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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NEW IMIDAZOPYRIDINE DERIVATIVES BASED ON 2,3-DIAMINOPYRIDINE

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Abstract: The present research work focuses on the synthesis and evaluation of antibacterial activity of newly developed imidazopyridine analogues arising from 2,3-aminopyridine substructure. Three new imidazopyridine derivatives have been synthesized from the reaction of 2,3-aminopyridine with ibuprofen, naproxen and etodolac employing HCl as both the solvent and the catalyst. The resulting compounds were identified by using (FTIR) and (^{1}H-NMR). The antibacterial activity of these newly synthesized compounds was carried out against a series of bacteria: *Escherichia coli, Klebsiella pneumoniae*, *Staphylococcus aureus* & *Streptococcus mutans* by microdilution method of 96 well microplates. These findings revealed that all synthesized compounds possessed pronounced antimicrobial effects against all the bacterial strains studied. The highest antimicrobial activity was registered for compound 1-((-[(1*H*-imidazo [4,5-*b*]pyridin-2-yl)methyl)-]-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indole that was most effective toward *Escherichia coli*. Based on these discoveries, it can be assumed that the newly imidazopyridine derivatives synthesized might be of interest for further study, targeting potential antibacterial activity.

***Keywords*:** Imidazopyridine, 2,3-Diaminopyridine, Antibacterial Activity, Synthesis

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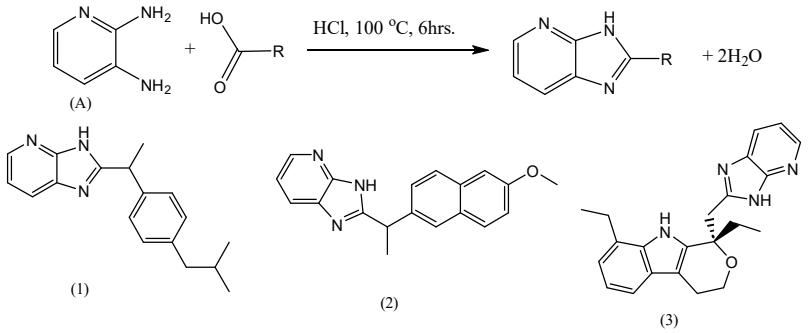
Introduction

Substituted pyridines are versatile scaffolds that have various biological activities: antipyretic/analgesic, antiprotozoal, antibacterial, antitumor, antifungal, anti-inflammatory, and antiapoptotic.[1] In these compounds, imidazopyridine and its derivatives are the most important heterocyclic compounds that have attracted great attention in natural products, medicinal chemistry and synthetic chemistry. Due to the specific structure of the imidazopyridine ring, it can bind to different enzymes and receptors through weak interactions, switching on a number of biological and pharmacological effects.[2] Imidazopyridines have been found in many investigations to display valuable therapeutic effects such as antimicrobial,[3] and antibacterial,[4] antifungals,[5] anticandidal,[6,7] kinase inhibitors,[8,9] antitubercular agents,[10] anti-plasmodial agents[11] and anticancer agents,[12] In addition, research on synthesized imidazopyridine derivatives has shown that they possess anti-inflammatory, antiviral, antiosteoporosis, anti-parasitic and antihypertensive properties.[13,14]

Results and Discussion

Synthesis

This study includes the synthesis of new imidazopyridine derivatives through the reaction of 2,3-diaminopyridine (A) with different carboxylic acids containing drugs NSAID (Naproxen, Ibuprofen and Etodolac) in the presence of HCl as solvent and catalyst as shown in Scheme 1. Thus, 2,3-diamino pyridine **A** (0.01 mole) was refluxed with etodolac, ibuprofen or naproxen (0.01 mole each) in the presence of 4N hydrochloric acid (HCl) for 6 hours at 100 °C to give the compounds **1-3**. Their structures were confirmed by FTIR and ^{1}H-NMR spectroscopy.



Scheme 1. Synthesis of imidazopyridine derivatives.

Antimicrobial activity of compounds 1-3 against E. coli

This study assessed the antibacterial efficacy of three novel compounds against *Escherichia coli* (*E. coli*), a prevalent gram-negative bacterium, as it is shown in **Figure 1**. According to the MIC, compound **1** exhibited a limited inhibitory effect on *E. coli.* (MIC = 906.1 µg/mL) since the highest MIC, the lowest the antimicrobial activity. However, compound **2** showed a significantly lower MIC value of 171.9 µg/mL indicating a stronger antimicrobial activity compared to compound **1**. On the other hand, compound **3** demonstrated remarkably low MIC value as the most potent antimicrobial activity with an MIC of 9.091 µg/mL indicating a highly effective antimicrobial agent against *E. coli.*

	Compound-1	Compound-2	Compound-3
MIC	906.1	171.9	9.091

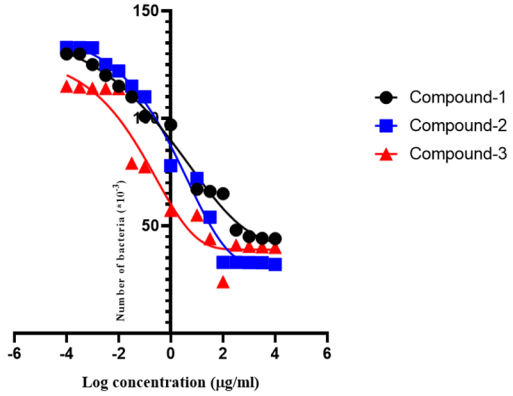


Figure 1. Antimicrobial activity of compounds **1**, **2** and **3** against *E. coli*.

Antimicrobial activity of compounds 1-3 against Klebsiella pneumoniae

In contrast to findings showed in the previous section, the antimicrobial activities of the three compounds evaluated against *Klebsiella pneumoniae*, another gram-negative bacterium showed that compound **1** exhibited the highest inhibitory effect on *Klebsiella pneumoniae* (MIC = 20.3 µg/mL) **Figure 2**. However, compound **2** showed a significantly higher MIC value of 581.6 µg/mL indicating a weaker antimicrobial activity compared to compound **1**. On the other hand, compound **3** demonstrated remarkably high MIC value as the least potent antimicrobial activity with an MIC of 994.2 µg/mL.

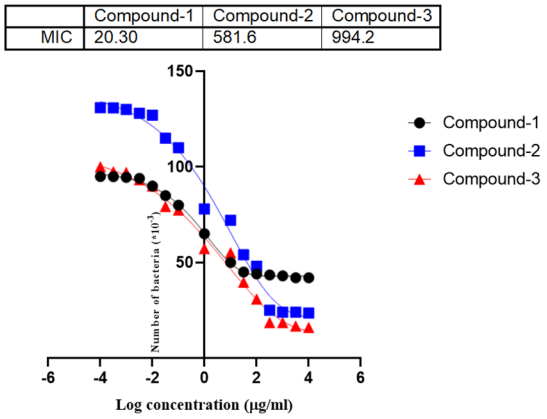


Figure 2. Antimicrobial activity of compounds **1**, **2** and **3** against *Klebsiella pneumoniae.*

Antimicrobial activity of compounds 1-3 against Staphylococcus aureus

When tested against *Staphylococcus aureus*, a common gram-positive bacterium compounds **1**, **2** and **3** displayed MIC of 843.2, 1270 and 566.4 µg/mL, repectively **Figure 3**. Although compound **3** showed the lowest MIC against *Staphylococcus aureus* which means it has the highest antimicrobial activity among the three compound against this species, it still represents a high MIC.

	Compound-1	Compound-2	Compound-3
MIC	843.2	1270	566.4

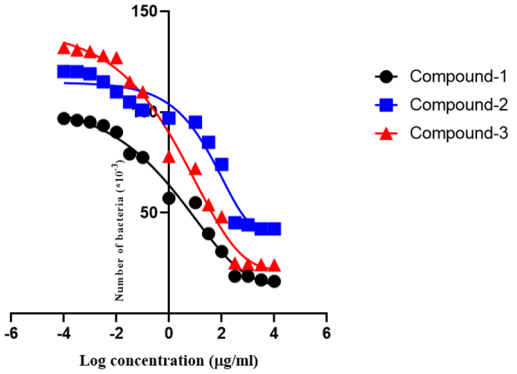


Figure 3. Antimicrobial activity of compounds **1**, **2**, and **3** against *Staphylococcus aureus.*

Antimicrobial activity of compounds 1-3 against Streptococcus mutans

The three compound were also tested against *Streptococcus mutans*, another gram-positive bacterium. Compounds **1**, **2** and **3** showed MIC of 1219, 581.6 and 843.2 µg/mL, respectively **Figure 4**. Again, although compound **2** showed the lowest MIC against *Streptococcus mutans* which means it has the highest antimicrobial activity among the three compound against this species, the value still represents a high MIC.

	Compound-1	Compound-2	Compound-3
MIC	1219	581.6	843.2

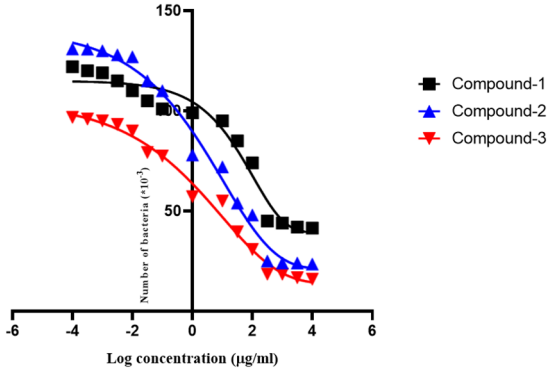


Figure 4. Antimicrobial activity of compounds **1**, **2**, and **3** against *Streptococcus mutans.*

Experimental

All reactions were performed in oven dried glassware. The solvents and reagents used in the commercial grade were distilled before use. Thin layer chromatography (TLC) on silica gel GF 254 plates was used in determining the reaction progress. Melting points were obtained using an electrothermal melting point apparatus in capillary tubes with the capillary being open at both ends. ^{1}H-NMR experiments were recorded at ambient temperature using Bruker Avance 400 MHz NMR spectrometer. Preparation of samples involved the dissolving of the compounds in deuterated chloroform ($CDCl_3$) with (TMS) as the internal reference. Chemical shifts are expressed in (ppm) abbreviated as δ.

General Procedure for Synthesis of Compounds 1-3[15]

2,3-Diamino pyridine (A) (0.01 mole) was refluxed with etodolac, ibuprofen or naproxen (0.01 mole each) in the presence of 4N hydrochloric acid (HCl) for 6 hours at 100 °C, with a stirring speed of 320 rpm using a reflux condenser. The reaction progress was monitored by TLC, employing a chloroform: methanol (9:1) as a mobile phase. After completion, the mixture was gradually neutralized with 10% NaOH (w/v) until the pH was shifted to 8-9. The resulted solution was further chilled in an ice bath for 5 minutes to facilitate precipitation. The precipitate was obtained through filtration and subsequently dried.

Synthesis and spectral data of 2-[1-(4-isobutylphenyl)ethyl]-1H--imidazo[4,5-b] pyridine 1 (figure 5)

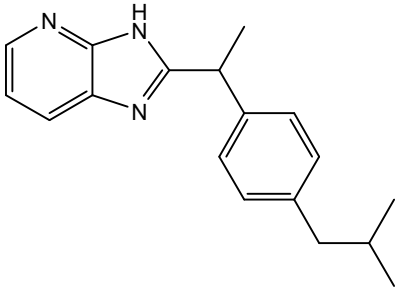


Figure 5. Structure of compound **1** derived from Ibuprofen.

It was obtained using the general procedure. A conversion was observed after 6 hours. The resulting product is a white powder, with a melting point of 98°C, yield 71 %. *FTIR (KBr)*, ν(cm^{-1}): N-H (3512), C-H aliphatic (2970-2850), C-H aromatic (3070), C=C (1590, 1454), C=N (1665), C-N (1340-1000). *^{1}H NMR* (400 MHz, $CDCl_3$) δ(ppm): 12.90 (1H, s, N-H), 8.32 (1H, d, *J* = 7.5 Hz, C-$H_{pyridine}$), 7.84 (1H, d, *J* = 7.5 Hz, C-$H_{pyridine}$), 7.15 (1H, t, *J* = 7.5 Hz, C-$H_{pyridine}$), 7.18, (2H, d , *J* =7.5, Hz, C-$H_{benzene}$), 7.04 (2H, d , *J* =7.5, Hz, C-$H_{benzene}$), 4.16 (1H, q, *J* = 6.8 Hz, C-H), 2.43 (2H, d, *J* = 7.0 Hz. CH_2), 1.82 (1H, heptet, *J* = 7.0 Hz, CH), 1.62 (3H, d, *J* = 6.8 Hz CH_3), 0.87 (6H, d, *J* = 6.8 Hz, 2 x CH_3).

Synthesis and spectral data of (S)-2-[1-(6-methoxynaphthalen-2--yl)ethyl]-1H-imidazo[4,5-b]pyridine 2 *(figure 6)*

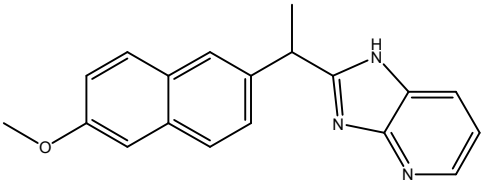


Figure 6. Structure of compound **2** derived from Naproxen.

It was obtained using the general procedure. A conversion was observed after 6 hours. The resulting product is a faint pink powder, with a melting point of 95°C, yield 65%. *FTIR (KBr)*, ν(cm^{-1}): N-H (3490), C-H aliphatic (2990-2860), C-H aromatic (3080), C=C (1587, 1470), C=N (1670), C-O (1290-990). *^{1}H NMR* (400 MHz, $CDCl_3$), δ(ppm): 12.80 (1H, s, N-H), 8.30 (1H, d, *J* = 7.5 Hz, C-$H_{pyridine}$) 7.83 (1H, d, *J* = 7.5 Hz, C-$H_{pyridine}$), 7.78 (1H, d, *J* = 7.5 Hz, C-$H_{benzene}$), 7.73 (1H, d, *J* = 7.5 Hz, C-$H_{benzene}$), 7.48 (1H, d, *J* = 1.5 Hz, C-$H_{benzene}$), 7.34 (1H, d, *J* = 1.5 Hz, C-$H_{benzene}$), 7.23 (1H, d , *J* = 1.5 Hz, C-$H_{benzene}$), 7.15 (1H, t, *J* = 7.5 Hz, C-$H_{pyridine}$), 7.06 (1H, d, *J* = 7.5 Hz, C-$H_{benzene}$), 4.15 (1H, q, *J* = 6.8 Hz, CH), 3.81 (3H, s, CH_3), 1.68 (3H, d, *J* = 6.8 Hz, CH_3).

Synthesis and spectral data of 1-[(1H-imidazo[4,5-b]pyridin-2--yl)methyl]-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indole3 *(figure 7)*

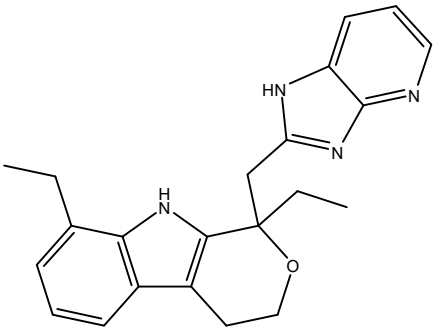


Figure 7. Structure of compound **3** derived from Etodolac.

It was obtained using the general procedure. A conversion was observed after 6 hours. The resulting product is a brown powder, with a melting point of 92°C, yield 59%. *FTIR (KBr)*, ν(cm^{-1}): N-H (3515-3490), C-H aliphatic (2997-2890), C-H aromatic (3087), C=C (1589,1480), C=N (1680), C-O (1300-995). *^1H NMR* (400 MHz, $CDCl_3$) δ (ppm): 12.88 (1H, s, N-H), 11.67 (1H, s, N-H_{indole}), 8.32 (1H, d, *J* = 7.5 Hz, C-$H_{pyridine}$), 7.84 (1H, d, *J* = 7.5 Hz, C-H $_{pyridine}$), 7.17 (1H, t, *J* = 7.5, Hz, C-H_{indole}), 7.15 (1H, t, *J* = 7.5 Hz, C-$H_{pyridine}$), 7.09 (1H, d, *J* = 7.5, Hz, C-H_{indole}), 7.07 (1H, d, *J* = 7.5, Hz, C- H_{indole}), 3.69 (2H, m, CH_2-O), 3.02 (2H, s, CH_2), 2.71 (2H, q, *J* = 8.0 Hz, C$\underline{H_2}$-CH_3), 2.62 (2H, t, *J* = 7.0 Hz, C$\underline{H_2}$-CH_2-O), 1.67 (2H, q, *J* = 8.0 Hz, C$\underline{H_2}$-CH_3), 1.18 (3H, t, *J* = 8.0 Hz, C$\underline{H_3}$-CH_2), 0.89 (3H, t, *J* = 8.0 Hz, C$\underline{H_3}$-CH_2).

Experimental procedure for antimicrobial testing preparation of bacterial cultures*:* Bacterial strains were cultured in Mueller-Hinton broth, a nutrient-rich medium suitable for supporting bacterial growth.

Microdilution method setup*:* The antimicrobial activity of the synthesized compounds was tested using the microdilution method in 96-well

microplates. This approach allows for precise measurement of bacterial inhibition at various concentrations of the test compounds.

Preparation of test compounds*:* Serial dilutions of the synthesized compounds were prepared. The concentration range of these dilutions spanned from 0.5 µg/mL to 1000 µg/mL.

Incubation*:* The microplates containing the bacterial cultures and test compounds were incubated for 24 hours at 37°C to allow interaction between the bacteria and the compounds.

Determination of minimum inhibitory concentration (MIC)*:* MIC was identified as the lowest concentration of the test compound at which no visible bacterial growth was observed after the incubation period. The absence of visible turbidity in the wells indicated effective bacterial inhibition.

Conclusions

The research paper focused on the synthesis of three novel imidazopyridine compounds derived from 2,3-diaminopyridine. The derivatives were tested for their antibacterial activity against several bacterial strains. The findings showed that all the synthesized compounds exhibited notable antimicrobial effects, with compound **3** demonstrating the strongest activity, particularly against *Escherichia coli*. This study suggests that these new derivatives are promising candidates for further investigation into their potential as antibacterial agents. The results highlight the importance of these compounds for future therapeutic applications.

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