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Synthesis of some new pyridine-acetamide hybrids as potential antibacterial and antioxidant agents

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ABSTRACT The present study reports the synthesis of two new series of pyridine-acetamide hybrid derivatives, **6a-e** and **13a-e** and their evaluation of antibacterial and antioxidant properties. The synthesis involved acylating pyridinylamines with chloroacetyl chloride to form corresponding 2-chloro-*N*-pyrdinylacetamides, which were then reacted with various secondary amines to yield pyridine-acetamide hybrid derivatives. Compounds **6b** and **6e** showed the highest efficacy comparable to the standard antibiotic, azithromycin. Compound **6b** showed the highest antioxidant potential, with IC_{50} of 66.2 µg/mL and compound **13e** showed an IC_{50} of 54.7 µg/mL. The findings show the influence of functional groups on the biological efficacy of pyridine-acetamide hybrids and their potential for further development in antimicrobial and antioxidant applications.

KEYWORDS Pyridine, Acetamide, Secondary amines, Antibacterial and Antioxidant activities.

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INTRODUCTION

Antibacterial chemotherapy is an essential component of contemporary medicine, addressing numerous infectious disorders. The emergence of antimicrobial resistance (AMR) complicates treatment regimens and diminishes the efficacy of these medications, resulting in heightened morbidity and death. The excessive utilization of antibiotics, frequently resulting from improper suggesting and patient requests, intensifies resistance, hindering treatment efficacy and highlighting the critical necessity for novel antimicrobial medicines.[1] In addition to addressing AMR, oxidative stress is a critical contributor to numerous pathological illnesses, such as cancer, cardiovascular diseases, and neurological disorders. Reactive oxygen species (ROS), including superoxide anions and hydroxyl radicals, exhibit a dual function in biological systems. Excessive accumulation of free radicals may result in cellular damage and facilitate disease progression. Under physiological settings, organisms sustain a fragile equilibrium between ROS and their intrinsic antioxidant mechanisms. During pathogenic conditions, this equilibrium is disturbed, resulting in oxidative stress and tissue injury.[2]

The development of compounds with antibacterial and antioxidant properties is promising for tackling AMR and oxidative stress. These compounds target bacterial pathogens while mitigating oxidative damage, offering a multi-faceted approach to infection control and therapeutic intervention, especially beneficial in clinical settings where patients are susceptible to infections and oxidative stress. In this context, heterocyclic compounds are crucial in drug design due to their diverse biological activities and structural versatility. Pyridine, a common component in both natural and synthetic pharmaceuticals, is a valuable precursor in the synthesis of

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various heterocyclic compounds. These compounds have shown effectiveness in various biological activities, including antibacterial, anticancer, antiinflammatory, and inhibitory effects on ketohexokinase and nitric oxide synthase shown in **Figure 1**. Pyridine derivatives are often found in vitamins such as B_5 and B_6, playing important roles in various physiological processes. These compounds have extensive applications in medicine, including antibacterial, antituberculosis, antidepressant, nootropic effects, and in agriculture as effective fungicides, herbicides, and growth stimulants.[3-6]

Combining multiple pharmacophore fragments in a single molecule is crucial for designing biologically active compounds. α-Chloroacetamides are useful for synthesizing complex heterocyclic structures and have potential biological effects such as antimalarial, anticancer, antidiabetic, anti-tuberculosis, and anti-inflammatory effects. They also find applications in industrial sectors such as stabilizers, plastic releasing agents, films, surfactants, soldering flux, organic fibers, and dyes.[7] Morpholine, a key synthon in medicinal chemistry, is used in various therapeutic applications such as analgesics, antioxidants, and antidepressants. Piperazine, a major molecule in veterinary medicine, is used in human pharmacology. The piperidine moiety is crucial in drug synthesis, aiding in anticancer, antibacterial, analgesic, anti-inflammatory, and antipsychotic treatments.[8-10] The combination of morpholine, piperazine, and piperidine with pyridine acetamides increases their biological activity, enhancing their therapeutic potential and efficacy, making them promising candidates for further development in various medical applications.

The present study aims to synthesize new aminopyridine-acetamide derivatives, followed by an assessment of their potential antibacterial and antioxidant activities.

RESULTS AND DISCUSSION

Chemistry

Synthesis of two new series of pyridine-acetamide hybrid derivatives **6a-e** and **13a-e** was achieved successfully through multistep synthetic routes as depicted in **Schemes 1** and **2**.

Scheme 1 summarizes a series of multistep processes involved in the synthesis of the pyridine-acetamide analogs **6a-e**. Initially, the nitropyridine derivative was reduced to aminopyridine (**2**) using B_2pin_2 in MeOH and subsequent nucleophilic substitution of cyanide at bromine of compound **2** produced compound **3** by adopting the literature procedure. Further, the addition of chloroacetyl chloride produced an intermediate amide compound **4** and the amination of acetyl chloride with various secondary amines **(5a-e)** produced targeted pyridine-acetamide hybrids **6a-e.**

Scheme 2 delineates the multistep synthesis of pyridine-acetamide derivatives **13a-e.** Pyridine phthalimide (**8**) was synthesized from pyridine phthalic acid *through* cyclic condensation. Compound **8** on Hoffmann bromamide degradation afforded 3-aminopicolinic acid (**9**). The carboxylic acid **9** group was esterified with ethanol, yielding compound **10** as an amino ester. The addition of chloroacetyl chloride resulted an intermediate amide compound **11**. In the last step, amination on acetyl chloride of compound **11** with various secondary amines (**12a-e**) yielded targeted pyridine acetamide hybrids **13a-e**. The structures of all the synthesized compounds were confirmed through Fourier transform infrared (FTIR), ^{1}H NMR, ^{13}C NMR and high-resolution mass spectrometry (HRMS) spectral data.

Biology

Antibacterial activity

The present study evaluated the antibacterial efficacy of newly synthesized pyridine-acetamide hybrids **6a-e** and **13a-e** against Gram-positive and Gram-negative bacterial strains, specifically, *Bacillus subtilis*, *Bacillus megaterium*, *Escherichia coli*, and *Klebsiella pneumoniae*. As illustrated in **Table 1**, the diameter of inhibition zones (DIZ) exhibited considerable variation across tested drugs and strains, signifying antibacterial characteristics. Compound **6b** (morpholine) has shown the highest

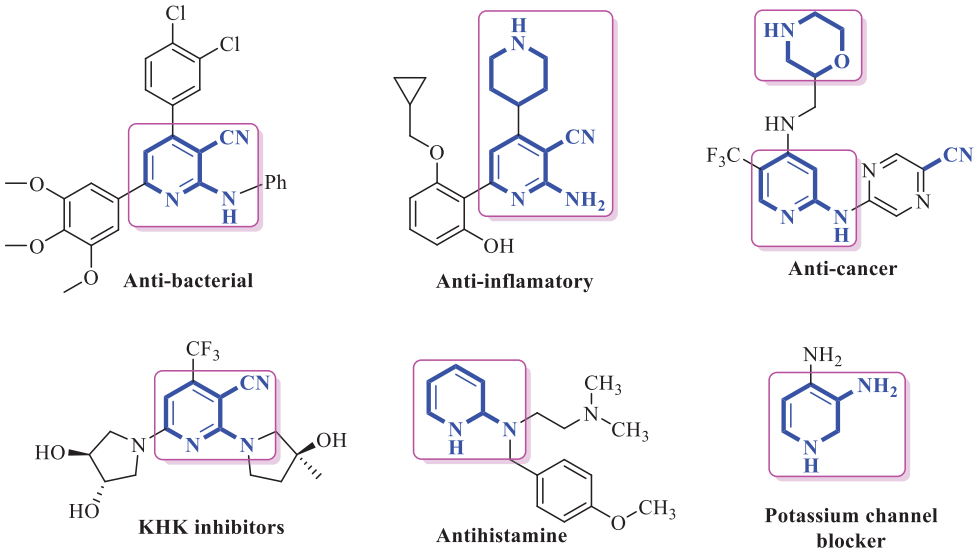
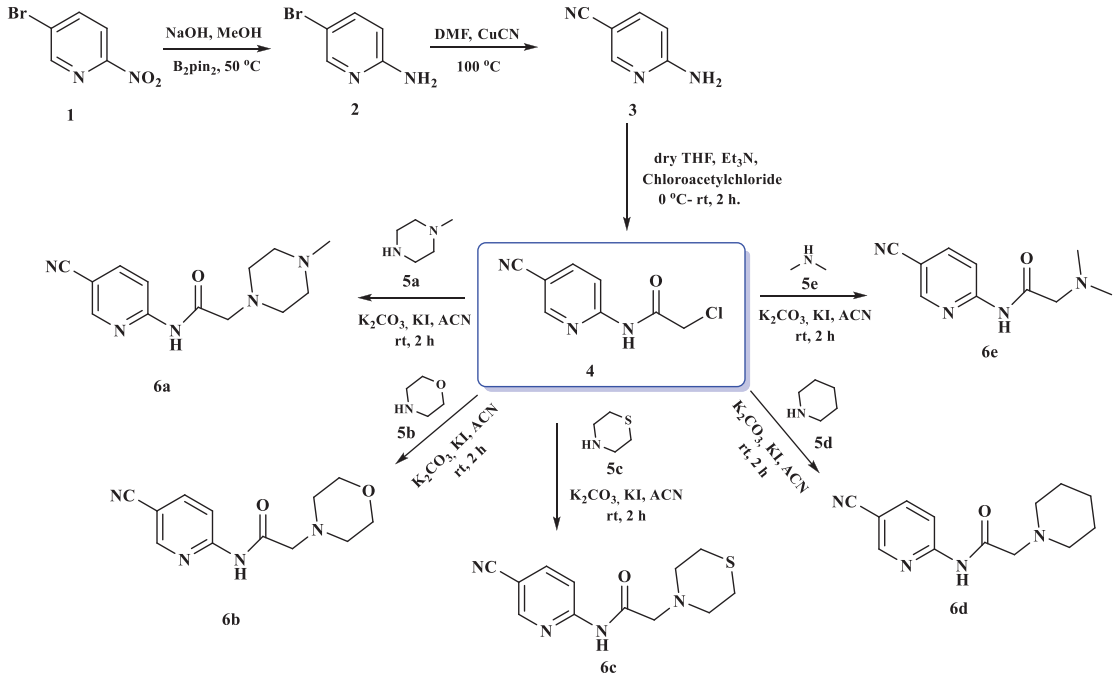
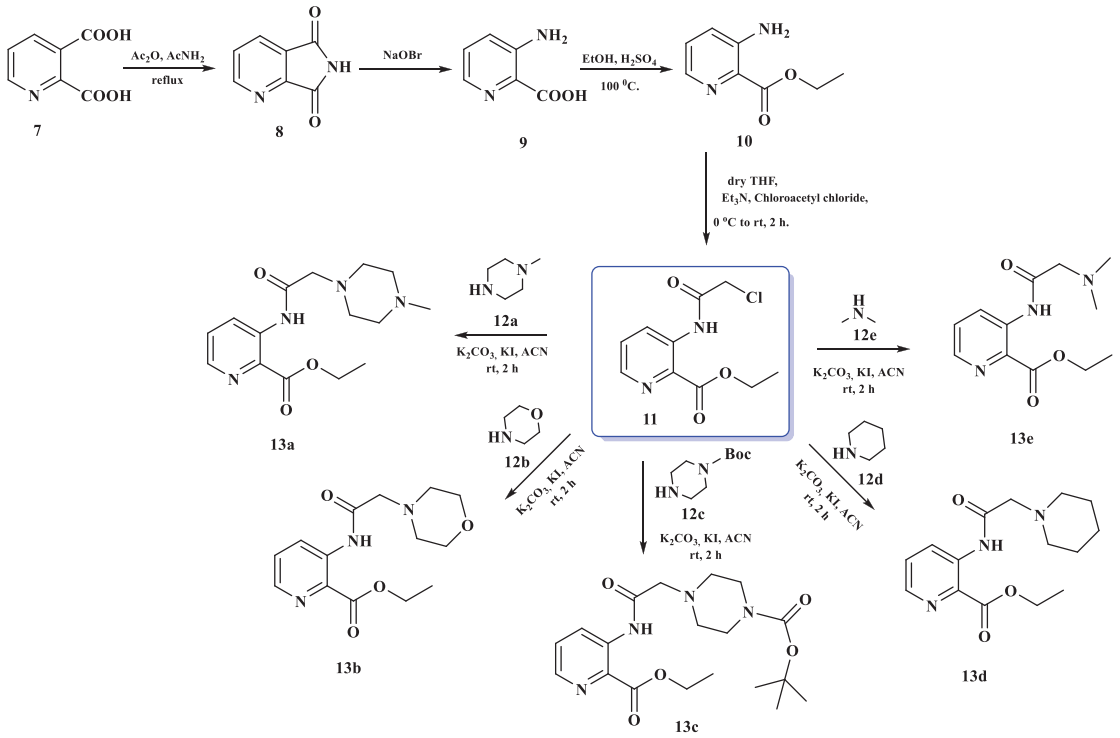


Figure 1: Biologically active drugs containing aminopyridine



Scheme 1: Synthesis of pyridine-acetamide hybrids 6a-e



Scheme 2: Synthesis of pyridine-acetamide hybrids 13a-e

efficacy, with DIZ values of 2.1 for *B. subtilis*, 1.9 for *B. megaterium*, 1.8 for *K. pneumoniae*, and 2.0 for *E. coli*. Compounds **6e** (dimethylamine) and **6c** (thiomorpholine) exhibited significant activity, with DIZ values of 2.1 and 1.8 respectively against *B. subtilis*. In contrast, compound **6a** (*N*-methyl piperazine) derivative has shown negligible effectiveness against all strains, indicating that structural alterations may be required for enhanced efficacy.

Compounds **13a-e** exhibited diverse antibacterial activities. Compound **13b** (morpholine) exhibited moderate inhibition, with DIZ values of 1.7 for *B. subtilis* and 1.6 for *B. megaterium*. Nevertheless, the N-Boc piperazine analogue **13c** diminished activity, suggesting that the protective N-Boc group may impede antibacterial efficacy. Moreover, the piperidine derivative **13d** and the dimethylamine analogue **13e** exhibited comparable inhibitory zones, indicating that

Table 1: Antibacterial activity of synthesized pyridine-acetamide hybrids 6a-e and 13a-e

	Diameter inhibition zone (in cm) at Conc. 200 µg/L			
S. No.	*Bacillus subtilis*	*Bacillus megaterium*	*Klebsiella pneumonia*	*Eschericha coli*
6a	1.1	0.8	1.0	0.9
6b	2.1	1.9	1.8	2.0
6c	1.8	1.7	1.6	1.8
6d	1.3	1.4	1.2	1.3
6e	2.1	2.5	1.9	1.7
Azithromycin	2.3	2.6	2.1	1.9
13a	0.6	0.7	1.1	0.5
13b	1.7	1.6	1.5	1.4
13c	1.3	1.0	1.2	0.7
13d	0.8	0.9	1.2	0.8
13e	1.8	1.6	1.7	1.6
Azithromycin	2.3	2.6	2.1	1.9

although they maintain some efficacy, they do not surpass their equivalents.

Antioxidant activity

The antioxidant activity of the newly synthesized pyridine-acetamide hybrids **6a-e** and **13a-e** was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The results are summarized in **Table 2**, reveals that compound **6b** (morpholine) showed the highest antioxidant activity with an IC_{50} value of 66.2, indicating as a potent free radical scavenger due to the presence of morpholine ring. In contrast, compound **6a** (*N*-methyl piperazine) exhibited the least antioxidant activity with an IC_{50} value of 127.8, suggesting that the piperazine structure comparatively least favour in radical scavenging among the tested derivatives. Other notable compounds **6e** (dimethylamine) and **6c** (thiomorpholine) showed potent activity with IC_{50} values of 82.7 and 89.7, respectively.

Compounds **13a-e** displayed varied antioxidant activities. Notably, compound **13b** (morpholine) showed IC_{50} value of 66.4. Conversely, compound **13d** (*N*-methyl piperazine) showed a weaker scavenging ability with an IC_{50} value of 119.9, suggesting that the piperidine moiety may not provide the same level of electron donation as the morpholine variant. The N-Boc piperazine analogue **13c** exhibited an IC_{50} value of 79.4. The dimethylamine analogue **13e** stood out with an IC_{50} value of 54.7, surpassing ascorbic acid, indicating its remarkable capability in scavenging DPPH radicals.

Table 2: DPPH free radical scavenging activity of synthesized pyridine-acetamide hybrids 6a-e and 13a-e

S. No.	IC_{50} values (µg/mL)
6a	127.8
6b	66.2
6c	89.7
6d	107.9
6e	82.7
Ascorbic acid	63.7
13a	134.7
13b	66.4
13c	79.4
13d	119.9
13e	54.7
Ascorbic acid	48.54

DPPH: 2,2-diphenyl-1-picrylhydrazyl

EXPERIMENTAL

Chemicals were obtained from vendors and used without purification. Reactions are monitored by TLC and synthesized compounds are purified using column chromatography. FTIR spectra were recorded on SHIMADZU-8400 spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker-500 spectrometers. Tetramethylsilane was used as an internal standard and the values are given in parts per million (δ, ppm) and hertz (J = Hz). HRMS were recorded using a Xevo TQD Quadrupole mass spectrometer. Melting points are determined in an open capillary method on GUNA melting point apparatus.

Scheme 1

The synthesis of 5-bromopyridin-2-amine (**2**) and 2-amino-5-cyanopyridine (**3**) was executed in strict adherence to the delineated experimental protocol.[11,12]

Synthesis of 5-bromopyridin-2-amine (2)

Literature m.p.: 136–141°C, observed m.p.: 135–139°C.

Synthesis of 2-amino-5-cyanopyridine (3)

Literature m.p.: 161–165°C, observed m.p.: 158–160°C.

Synthesis of 2-chloro-N-(5-cyanopyridin-2-yl)acetamide (4)

A solution of compound **3** (10 mmol) in 20 mL of THF was cooled to 0°C, chloroacetyl chloride (12 mmol) was added dropwise to the solution and continued the reaction for 2 h. The solution was concentrated under vacuum and the obtained crude was extracted with ethyl acetate (EtOAc) and water. Subsequently, the organic layer was washed with

satd. $NaHCO_3$ and brine followed by dried over with anhyd. Na_2SO_4 and compound **4** was purified by using column chromatography.

General procedure of synthesis of N-(5-cyanopyridin-2-yl)-2-(dimethylamino)acetamides (6a-e)

The compound **4 (**10 mmol), secondary amines **5a-e** (15 mmol), K_2CO_3 (20 mmol), KI (0.01 mmol), and 30 mL of acetonitrile (CH_3CN) were stirred for 2 h. After completing the reaction, ethyl acetate (3 × 15 mL) was used to extract the solution. Then, the organic layer was washed with satd. $NaHCO_3$ and brine followed by dried over with anhyd. Na_2SO_4. The solvent was removed by rotaevaporator and further purified the compounds **6a-e** using column chromatography.

N-(5-Cyanopyridin-2-yl)-2-(4-methylpiperazin-1-yl) acetamide (6a)

Off-white solid: Yield: 67%, m.p.: 189–191°C, FTIR (KBr Pellet, υ, cm^{-1}): 3230, 2974, 2922, 2855, 2821, 1689, 1576, 1498, 1367; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 9.76 (s, 1H), 8.23 (s, 1H), 8.54–8.52 (m, 1H), 7.96–7.94 (m, 1H), 2.97–2.94 (m, 4H), 3.21 (s, 2H), 2.46–2.38 (m, 4H), 2.24 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 169, 153, 151, 141, 116, 113, 105, 66, 62, 53; HRMS: Calc. for $C_{13}H_{17}N_5O$ 259.3130 found 260.4658 $[M+H]^+$.

N-(5-Cyanopyridin-2-yl)-2-morpholinoacetamide (6b)

Off-white solid: Yield: 62%, m.p.: 168–170°C; FTIR (KBr Pellet, υ, cm^{-1}): 3457, 3228, 2922, 2856, 2819, 1692, 1498, 1307; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 9.80 (s, 1H), 8.59 (s, 1H), 8.40–8.37 (m, 1H), 7.97–7.94 (m, 1H), 3.82–3.80 (m, 4H), 3.21 (s, 2H), 2.65–2.63 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 169, 153, 151, 141, 116, 113, 105, 66, 62, 53; HRMS: Calc. for $C_{12}H_{14}N_4O_2$ 246.2700 found 247.1194 $[M+H]^+$.

N-(5-Cyanopyridin-2yl)-2-thiopmorpholinoacetamide (6c)

Pale-brown solid: Yield: 70%; m.p.: 155–157°C; FTIR (KBr Pellet, υ, cm^{-1}): 3295, 2922, 2851, 2787, 2229, 1708, 1595; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 9.77 (s, 1H), 8.59 (s, 1H), 8.39–8.36 (m, 1H), 7.96–7.94 (m, 1H), 3.20 (s, 2H), 2.89–2.87 (m, 4H), 2.79–2.77 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 169, 153, 151, 141, 116, 113, 105, 62, 55, 27; HRMS: Calc. for $C_{12}H_{14}N_4OS$ 262.3310 found 263.0964 $[M+H]^+$.

N-(5-Cyanopyridin-2yl)-2-(piperidin-1y)acetamide (6d)

Off-white solid: Yield: 67%; m.p.:176–178°C; FTIR (KBr Pellet, υ, cm^{-1}): 3420, 3230, 2934, 2817, 1694, 1500, 1308; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 9.72 (s, 1H), 8.56 (s, 1H), 8.39–8.36 (m, 1H), 7.96–7.94 (m, 1H), 3.20 (s, 2H) 2.69–2.67 (m, 4H), 2.59–2.57 (m, 4H), 1.52–1.42 (m, 2H); ^{13}C NMR ((125 MHz, $CDCl_3$, δ ppm): 169, 153, 151, 141, 116, 113, 105, 62, 55, 27; HRMS: Calc. for $C_{13}H_{16}N_4O$ 244.1654 found 245.3324 $[M+H]^+$.

N-(5-Cyanopyridin-2yl)-(dimethylamino)acetamide (6e)

Brown solid: Yield: 73%; m.p.: 169–171°C; FTIR (KBr Pellet, υ, cm^{-1}): 3293, 2919, 2850, 2229, 1705, 1593, 1501, 1382; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 9.94 (s, 1H), 8.59 (s, 1H), 8.41–8.39 (m, 1H), 8.38–8.36 (m, 1H), 7.96–7.93 (m, 1H), 3.41 (s, 2H), 2.40 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 170, 153, 151, 141, 140, 116, 113, 108, 105, 63, 45; HRMS: Calc. for $C_{10}H_{12}N_4O$ 204.2330 found 205.1085 $[M+H]^+$.

Scheme 2

The synthesis of 5*H*-pyrrolo[3,4-b]pyridine-5,7(6*H*)-dione (**8**), 3-aminopicolinic acid (**9**), and ethyl-3-aminopicolinate (**10**) was performed using previously reported methodologies.[13]

Synthesis of 5H-pyrrolo[3,4-b]pyridine-5,7(6H)-dione (8)

Literature m.p.: 232–235°C, observed m.p.: 230–232°C.

Synthesis of 3-aminopicolinic acid (9)

Literature m.p.: 195–201°C, observed m.p.: 193–198°C.

Synthesis of ethyl-3-aminopicolinate (10)

Literature m.p.: 126–127°C, Observed m.p.: 124–126°C.

Synthesis of ethyl 3-(2-chloroacetamido)picolinate (11)

A solution of compound **10 (**10 mmol) in THF (20 mL) was cooled to 0°C, chloroacetyl chloride (12 mmol) was added dropwise to the solution and continued the reaction for 2 h. The solution was concentrated under vacuum and the obtained crude was extracted with EtOAc and water. Subsequently, the organic layer was washed with satd. $NaHCO_3$ and brine followed by dried over with anhyd. Na_2SO_4 and purified by using column chromatography.

General procedure of synthesis of ethyl 3-(2-(dimethylamino)acetamido)picolinates (13a-e)

The compound **11 (**10 mmol), secondary amines **12a-e (**15 mmol), K_2CO_3 (20 mmol), KI (0.01 mmol), and 30 mL of CH_3CN were stirred for 2 h. After completion of the reaction, ethyl acetate (3 × 15 mL) was used to extract the organic portion, which was then washed with water, brine and satd. $NaHCO_3$. Then, the organic layer was dried over with anhyd. Na_2SO_4 and purified the compounds **13a-e** using column chromatography.

Ethyl 3-(2-(4-methylpiperazin-1yl)acetamido)picolinate (13a)

Pale-brown solid: Yield: 65%; m.p.: 193–195°C; FTIR (KBr Pellet, υ, cm^{-1}): 3294, 2920, 2853, 2229, 1706, 1593, 1501, 1981; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 12.07 (s, 1H), 9.20–9.17 (m, 1H), 8.45–8.43 (m, 1H), 7.50–7.46 (m, 1H), 4.56–4.49 (m, 4H), 3.22 (s, 2H), 2.69–2.63 (d, 4H), 2.37 (s, 2H), 1.94 (s, 3H), 1.52–1.47 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 171, 166, 143, 138, 133, 128, 127, 62, 54, 53, 45; HRMS: Calc. for $C_{15}H_{22}N_4O_3$ 306.3601 found 307.1764 $[M+H]^+$.

Ethyl-3-(2-morpholinoacetamido)picolinate (13b)

Off-white solid: Yield: 79%; m.p.: 160–162°C; m.f.: $C_{14}H_{19}N_3O_4$ (*m.w.* 293.35 g/mol); FTIR (KBr Pellet, υ, cm^{-1}): 3289, 2953, 2858, 2812, 2229, 1705, 1594, 1497, 1380; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 12.16 (s, 1H), 9.20–9.17 (m, 1H), 8.46 (s, 1H), 7.50–7.48 (m, 1H), 4.55–4.50 (m, 2H), 3.90 (s, 4H), 3.21 (s, 2H), 2.67 (s, 4H), 1.50–1.47

(m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 170, 166, 143, 138, 132, 128, 127, 66, 62, 62, 53; HRMS: Calc. for $C_{14}H_{19}N_3O_4$ 293.3568 found 294.1458 $[M+H]^+$.

Tert-butyl-4-(2-((2-(ethoxycarbonyl)pyridine-3yl)amino-2-oxoethyl)piperaz-ine-1-carboxylate (13c)

White-solid: Yield: 69%; m.p.: 157–159°C; FTIR (KBr Pellet, υ, cm^{-1}); 3298, 2953, 2834, 2230, 1705, 1592, 1501, 1381; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 12.14 (s, 1H), 9.20–9.17 (m, 1H), 8.46 (s, 1H), 7.51–7.47 (m, 1H), 4.54–4.48 (m, 2H), 3.63 (s, 4H), 3.22 (s, 2H), 2.61 (s, 4H), 1.49 (m, 9H); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 170, 166, 154, 143, 138, 132, 128, 127, 62, 62, 53, 28, 14; HRMS: Calc. for $C_{19}H_{28}N_4O_5$ 392.4589 found 393.2138 $[M+H]^+$.

Ethyl-3-(2-(piperidin-1-yl)acetamido)picolinate (13d)

Brown solid: Yield: 65%; m.p.: 196–198°C; m.f.: FTIR (KBr Pellet, υ, cm^{-1}); 3449, 2922, 2863, 1695, 1616, 1500, 1457, 1371; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 12.16 (s, 1H), 9.20–9.17 (m, 1H), 8.46 (s, 1H), 7.50–7.48 (m, 1H), 4.55–4.50 (m, 2H), 3.90 (s, 4H), 3.21 (s, 2H), 2.67 (s, 4H), 1.50–1.47 (m, 2H), 1.36 (t, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 172, 168, 143, 138, 132, 128, 127, 66, 62, 62, 53; HRMS: Calc. for $C_{15}H_{21}N_3O_3$ 290.3576 found 291.4509 $[M+H]^+$.

Ethyl-3-(2-(dimethylamino)acetamido)picolinate (13e)

Off-white solid: Yield: 61%; m.p.: 156–158°C; FTIR (KBr Pellet, υ, cm^{-1}); 3238, 2980, 2833, 2783, 1693, 1577, 1499, 1411, 1307; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 12.16 (s, 1H), 9.20–9.17 (m, 1H), 8.46 (s, 1H), 7.50–7.48 (m, 1H), 4.55–4.50 (m, 2H), 3.21 (s, 2H), 2.58 (s, 6H), 1.50–1.47 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 170, 166, 143, 138, 132, 128, 127, 66, 62, 62, 53; HRMS: Calc. for $C_{12}H_{17}N_3O_3$ 250.2814 found 251.3958 $[M+H]^+$.

Biological assays

Gram-positive bacteria, *B. subtilis* (ATCC 6633), *B. megaterium* (ATCC 14581), and Gram-negative bacteria, *K. pneumoniae* (ATCC BAA-2146), *Eschericha coli* (*ATCC 25922*) were obtained from IMTECH and cultured in nutrient broth at 37°C for 24 h. Himedia Nutrient Broth, Nutrient Agar, DMSO, and plant culture tested were purchased from Himedia. All other chemicals used for the study were of analytical grade.

Antibacterial activity

The antibacterial activity of the pyridine-acetamide hybrids **6a-e** and **13a-e** were was evaluated using the agar diffusion method on nutrient agar plates against Bacterial strains, *B. subtilis*, *B. megaterium*, *E. coli*, and *K. pneumoniae*. Compounds were applied at a concentration of 5 μL on sterile filter paper discs, with azithromycin as standard. Nutrient Agar was prepared by dissolving 3.25 g in distilled water and sterilizing in an autoclave. The medium was then poured into pre-sterilized 90 mm petri dishes, and bacterial suspensions were prepared. The plates were inoculated with 100 μL of the bacterial suspension (approximately 10^4–10^6 colony-forming units/μL) and dried for 1 h. The plates were incubated at 37°C for 24 h, and visible inhibition zones around the discs indicated antibacterial activity. The DIZ was recorded, and experiments were performed in triplicate for reproducibility. The results are summarized in **Table 1** and showed significant antibacterial activity against the tested strains, highlighting the effectiveness of the compounds.[14,15]

Antioxidant assay: DPPH radical scavenging activity

The antioxidant activity of synthesized compounds was assessed using the DPPH radical scavenging assay. A 0.004% methanol solution of DPPH was prepared as the stable radical reagent. In each assay, 4 mL of this DPPH solution was mixed with 1 mL of test compounds, dissolved in methanol at varying concentrations of 25, 50, 75, and 100 µg/mL. The absorbance of the resulting solutions was measured at 517 nm using a ultraviolet-Visible spectrophotometer, with a blank solution serving as a control. The percentage of DPPH inhibition (I%) was calculated using the following formula.

$$I\% = \frac{A\,control - A\,sample}{A\,control} \times 100$$

Where A sample is the absorbance of the test compound and A control is the absorbance of the control reaction (including all reagents but the test compound). Testing was done in triplicate. For each compound, IC_{50} was calculated, along with the standard compound ascorbic acid, and the results are summarized in **Table 2**. The IC_{50} values for compounds and standard ascorbic acid were determined by graphing concentration versus percent of scavenging activity. The concentration of a substance needed to scavenge 50% of DPPH free radicals is known as the IC_{50} value.[16,17]

CONCLUSION

The study demonstrated that compound **6b** (morpholine) exhibited the highest antibacterial activity and antioxidant potential, with an IC_{50} value of 66.2 µg/mL, comparable to ascorbic acid. The dimethylamine derivative **13e** also showed promising antioxidant properties, surpassing ascorbic acid. These findings highlight the potential activity of pyridine-acetamide hybrids for antibacterial and antioxidant applications, suggesting their future use in pharmaceuticals. Future research focuses on optimizing these compounds and exploring their broader biological activities through *in vivo* studies to assess their pharmacokinetics and therapeutic potential, while further investigation into their molecular mechanisms could lead to more targeted drug development for oxidative stress-related diseases and infection control.

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