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# SYNTHESIS OF 2-((5-2-METHYLIMIDAZO[1,2-*A*]PYRIDINE-3-YL)-1,3,4-OXADIAZOL-2-YL)THIO)-*N*-PHENYLACETAMIDE DERIVATIVES AND THEIR BIOLOGICAL EVALUATION

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**ABSTRACT**

In this work, a novel series of compounds based on 2-((5-(2-methylimidazo[1,2-*a*]pyridine-3-yl)-1,3,4-oxadiazol-2-yl)thio)-*N*-phenylacetamide derivatives was meticulously synthesized and thoroughly characterized through infrared spectroscopy, $^{1}$H NMR, $^{13}$C NMR, and mass spectroscopies. The systematic synthesis and characterization of these compounds aimed to establish a foundation for investigating their potential antibacterial and antifungal activities. The research methodology involved a multi-faceted approach, wherein the synthesized compounds were subjected to rigorous testing to evaluate their effectiveness against bacterial and fungal strains. The experimental design included a range of concentrations to provide a comprehensive understanding of the compounds' inhibitory effects.

**Keywords:** Imidazo[1,2-*a*]pyridine, 1,3,4-Oxadiazole, Antifungal, Antibacterial, Evaluation.
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## INTRODUCTION

Nitrogen-containing heterocycles have long been of interest in synthetic organic chemistry as well as in medicinal chemistry.[1] Imidazo[1,2-*a*]pyridine stands out prominently among the nitrogen-fused azoles in scholarly discourse, owing to their diverse range of applications across multiple fields such as medicinal chemistry, organometallics, and material science.[2,3] It has long been recognized that imidazo[1,2-*a*]pyridine derivatives have a wide range of biological actions[4,5] and are used as antiviral[6], antiulcer[7], antibacterial[8], antifungal[9], antiprotozoal[10,11], Antiherpes[12] and anti-inflammatory[13] agents. These compounds exhibit multifunctional properties, serving as inhibitors of α-amyloid formation, activators of GABA and benzodiazepine receptors, and agents with cardiotonic effects.[14,15,16] 1,3,4-Oxadiazole is another type of heterocyclic molecule that consists of a five-membered ring structure including one oxygen atom and two nitrogen atoms[17]. Compounds having the 1,3,4-oxadiazole nucleus have a distinct place in medicinal chemistry and play an important role due to their high biological activity[18,19,20]. The small and modest 1,3,4-oxadiazole core is found in derivatives convoluted in research designed at evaluating novel derivatives with stimulating therapeutic activities such as anti-inflammatory[21,22], antimicrobial[23,24], anticancer[25,26], antituberculosis[27], antidiabetic[28]and analgesic[29,30] agents. The significant biological efficacies and the versatile applications of 1,3,4-oxadiazoles, especially those featuring 2,5-disubstituted configurations, have ignited substantial interest within the scientific community. As a continuation of our interest in the high efficacy of antibacterial research, we design, synthesize, and bioactive evaluation of novel 2,5-disubstituted 1,3,4-oxadiazole derivatives. Our approach focuses on the incorporation of imidazo[1,2- *a*]pyridine and phenylacetamide substitutes into the molecular structure of these compounds. These structural modifications are strategically chosen to enhance the antimicrobial properties of the compounds.

## EXPERIMENTAL

### Materials and Methods

In this work, all the reagents and chemicals were sourced from reputable suppliers such as Sigma-Aldrich and Spectrochem chemicals and employed directly without purification. To monitor the progress of the reactions, thin-layer chromatography (TLC) using silica gel-G plates (G60 $F_{254}$, E-Merck Co.) was employed. Visualization under UV light allowed for real-time observation of reaction progress, facilitating efficient optimization of the reaction conditions. Melting points of the synthesized derivative were determined in open capillaries and were uncorrected. Infrared spectra were recorded using an FT-IR-8400


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instrument with KBr pallets. Nuclear magnetic resonance (NMR) spectroscopy, including both $^{1}H$ and $^{13}C$ NMR, was performed proceeding a Bruker Avance 400 MHz spectrometer as well as a 101 MHz spectrometer, individually. The chemical shifts are described in ppm compared to the solvent DMSO-$d_6$, with tetramethylsilane (TMS) serving as the internal reference. The mass spectra were obtained using ultraperformance liquid chromatography coupled with mass spectrometry (LC/MS), employing electrospray ionization in positive ion mode. The LC/MS analysis provided the identification of molecular weights and fragmentation patterns of the compounds, covering a mass range of 100 to 1500 Da with a cone voltage of 30 V.

## General Procedures

### Synthesis of ethyl 2-methylimidazo[1,2-*a*]pyridine-3-carboxylate (3)

Ethyl acetoacetate (10mmol) was dissolved in 40 mL of methanol and cooled to 0-5 ºC with stirring for 10-15 min. N-Bromo succinimide (NBS) (10 mmol) was added portion-wise and the reaction mass was stirred in cooling for 15-20 min. Subsequently, the mixture was stirred at room temperature for 45-50 min until it became clear. 2-Aminopyridine (10 mmol) remained then further portion and the reaction mass was refluxed about 2-3 h at 80ºC. The improvement of the reaction mass was monitored via TLC until the starting material was fully consumed. Upon completion, the solvent was evaporated under reduced pressure and the residue was poured onto crushed ice. The resulting solid was recrystallized using methanol to afford the desired product.[31]

### Synthesis of 2-methylimidazo-[1,2-*a*]pyridine-3-carbohydrazide (4)

Ethyl 2-methylimidazo[1,2-*a*]pyridine-3-carboxylate (10mmol) dissolved in 10 mL of methanol was added to hydrazine hydrate (99%) (10mmol) dropwise and the reaction mass was heated 70 ℃ for 2-3 h. The improvement of the reaction mass was monitored via TLC and subsequently, the completion, of the reaction mass was cooled to rt and poured into crushed ice-cold water. The resulting solid was collected and recrystallized using ethyl acetate to obtain the product.[32]

#### Synthesis of 5-(2-methylimidazo-[1,2-*a*]pyridine-3-yl)-1,3,4-oxadiazole-2-thiol (5)

A mixture of 2-methylimidazo-[1,2-*a*]pyridine-3-carbohydrazide (10 mmol) and KOH (10 mmol) in 20 mL of methanol was stirred at 0ºC until the solution became clear followed by the addition of $CS_2$ (10 mmol) dropwise and then the reaction mass was refluxed at 80 ºC about 6-7 h. The progress of the reaction mass was monitored by TLC and after completion; the reaction mass was cooled to rt and poured into crushed ice-cold water. The resulting mixture remained then acidified by 1 N HCl. The solid product was collected by filtration, washed with water, and dried at room temperature overnight. It was observed that the compound exists in thione-thiol tautomeric forms, but the investigation revealed a specific tautomeric form in this case.[33]

#### Synthesis of 2-((5-2-methylimidazo[1,2-*a*]pyridine-3-yl)-1,3,4-oxadiazol-2-yl)thio)-*N*-phenylacetamide (6a-o)

A mixture of 5-(2-methylimidazo[1,2-*a*]pyridine-3-yl)-1,3,4-oxadiazole-2-thiol (10 mmol) and $K_2CO_3$ (10 mmol) in 10 mL of DMF was stirred for 15-30 min until a color change was observed. Subsequently, 2-chloro-*N*-phenylacetamide derivative (10 mmol) remained additional portion-wise and the reaction mass was stirred on behalf of 2-3 h at RT. The progress of the reaction mass was checked by TLC and after the completion; the reaction mass was poured into crushed ice, resulting in the formation of a hazy solution. A few drops of aqueous HCl were added to the solution. The solid product was collected by vacuum filtration and dried overnight in a refrigerator (**Scheme-1**). The newly formed compounds were purified through recrystallization using either methanol or ethanol as solvents. The reactions proceeded smoothly, yielding products with yields ranging from 41% to 81% (**6a-o**).

## Spectral Data

### 2-((5-(2-methylimidazo[1,2-a]pyridin-3-yl)-1,3,4-oxadiazol-2-yl)thio)-N-phenylacetamide(6a)

Oyster white, yield:81%, M.P.:207-209ºC, IR(KBr,$\upsilon_{max}$,$cm^{-1}$): 3352.39, 3136.36, 2982.05, 1728.28, 1658.84, 1604.83, 1500.67, 1446.66, 856.42, MS(*m/z*): 366.3($M^+$). Anal. Calcd. For $C_{18}H_{15}N_5O_2S$: C, 59.17; H, 4.14; N, 19.17; found: C, 59.15; H, 4.12; N, 19.15.

**2-((5-(2-methylimidazo[1,2-a]pyridin-3-yl)-1,3,4-oxadiazol-2-yl)thio)-N-(p-tolyl)acetamide(6b)**
Oyster white, yield:78%, M.P.:213-215°C, IR(KBr,$\upsilon_{max}$,$cm^{-1}$): 3309.96, 3140.22, 2974.33, 1716.70, 1662.69, 1600.97, 1504.53, 1446.66, 860.28, $^1$H NMR (400 MHz, DMSO-*d*$_6$) δ 2.38 (s, 3H), 2.62 (s, 3H), 4.21 (s, 2H), 7.03 (t, *J* = 6.9 Hz, 1H), 7.25 (d, *J* = 7.9 Hz, 2H), 7.34 (d, *J* = 7.9 Hz, 2H), 7.40 (t, *J* = 7.9 Hz, 1H), 7.59 (d, *J* = 8.9 Hz, 1H), 8.90 (d, *J* = 7.0 Hz, 1H), 10.32 (s, 1H),$^{13}$C NMR (101 MHz, DMSO-*d*$_6$) δ 16.33, 21.23, 33.34, 49.08, 113.57, 115.39, 116.68, 127.24, 127.38, 128.44, 130.06, 132.80, 138.72, 145.68, 146.63, 159.23, 171.94. MS(*m/z*):380.3($m^+$). Anal. Calcd. For $C_{19}H_{17}N_5O_2S$: C, 60.14; H, 4.52; N, 18.46; found: C, 60.12; H, 4.51; N, 18.45.

**N-(4-fluorophenyl)-2-((5-(2-methylimidazo[1,2-a]pyridin-3-yl)-1,3,4-oxadiazol-2-yl)thio)acetamide(6c)**
Oyster white, yield:62%, M.P.:206-208°C, IR(KBr,$\upsilon_{max}$,$cm^{-1}$): 3498.99, 3147.93, 3070.78, 1728.28, 1658.84, 1612.54, 1573.97, 1481.38, 837.13, $^1$H NMR (400 MHz, DMSO-*d*$_6$) δ 2.59 (s, 3H), 4.34 (s, 2H), 7.18 (dt, *J* = 17.9, 7.9 Hz, 3H), 7.52 (t, *J* = 7.9 Hz, 1H), 7.60 (dd, *J* = 8.8, 5.0 Hz, 2H), 7.71 (d, *J* = 8.9 Hz, 1H), 9.16 (d, *J* = 6.8 Hz, 1H), 10.53 (s, 1H), $^{13}$C NMR (101 MHz, DMSO-*d*$_6$) δ 15.64, 37.25, 107.13, 114.79, 115.82, 116.04, 117.01, 121.37, 127.68, 128.09, 135.57, 146.58, 148.31, 157.45, 159.36, 159.84, 161.68, 165.29. MS(*m/z*):384.3($m^+$). Anal. Calcd. For $C_{18}H_{14}FN_5O_2S$: C, 56.39; H, 3.68; N, 18.27; found: C, 56.36; H, 3.66; N, 18.24.

**N-(4-chlorophenyl)-2-((5-(2-methylimidazo[1,2-a]pyridin-3-yl)-1,3,4-oxadiazol-2-yl)thio)acetamide(6d)**
Oyster white, Yield:60%, M.P.:228-230°C, IR(KBr,$\upsilon_{max}$,$cm^{-1}$): 3456.55, 3117.07, 2931.90, 1894.16, 1689.70, 1608.69, 1546.96, 1481.38, 829.42, $^1$H NMR (400 MHz, DMSO-*d*$_6$) δ 2.60 (s, 3H), 4.36 (s, 2H), 7.22 (td, *J* = 6.9, 1.3 Hz, 1H), 7.35 – 7.43 (m, 2H), 7.53 (ddd, *J* = 8.8, 7.0, 1.3 Hz, 1H), 7.58 – 7.69 (m, 2H), 7.72 (dt, *J* = 9.0, 1.3 Hz, 1H), 9.18 (dt, *J* = 6.9, 1.3 Hz, 1H), 10.59 (s, 1H), $^{13}$C NMR (101 MHz, DMSO-*d*$_6$) δ 15.18, 36.86, 106.67, 114.34, 116.56, 120.71, 127.22, 127.25, 127.64, 128.81, 137.64, 146.13, 147.85, 158.91, 161.19, 165.09, MS(*m/z*):400.3($m^+$). Anal. Calcd. For $C_{18}H_{14}ClN_5O_2S$: C, 54.07; H, 3.53; N, 17.52; found: C, 54.04; H, 3.51; N, 17.50.

**N-(4-bromophenyl)-2-((5-(2-methylimidazo[1,2-a]pyridin-3-yl)-1,3,4-oxadiazol-2-yl)thio)acetamide(6e)**
Oyster white, Yield:58%, M.P.:244-246°C, IR(KBr,$\upsilon_{max}$,$cm^{-1}$): 3498.99, 3109.35, 2935.76, 1890.30, 1689.70, 1604.83, 1543.10, 1485.24,829.42, $^1$H NMR (400 MHz, DMSO-*d*$_6$) δ 3.35 (s, 3H), 4.35 (s, 2H), 7.20 (t, *J* = 6.8 Hz, 1H), 7.50 (d, *J* = 8.5 Hz, 3H), 7.56 (d, *J* = 8.6 Hz, 2H), 7.70 (d, *J* = 8.9 Hz, 1H), 9.14 (d, *J* = 6.9 Hz, 1H), 10.58 (s, 1H), $^{13}$C NMR (101 MHz, DMSO-*d*$_6$) δ 15.63, 37.37, 107.10, 114.76, 115.76, 116.99, 121.53, 127.65, 128.06, 132.16, 138.51, 146.57, 148.29, 159.34, 161.63, 165.56. MS(*m/z*) :446.3($m^+$). Anal. Calcd. For $C_{18}H_{14}BrN_5O_2S$: C, 48.66; H, 3.18; N, 15.76; found: C, 48.64; H, 3.16; N, 15.75.

**Antimicrobial Activity**
All microbial cultures are subjected to testing against both recognized and unidentified standard medicines. Mueller Hinton Broth serves as the nutrient standard for cultivating and diluting the drug interruption on behalf of the examination. All essential controls, including Drug, Vehicle, Agar, Organism, and known antibacterial drugs control, have been implemented. The "Broth Dilution Method" is utilized for MIC determination, with serial dilutions prepared in both primary and secondary screenings. Mueller Hinton broth serves as the nutrient medium for cultivating and diluting the drug suspension for the test. Test organisms are cultured in broth on behalf of 24 hours for microorganisms and 48 hours for molds at 37°C. Serial watering down of test compound solutions is prepared, inoculated with test organisms, and then incubated at 37°C for 48 hours. The tubes are subsequently examined for the presence or absence of bacteriological development. The lowest concentration displaying no growth is identified as the minimum inhibitory concentration (MIC). This technique involves preparing serial dilutions of the test compounds in a suitable growth medium, followed by inoculation with standardized bacterial or fungal cultures. The dilutions are then incubated under optimal conditions for microbial growth, allowing the assessment of the compounds' inhibitory effects. The minimum inhibitory concentration (MIC) of each compound is

determined, representing the lowest concentration at which microbial growth inhibition is observed. Each synthesized drug and the standard drug were liquefied in a DMSO-water mixture at a concentration of 2 mg/mL. Now the main screening, concentrations of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, and 62.5 µg/mL of the produced drugs were tested. Data for the initial solution were not recorded due to the high concentration of DMSO (10%). The active synthesized drugs identified in the primary screening were further diluted to concentrations of 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, and 12.5 µg/mL for secondary screening. The highest dilution that showed at least 99% inhibition was determined as the minimum inhibitory concentration (MIC). Each dilution is inoculated with the microbial cultures and incubated for a specified period to allow for microbial growth. Following incubation, the MIC of each compound is determined based on visual inspection of microbial growth or using automated methods, with the lowest concentration exhibiting significant inhibition considered as the MIC. Compounds demonstrating promising activity in the primary screening undergo further evaluation in a secondary screening phase. In this stage, active compounds identified in the primary screening are subjected to additional dilutions to obtain a more refined concentration range. The MIC is again determined for each compound to confirm its inhibitory activity against the target microorganisms.

**Test Culture**

Gram Positive: *Staphylococcus aureus*, *Streptococcus pyogenus*
Gram Negative: *Escherichia coli*, *Pseudomonas aeruginosa*
Fungi: *Aspergillus niger*, *Candida albicans*
Standard Drugs: The standard drugs used in the present study are.;
Chloramphenicol and Ampicillin: Antibacterial activity
Nystatin: Antifungal activity

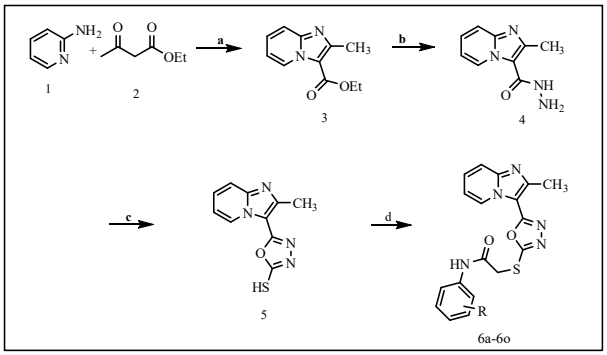
Table-1: Antibacterial and Antifungal Activity of 1,3,4-Oxadiazole Derivatives (6a-o):

| Compound Code | Antibacterial MIC(µg/mL) | | | | Antifungal MIC (µg/mL) | |
|---|---|---|---|---|---|---|
| | *S. aureus* MTCC737 | *S. pyogenus* MTCC1925 | *E. coli* MTCC443 | *P. aeruginosa* MTCC5210 | *A. niger* MTCC282 | *C.albicans* MTCC183 |
| Chloramphenicol | - | - | 50 | 50 | - | - |
| Ampicillin | 50 | 50 | - | - | - | - |
| Nystatin | - | - | - | - | 50 | 100 |
| 6a | 1000 | 1000 | 250 | 250 | 1000 | 1000 |
| 6b | 250 | 250 | 1000 | 1000 | 250 | 500 |
| 6c | 125 | 125 | 250 | 250 | 250 | 250 |
| 6d | 250 | 125 | 250 | 250 | 250 | 125 |
| 6e | 1000 | 500 | 1000 | 500 | 1000 | 500 |
| 6f | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 6g | 500 | 500 | 1000 | 1000 | 500 | 1000 |
| 6h | 1000 | 1000 | 500 | 500 | 500 | 1000 |
| 6i | 500 | 500 | 1000 | 1000 | 500 | 1000 |
| 6j | 500 | 500 | 500 | 500 | 500 | 1000 |
| 6k | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 6l | 1000 | 1000 | 500 | 500 | 1000 | 500 |
| 6m | 500 | 500 | 1000 | 1000 | 500 | 1000 |
| 6n | 500 | 500 | 250 | 250 | 500 | 250 |
| 6o | 250 | 250 | 500 | 500 | 500 | 250 |

## RESULTS AND DISCUSSION

The synthesis of the target molecules followed a systematic procedure outlined in Scheme-1. The first step involved the reaction of 2-amino pyridine **1** with ethyl acetoacetate **2**, facilitated by *N*-bromosuccinimide (NBS) in situ, resulting in an excellent yield of compound **3**. Subsequently, compound **3** underwent a

transformation into its corresponding carbohydrazide **4** through a reaction with hydrazine hydrate. Further cyclization of compound **4** was achieved by utilizing $CS_2$ in the presence of a basic medium, leading to the formation of a five-membered ring with heteroatoms oxygen and nitrogen, resulting in compound **5**. The use of $CS_2$ as a cyclizing reagent proved highly effective, yielding the desired product efficiently. The next synthetic step involved the reaction of compound **5** with derivatives of 2-chloro-*N*-phenylacetamide in the presence of a basic medium, ultimately yielding the novel target compound **6a-o**. Significantly, the overall synthetic pathway demonstrated efficient and high-yielding transformations at each step. All the synthesized compounds were subjected to thorough spectral analysis to confirm their structural integrity and composition.




a. NBS, methanol, 80°C, b. $NH_2NH_2.H_2O$, MeOH, 80-90°C, c. KOH, $CS_2$, methanol, 80°C, d. $K_2CO_3$, DMF, Substituted 2-chloro-N-phenylacetamide, RT

Scheme-1: Synthetic Pathway for the Desired Compounds

The synthesized compounds **6a-o** were characterized through $^1$H NMR spectroscopy employing DMSO-$d_6$ as a solvent and TMS as the internal standard reference. The proton signals attributed to the methyl group of imidazo[1,2-*a*]pyridine was observed at 4.36 ppm. Additionally, the 2º amine of 2-chloro-*N*-phenylacetamide exhibited a distinctive signal at 10.59 ppm. The $^{13}$C NMR spectrum revealed the methyl group of imidazo[1,2-*a*]-pyridine at 15.18 ppm, the (C=N) carbon at 147.84 ppm, the (C=O) carbon at 165.07 ppm, and the C-S carbon at 161.18 ppm. These chemical shifts revealed the carbon atom environments and confirmed the structure of the synthesized compounds **6a-o**. Moreover, the mass spectrometry further supported the characterization, yielding an observed mass of *m/z* 366.3. The MIC values obtained from the evaluation indicated varying degrees of inhibition for the newly synthesized compounds. Compound 6a demonstrated moderate activity against *S. pyogenes* and *E. coli*. Compound 6b exhibited moderate activity against *S. aureus*, *P. aeruginosa*, and *A. niger* strains. Notably, compound 6c displayed good to moderate activity against both Gram-positive and Gram-negative bacteria, including *S. aureus*, *S. pyogenes*, *E. coli*, and *P. aeruginosa*, as well as against the fungal strains *A. niger* and *Candida albicans*. Compound 6d showed good to moderate activity against both Gram-positive and Gram-negative bacterial strains.

Table-2: Physical Examination of the Synthesized Compounds

| S. No. | Compound code | R | M.P. (°C) | Yield% |
|---|---|---|---|---|
| 1 | 6a | H | 207-209 | 81 |
| 2 | 6b | 4-$CH_3$ | 213-215 | 78 |
| 3 | 6c | 4-F | 206-208 | 62 |
| 4 | 6d | 4-Cl | 228-230 | 60 |
| 5 | 6e | 4-Br | 244-246 | 58 |
| 6 | 6f | 4-$OCH_3$ | 219-221 | 42 |
| 7 | 6g | 2,4-di$CH_3$ | 210-212 | 61 |
| 8 | 6h | 3-Cl | 224-226 | 41 |
| 9 | 6i | 2-$OCH_3$ | 201-203 | 40 |
| 10 | 6j | 2-$CH_3$ | 199-201 | 46 |
| 11 | 6k | 3-$CH_3$ | 202-204 | 49 |
| 12 | 6l | 3-$OCH_3$ | 226-228 | 42 |
| 13 | 6m | 3-Cl,4-F | 222-224 | 43 |
| 14 | 6n | 2,4-diF | 186-188 | 51 |
| 15 | 6o | 3,4-diCl | 231-233 | 53 |

Compound 6e demonstrated moderate activity overall against S. pyogenes, P. aeruginosa, and Candida albicans bacterial strains. 6i shows moderate towards S. aureus, S. pyogenes, and A.niger.6j shows moderate activity towards all bacterial and fungal strains. 6n shows good activity towards gram-negative

bacterial strains and also fungal strain Candida albicans. 6o shows good activity towards gram-positive bacterial strains and also fungal strain Candida albicans. Conversely, all other compounds exhibited poor activity against both bacterial and fungal strains. These findings provide valuable insights into the potential effectiveness of the synthesized compounds against specific microbial targets. Chloramphenicol and ampicillin, a standard antibacterial drug, exhibited moderate activity in inhibiting microbial growth, while nystatin, employed for antifungal purposes, demonstrated similar moderate activity in impeding fungal proliferation.

## CONCLUSION

In conclusion, the investigation into combining imidazo[1,2-*a*]pyridine with 1,3,4-oxadiazole derivatives has revealed a highly versatile molecular framework achievable through diverse synthetic approaches. The antimicrobial efficacy of these compounds against various bacterial and fungal strains highlights their potential as potent antimicrobial agents, representing a significant advancement in combating infectious diseases. This research underscores the compound's potential to provide valuable insights and drive progress across multiple domains, with promising implications for human health and well-being.

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## CONFLICT OF INTEREST

The authors declare that they have no conflicts 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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