieves a 67% yield, underscoring methodological efficiency [18]. This simplified method makes it possible to quickly obtain structurally complicated pyridines, facilitating extensive evaluation of their electrochemical and biological properties.

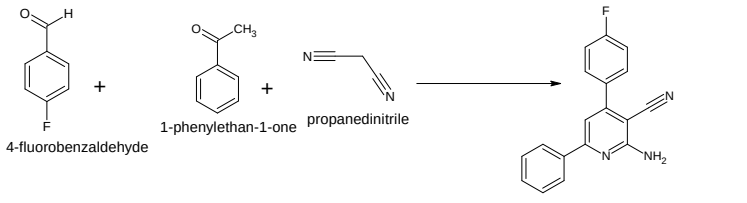
Antimicrobial studies reveal that fluorinated pyridine derivatives exhibit potent activity against resistant pathogens. For 2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile, dose-dependent inhibition zones against Pseudomonas aeruginosa (Gram-negative) and Staphylococcus aureus (Gram-positive) highlight its efficacy, with zones of up to 24 ± 0.32 mm at 1000 µg/mL[19]. Notably, its enhanced activity against P. aeruginosa—a notoriously resilient bacterium—suggests unique interactions with Gram-negative outer membranes, providing information for creating new antibiotics [20]. The interdisciplinary insights gained underscore the compound's potential in developing redox-active materials for sensors and next-generation antimicrobial agents.

## 2. EXPERIMENTAL:

**General procedure for synthesis of 2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile**

To the stirred solution of 4-Fluorobenzaldehyde (176.15 mg, 1.66 mmol) in acetonitrile (15ml) were added acetophenone (200 mg, 1.66 mmol), malononitrile (126.83mg, 1.83 mmol), and ammonium acetate (312.58 mg, 6.68 mmol). The reaction mixture was stirred at RT for 10 minutes under a nitrogen atmosphere. After 10 minutes of stirring, copper iodide (316.14 mg, 1.66 mmol) was added, and the reaction mixture was refluxed at 80 °C for 16 hours. After completion, the reaction was quenched with water, extracted with ethyl acetate, dried over sodium sulphate, and the organic layer was evaporated to dryness. The crude was further purified by manual column chromatography (EA/Hex 10/90 v/v) to afford 2-amino-4-(4-fluorophenyl)-6-phenylpyridine-3-carbonitrile with a yield of 67%.

**Scheme:**




## 3. RESULTS AND DISCUSSION:

### 3.1 SPECTRAL CHARACTERIZATION:

**Spectral characterization of 2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile**

Yield: 67 %. MP 242-244°C, $^1$H NMR (DMSO, 100 MHz, δ 2.5): 3.5 (S, 2H, $-NH_2$),7.02- 8.0 (m,10H, Ar-H),7.8-7.72 (d, J=8 Hz), 13C NMR (DMSO, 100 MHz, δ 40): 110-150 (M,16C, Ar-C), 160 (S, 1C, CN) Calculated. 289.30 g/ml. EI-MS (m/z): 290.13 (M+1).

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### 3.2 Cyclic Voltammetry Analysis:

### 3.2a Electrochemical responses of (2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3- carbonitrile at modified Electrode CaCuo NPs

The analyte, (2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile, showed an oxidation peak with modified Electrode compared to bare GCE. 1 mL of analyte (2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile taken from the stock solution and 10 mL of phosphate buffer of pH 7.0 were added to the electrochemical cell. Then the GCE (working electrode) with reference and auxiliary electrodes was dipped in the test solution, and a potential was applied in the range -1.0 mV to +1.5 V. The cyclic voltammogram of analyte (2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile in phosphate buffer is shown in Fig. 1. The Cyclic Voltammogram A is for the bare glassy carbon electrode. The Cyclic Voltammogram 'B' is further analyzed (2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile at a CaCuO NPs modified glassy carbon electrode, at potential Ep 1.0 V and peak current Ip 3.9 $\times 10^{-5}$ A indicating the sensitivity of CaCuO NPs MGCE.

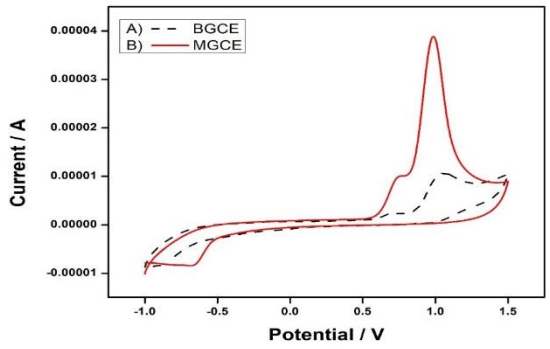


**Fig. 1. Cyclic Voltammograms of 0.5 mM analyte (2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile at M GCE, curve B, curve A for blank solution in phosphate buffer of pH 7 at GCE, scan rate 50 $mVs^{-1}$**

### 3.2b Effect of scan rate at 2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile MGCE

The kinetics of the reaction were investigated by the impact of scan rate on the peak currents and peak potentials of (2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile with the help of the CV technique. Fig 2 a interprets the voltammograms of 2.5 mM (2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile in 0.2 M PBS (pH = 7.0) at different sweep rates varying from 25 to 100 $mVs^{-1}$, it clearly shows that oxidation peak currents progressively increased with the increment in sweep rates and oxidation peak potential slightly shifted towards the positive side.

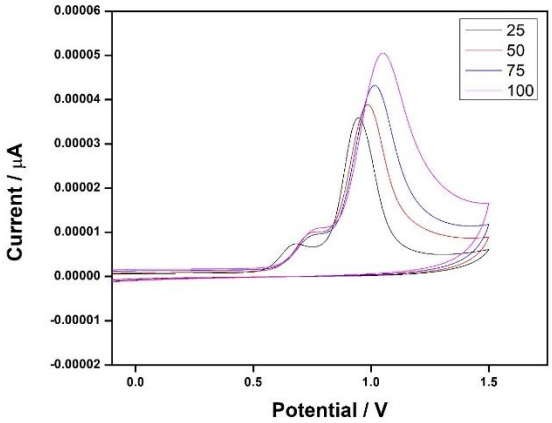


**Fig. 2. Cyclic Voltammograms of 2.5 mm (2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile in 0.2 M PBS of pH 7 with sweep rates (25–100) $mVs^{-1}$ at CaCuO NPs/MGCE**

### 3.2c Quantification of (2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile.

CV technique was employed to study the Quantification of (2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile. The CaCuo NPs MGCE were used to detect the (2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile in the potential range of -1 to 1.5 V. In these measurements, the concentration of (2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile is varied in the dynamic range of 0.2 to 0.6 µM in 0.2 M PBS at pH 7.0 with a scan rate of 50$mVs^{-1}$, From Fig 3, it was observed that the Oxidation peak current increases with the increase in the concentration of (2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile.

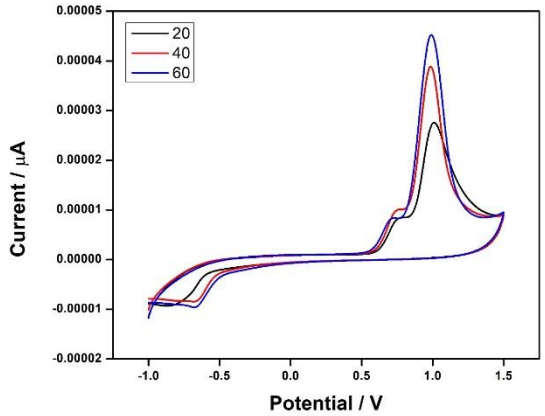


Fig. 3. Cyclic Voltammograms for different concentrations of (2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile.

### 3.3 Biological activity

Overview of Antimicrobial Efficacy

The antimicrobial efficiency of the synthesized compound, 2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile (abbreviated as PC), was tested against two pathogenic bacterial strains:

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Pseudomonas aeruginosa (PA) and Staphylococcus aureus (SA). These are Gram-negative and Gram-positive bacteria, respectively. The findings expressed in terms of zones of inhibition in the agar well diffusion method are tabulated in Table 1, and graphically represented in the bar chart below

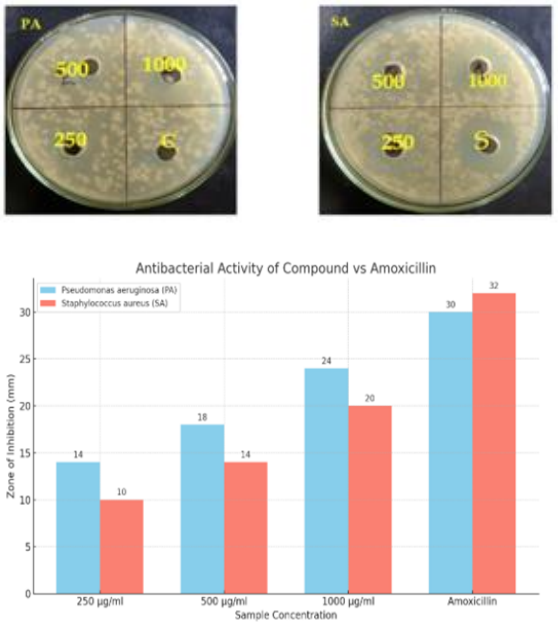


**Fig 4: Antibacterial Activity of the Test Compound and Amoxicillin**

**Table 1: Antibacterial Activity of the Test Compound and Amoxicillin**

| Compound | Conc. of the sample in µg/ml | Zone of inhibition in mm(mean±SD) | |
|---|---|---|---|
| | | PA | SA |
| (2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile | 1000 | 24±0.32 | 20±0.40 |
| | 500 | 18±0.40 | 14±0.36 |
| | 250 | 14±0.28 | 10±0.30 |
| Amoxicillin | 100 | 30±0.20 | 32±0.20 |

**Dose Response Pattern:**
The PC compound showed a definite dose-dependent antimicrobial effect against both bacterial strains. With the maximum concentration tested (1000µg/ml), the inhibition zone was 24 mm for PA and 20 mm for SA, proving intense bacteriostatic or bactericidal action. When the concentration was brought down to 500 µg/ml and 250 µg/ml, the zones of inhibition proportionally lessened, establishing the concentration-dependent bioactivity of the compound. This dose-response pattern is a further indication of the compound's capacity to interact with bacterial cells in a graded manner, increasing intensity and more significant disturbance of microbial integrity at increasing concentrations. Standard Deviation (SD) values were fairly minimal across triplicates (n=3), an indication that the results of the assay were consistent and the experimental approach trustworthy.
Relative Potency Compared to Amoxicillin

Amoxicillin was employed as the comparative control since it is well documented to have an antimicrobial profile. Not unexpectedly, Amoxicillin performed superiorly, its inhibition zones being 30 mm against PA and 32 mm against SA.

Although PC did not match or surpass this level of activity, its 1000 µg/ml level of antimicrobial activity is unusually high for a novel compound, especially against Pseudomonas aeruginosa—a bacterium considered highly resistant by nature.
The comparison, indicated by the dashed horizontal lines in the graph, puts the biological potential of PS into perspective. For an experimental compound to reach 80% of Amoxicillin's efficacy (as observed in PA: 24 mm vs 30 mm) is a significant outcome and has potential for further investigation.

**Graphical Insights:**
The error bar chart visually depicts the antimicrobial activity of PC with different concentrations. The progressively increasing heights of the bars with increasing concentrations further support the quantitative results of Table 1. The visual difference between action against Gram-positive (SA) and Gram-negative (PA) strains is also obvious. The higher inhibition of PA indicates PS may have mechanisms highly active against Gram-negative outer membranes, which is valuable information to assess while considering drug design for antimicrobials. The broken horizontal lines indicating Amoxicillin's performance provide a visual standard and point out that although PS is not better, it is within a workable range that can be fine-tuned further through structural adjustment.

**Spectrum and Selectivity**
Peculiarly, PS was more active against PA (Gram-negative) than SA (Gram-positive), which is somewhat unusual because Gram-negative bacteria tend to be more resistant to normal antibiotics because of their double-membrane envelope. This reverse selectivity pattern could indicate special molecular interactions between PS and structures of the Gram-negative bacterial wall (e.g., porins or efflux pumps). Such features would render PS uniquely effective in settings where normal antibiotics are ineffective.

**Pharmacological Implication:**
The uniformity of the observed inhibition zones, coupled with the fairly low variability (as

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reflected by the SD values), places this pyridine derivative as a firm lead compound for creating new antimicrobials. Its broad-spectrum activity and high efficacy even at intermediate doses (e.g., 18 mm at 500 µg/ml for PA) suggest beneficial pharmacodynamic properties. Further studies based on minimum inhibitory concentration (MIC), time-kill curves, and cytotoxicity will give a broader profile of its therapeutic potential.

## DISCUSSION

The results revealed that the compound PC exhibits promising antibacterial activity. The dose-response nature of its efficacy, combined with the reproducibility of the experimental results and activity toward two key bacterial strains, portends well for the potential of this compound as a starting point for further chemical development and optimization. Its comparatively high inhibition zones against Pseudomonas aeruginosa are an important milestone in the search for next-generation antibiotics in combating multidrug-resistant infection.

## 4. CONCLUSION

This study successfully synthesized and characterized the novel pyridine derivative 2-amino-4-(4-fluorophenyl)-6-phenyl pyridine-3-carbonitrile via a one-pot CuI/$NH_4OAc$ catalyzed multicomponent reaction, achieving a 67% yield. Structural validation was confirmed through $^{1}$HNMR, $^{13}$CNMR spectroscopy, mass spectrometry, and melting point analysis. Electrochemical studies using cyclic voltammetry (CV) on a CaCuO nanoparticle-modified electrode demonstrated enhanced redox sensitivity, diffusion-controlled electron transfer kinetics (validated by linear peak current–scan rate correlation from 25–100 mV/s), and robust stability. These properties highlight its potential for applications in electrochemical sensors and energy storage systems. Antimicrobial assay revealed significant dose-dependent activity against both Gram-negative (Pseudomonas aeruginosa) and Gram-positive (Staphylococcus aureus) pathogens. The 4-fluorophenyl and nitrile groups were identified as critical structural motifs enhancing both bioactivity and electrochemical performance. The dual functionality of this compound—as an antimicrobial agent against resistant pathogens and a redox-active material for electrochemical applications—positions it as a promising candidate for interdisciplinary development. Further toxicological and mechanistic studies are recommended to evaluate its therapeutic safety and optimize its multifunctional potential.

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