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Synthesis and Antimicrobial Activity of Chalcone Derivatives Containing Imidazo[1,2-a]pyridine Nucleus

Déto U. J–P N'GUESSAN[1], Songuigama COULIBALY[1], Mahama OUATTARA[1]*, Denise A. B. GUINA[3], Drissa SISSOUMA[2], Nanga Y. ZINZENDORF[3]

[1]Département de Chimie Thérapeutique et Chimie Organique, UFR Sciences Pharmaceutiques, Université Félix Houphouët-Boigny, 01 BP V34 Abidjan, Côte d'Ivoire
[2]Laboratoire de Chimie Organique Structurale, UFR SSMT, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan, Côte d'Ivoire
[3]Département de bactériologie - virologie, UFR Sciences Pharmaceutiques, Université Félix Houphouët-Boigny, 01 BP V34 Abidjan, Côte d'Ivoire

Abstract A series of imidazo[1,2-*a*]pyridinyl-chalcones (**5a–k**) have been synthesized by claisen chimdt condensation between 3-acetyl-2-methylimidazo[1,2-*a*]pyridine (**3**) and various benzaldehydes (**4a–k**). The structures of all the synthesized compounds have been confirmed by ^{1}H NMR, ^{13}C NMR and mass spectral data. The antibacterial activities of these synthesized compounds were tested against sensible and clinical strains of Gram-positive and Gram-negative bacteria, using commercially available antibiotics, Azithromycin, Cefotaxim and Ciprofloxacin as reference drugs. The antibiogram showed that the clinical strains used were either intermediate or resistant to the antibiotics tested. The range of minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) was 200 – 3.125 µg /mL against ATCC and clinical strains. With MICs values of 6.25 µg / mL and 3.125µg / mL, compound **5h** was found to be the most potent of all, respectively against clinical and reference strains of Gram-positive bacterium (*S. aureus)*. Structure activity relationship studies revealed that the presence of electron-withdrawing substituent or of bulkier group at phenyl ring is favorable to low bactericidal activities and a shrinking of the spectrum of action toward *P. aeruginosa*. On the contrary, the presence of an electron donor substituent on the same nucleus is closely related to the broadening of the spectrum of activity against Gram-positive and Gram-negative bacteria, including the drug-resistant species of *E. coli.*

Keyword: imidazo[1,2-*a*]pyridinchalcones, *S. aureus, P. aeruginosa, E. coli*

1. Introduction

After years of misuse of antibiotics, in recent decades several pathogenic bacteria have acquired artful mechanisms of resistance to challenge the efficacy of many currently available antibiotics [1]. In fact, *Pseudomonas aeruginosa* has become a major concern of healthcare professionals in a hospital setting [2]. In addition, the constant isolation of *Escherichia coli* strains resistant to several classes of antibiotics such as betalactamins and fluoroquinolones [3], threatens the effectiveness of treatment of an ever-expanding range of infections. Consequently, there is a dire need to develop novel antibacterial agents with better bactericidal properties, thereby overcoming such resistance [4]. Thus, attention has been paid to small heterocyclic molecules especially to the derivatives of imidazo[1,2-*a*]pyridine which are biologically important compounds and are useful antimicrobial and anthelminthic agents [5-7]. In previous work on imidazo[1,2-*a*]pyridine, it was highlighted that imidazo[1,2-*a*]pyridinyl-chalcones were

paradoxically more effective on resistant strain than on the sensitive strain of *E. faecalis* [7]. As a continuation of our project on searching for new antibacterial molecules the herein work was carried out to extend antimicrobial evaluation not only to other Gram-positive bacteria but also Gram-negative bacteria. Specifically, we envisaged here to screen the antibacterial activities of a series of imidazopyridinyl-chalcone, then to determine their minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) against sensitive and clinical strains of *E. Coli*, *P. Aeriginosa* and *S aureus.*

2. Materials and methods

2.1. Chemistry

For all compounds, the ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Brucker Avance 300. The tetramethylsilane (TMS) is used as internal standard and chemical shift values were recorded in ppm on the δ scale. The description of the NMR spectra uses the following symbols: S = singlet; d = doublet; dd = doublet of doublets; t = triplet; q = quadruplet; quint = quintet; m = multiplet. Mass spectra were recorded on a JEOL JMS DX300 spectrometer in ESI (electrospray ionization / quadrupole). All melting points (mp) were determined using a Kofler bench and are uncorrected. The thin layer chromatographies (TLC) were performed on silica plates Macherey-Nagel Sil G/UV254 or aluminaMacherey-Nagel ALOX N/UV254. Revealation of products was performed under UV light source (254 nm). The solvents and reagents including benzaldehydes used, were purchased from Acros Organics (France) or Aldrich (France). Melting points were determined on a Köfler bench. Azithromycin (AZM), Cefotaxim (CTX) and Ciprofloxacin (CIP) standard antibiotic discs were purchased from SIGMA Chimical Co. (USA).

2.1.1. General method for synthesis of 3-acetyl-2-methylimidazo[1,2-*a*]pyridine (3) [8].

To a solution of 2-aminopyridine (4.8 g, 0,042 mol) in absolute ethanol (50 mL) was added 1.05 equivalents of 3-chloro pentan-2,4-dione (14.4 g, 0,107 mol). The reaction mixture was stirred under reflux for 6 hours. After evaporating the solvent under vacuum, 125 mL of water was added then neutralized with a saturated solution of sodium bicarbonate. The resulting solution was stored in a refrigerator for 5 hours. The precipitate obtained is filtered and recrystallized from water to afford, 3.8 g of **3** as white solid.

M.p. : 155-159°C ; yield 52%. ^{1}H NMR (300MHz, DMSO-d6) δ: 9.53 (d, 1H, *J* = 5.8 Hz, H_5); 7.63 (m, 1H, H_8); 7.54 (m, 3H, H_7); 7.12 (m, 1H, H_6); 2.65 (s, 3H, C<u>H</u>$_3$); 2.51 (s, 3H, CO-C<u>H</u>$_3$). ^{13}C NMR (75MHz, DMSO-d6) δ: 187.5 (<u>C</u>=O), 152.6 (C_2), 146.1 (C_{8a}), 129.3 (C_2), 126.2 (C_7), 121.1 (C_6), 116.2 (C_5), 114.8 (C_3), 30.1 (CO-<u>C</u>H$_3$), 17.9 (<u>C</u>H$_3$). MS: for $C_{10}H_{10}N_2O$, calcd. 174.08 (M^++1), found 175.08.

2.1.2. General procedures for synthesis of imidazo[1,2-*a*]pyridinyl-chalcones (5a-k)

To a stirred solution of **3** (1.5 g, 8.62 mmol) in an ethanolic solution of sodium hydroxide (64.6 mmol of NaOH in 40 mL of ethanol), was added the appropriate benzaldehyde (10.35 mmol, 1.2 mol equivalent). The mixture was kept under stirring at room temperature for 5 h. After neutralization with a CH_3COOH (30%), the resulting precipitate was dried. The corresponding solid products **5a-k** were further purified by recrystallization with a yield of 30 to 85%.

1-(2-methylimidazo[1,2-*a*]pyridin-3-yl)-3-phenylprop-2-en-1-one (5a)

Pale yellow solid, m.p.: 155-159°C, yield 80%. ^{1}H NMR (300MHz, DMSO-d6) δ: 9.78 (d, 1H, *J* = 5.8 Hz, H_5); 7.80 (d, 1H, *J* = 15.6 Hz, CH=C<u>H</u>); 7.60 - 7.68 (m, 3H, Ph-2,6 and H_8); 7.50 (d, 1H, *J* = 15.6 Hz, C<u>H</u>=CH); 7.46 (m, 1H, H_7); 7.15 - 7.44 (m, 3H, Ph-3,5 and H_6); 7.06 (m, 1H, Ph-4); 2.89 (s, 3H, C<u>H</u>$_3$). ^{13}C NMR (75MHz, DMSO-d6) δ: 179.5 (<u>C</u>=O), 152.0 (C_{8a}), 146.5 (C_2), 141.6 (CH=<u>C</u>H), 132.0 (C_5), 128.4 (Ph-1), 125.4 (C_7), 124.2 (Ph-2,6), 122.5 (Ph-3,5), 121.7 (<u>C</u>H=CH), 120.1 (C_8), 117.0 (C_6), 114.7 (C_3), 18.4 (<u>C</u>H$_3$). One carbon is missing. MS: for $C_{17}H_{14}N_2O$, calcd. 262.11 (M^++1), found 263.11. Recrystallization from MeCN / H_2O (1: 1).

1- (2-methylimidazo[1,2-*a*]pyridin-3-yl)-3-p-tolylprop-2-en-1-one (5b)

Yellow pale Solid, m.p.: 151-153°C, yield 68% : ^{1}H NMR (300MHz, DMSO-d6) δ: 9.66 (d, 1H, *J* = 4.5 Hz, H_5); 7.75 (d, 1H, *J* = 16 Hz, CH=C<u>H</u>); 7.52-7.69 (m, 4H, CH_3-Ph-2,6, H_7 and H_8);; 7.48 (d, 1H, *J* = 16 Hz, C<u>H</u>=CH);

7.09-7.30 (m, 3H, CH_3-Ph-3,5 and H_6); 2.89 (s, 3H, C$\underline{H}_3$), 2.33 (s, 3H, C$\underline{H}_3$-Ph). ^{13}C NMR (75MHz, DMSO-d6) δ: 179,4 (C=O), 152.0 (C_{8a}), 146.5 (C_2), 141.6 (CH=CH), 140.4 (CH_3-Ph-4), 131.9 (C_5), 128.4 (CH_3-Ph-1), 125.4 (C_7), 124.9 (CH_3-Ph-3,5), 122.5 (CH_3-Ph-2,6), 121,7 (CH=CH), 117,1 (C_6), 114,7 (C_3), 21,1 (C$\underline{H}_3$-Ph), 18,1 (C$\underline{H}_3$). One carbon is missing. MS: for $C_{18}H_{16}N_2O$, calcd. 276.13 (M^+), found 276.14. Recrystallization from cyclohexane.

3- (2-hydroxyphenyl)-1-(2-methylimidazo[1,2-*a*]pyridin-3-yl) prop-2-en-1-one (5c)

Yellow solid, m.p. > 260°C, yield 56%. ^{1}H NMR (300MHz, DMSO-d6) δ: 9.78 (d, 1H, *J* = 5.8 Hz, H_5); 7.80 (d, 1H, *J* = 15.6 Hz, CH=C$\underline{H}$); 7.60 (m, 1H, H_8); 7.53 (m, 1H, H_7); 7.38 (d, 1H, *J* = 15.6 Hz, C$\underline{H}$=CH); 7.29-7.33 (m, 3H, OH-Ph-4,5,6); 7.13 (1H, m, H_6), 6.86 (d, 1H, *J* = 8.4 Hz, OH-Ph-3); 2.78 (s, 3H, C$\underline{H}_3$), ^{13}C NMR (75MHz, DMSO-d6) δ: 179.5 (C=O), 162.1 (OH-Ph-2), 152.0 (C_{8a}), 146.7 (C_2), 141.6 (CH=CH), 131.1 (C_5), 129.2 (OH-Ph-1), 125.4 (C_7), 124.2 (OH-Ph-6), 121,8 (OH-Ph-4), 121.5 (OH-Ph-5), 120.9 (CH=CH), 120.0 (C_8), 118.4 (OH-Ph-3), 117.0 (C_6), 114.7 (C_3), 18.3 (C$\underline{H}_3$). One carbon is missing. MS: for $C_{17}H_{14}N_2O_2$ calcd. 278.11 (M^++1), found 279.11. Recrystallization from ethanol.

3-(3,4-dimethoxyphenyl)-1-(2-methylimidazo[1,2-*a*]pyridin-3-yl)prop-2-en-1-one (5d)

Yellow pale solid, yield 70%, m.p.: 169-171°C ^{1}H NMR (300MHz, DMSO-d_6) δ: 9.66 (d, 1H, *J* = 7.5 Hz, H_5) ; 7.66 (d, 1H, *J* = 15.6 Hz, CH=C$\underline{H}$); 7.50 (m, 1H, H_8); 7.42 (m, 1H, H_7) ; 7.33 (d, 1H, *J* = 15.4 Hz, C$\underline{H}$=CH) ; 7.15 (1H, m, H_6) ; 6.88 (d, 1H, *J* = 15.6 Hz, diOCH_3-Ph-6) ; 6.76 (s, 1H, diOCH_3-Ph-2); 6.64 (d, 1H, *J* = 8.4, diOCH_3-Ph-5), 3.84 (s, 3H, 3-OC$\underline{H}_3$-Ph), ; 3.81 (s, 3H, 4-OC$\underline{H}_3$-Ph), 2.81 (s, 3H, C$\underline{H}_3$). ^{13}C NMR (75MHz, DMSO-d_6) δ: 179.4 (C=O), 151.7 (diOCH_3-Ph-3) ; 150.9 (diOCH_3-Ph-4), 148.9 (C_{8a}), 147.9 (C_2), 141.9 (CH=CH), 129.3 (C_6), 128.3 (diOCH_3-Ph-1), 127.5 (C_8), 122.6 (OCH_3-Ph-6), 121.9 (CH=CH), 120.4 (C_5) ; 119.6 (diOCH_3-Ph-2) ; 118.4 (diOCH_3-Ph-5), 116.4 (C_7), 114.7 (C_3), 55.2 (3-O$\underline{C}H_3$-Ph), 55.1 (4-O$\underline{C}H_3$-Ph), 18.0 (C$\underline{H}_3$). MS: for $C_{19}H_{18}N_2O_3$, calcd. 322.13 (M^++1), found 323.13. Recrystallization from hexane / DCM (3:1).

3-(4-nitrophenyl)-1-(2- methylimidazo[1,2-*a*]pyridin -3-yl)prop-2-èn-1-one (**5e**)

Yellow solid, m.p. > 260°C; yield 80%.^{1}H NMR (300MHz, DMSO-d_6) δ: 9.68 (d, 1H, *J* = 6,9 Hz, H_5), 7.98 (d, 2H, *J* = 4,8 Hz, NO_2-Ph-3,5), 7.73 (d, 2H, *J* = 4,8 Hz, NO_2-Ph-2,6), 7.68 (d, 1H, *J* = 15.3 Hz, CH=C$\underline{H}$), 7.50 (d, 2H, *J* = 15.3 Hz, C$\underline{H}$=CH); 7.36 (m, 1H, H_8), 7.29 (m, 1H, H_7), 7.27 (m, 1H, H_6), 2.83 (s, 3H, C$\underline{H}_3$). ^{13}C NMR (75MHz, DMSO-d_6) δ: 178,1 (C=O), 153,0 (C-N), 139,1 (C_2), 136,1 (C_5), 134,3 (CH=CH), 130,5 (NO_2-Ph-4), 130,9 (NO_2-Ph-1), 128,5 (NO_2-Ph-3,5), 127,9 (C_7), 123,1 (NO_2-Ph-2,6), 121,0 (CH=CH), 120,1 (C_8), 116,5 (C_6), 115,2 (C_3), 18,2 (C$\underline{H}_3$). MS: for $C_{17}H_{13}N_3O_3$, calcd. 307.10 (M^+), found 307.11. Recrystallization from butanol.

3- (4-chlorophenyl)-1-(2-methylimidazo[1,2-*a*]pyridin-3-yl)prop-2-en-1-one (5f)

Yellow solid, m.p.: 173-175°C, yield 72%. ^{1}H NMR (300MHz, DMSO-d_6) δ: 9.79 (d, 1H, *J* = 5.8 Hz, H_5), 7.80 (d, 1H, *J* = 15.6 Hz, CH=C$\underline{H}$), 7.68 (m, 1H, H_8), 7.66 (m, 2H, Cl-Ph-2,6); 7.55 (m, 1H, H_7), 7.51 (d, 1H, *J* = 15,9 Hz, C$\underline{H}$=CH), 7.43 (m, 2H, Cl-Ph-3,5); 7.28 (m, 1H, H_6), 2,79 (s, 3H, C$\underline{H}_3$) ^{13}C NMR (75MHz, DMSO-d_6)δ: 180.0 (C=O), 152,6 (C_{8a}), 146.7 (C_2), 139.9 (CH=CH), 137.2 (Cl-Ph-4), 133.5 (C_5), 130.8 (Cl-Ph-1), 128.6 (C_7), 128.3 (Cl-Ph-2), 128.0 (Cl-Ph-4), 127.1 (Cl-Ph-6), 122.8 (Cl-Ph-3,5), 120.9 (CH=CH), 120.0 (C_8), 116.4 (C_6), 115.0 (C_3), 18.1 (C$\underline{H}_3$). MS: for $C_{17}H_{13}ClN_2O$ calcd. 296.07 (M^+), found 296.09). Recrystallization from hexane / DCM (3: 1).

3- (2,6-dichlorophenyl)-1-(2-methylimidazo[1,2-*a*]pyridin-3-yl)prop-2-en-1-one (5g)

Yellow solid, m.p.:180-183°C, yield 55%.^{1}H NMR (300MHz, DMSO-d_6) δ: 9.80 (d, 1H, *J* = 5.8 Hz, H_5), 7.86 (d, 1H, *J* = 16 Hz, CH=C$\underline{H}$), 7.68 (m, 1H, H_8), 7.59 (m, 1H, H_7), 7.54 (d, 1H, *J* = 16 Hz, C$\underline{H}$=CH), 7.31-7.46 (m, 4H, Cl-Ph-2,3,4,5), 7.28 (m, 1H, H_6), 2,79 (s, 3H, C$\underline{H}_3$) ^{13}C NMR (75MHz, DMSO-d_6) δ: 179.3 (C=O), 152.8 (C_{8a}), 146.6 (C_2), 139.9 (CH=CH), 137.3 (Cl-Ph-2,6), 133.9 (C_5), 132.8 (Cl-Ph-1), 128.4 (C_7), 128.0 (Cl-Ph-3,5), 127.8 (Cl-Ph-4), 120.9 (CH=CH), 120.0 (C_8), 116.4 (C_6), 115.2 (C_3), 18.2 (C$\underline{H}_3$). MS: for $C_{17}H_{12}Cl_2N_2O$ calcd. 330.03 (M^++2), found 332.03 Recrystallization from ethyl acetate.

3-(2,4-dichlorophenyl)-1-(2-methylimidazo[1,2-*a*]pyridin-3-yl)prop-2-en-1-one (5h)

Yellow pale solid, m.p.: 197-200°C, yield 90%. ^{1}H NMR (300MHz, DMSO-d_6) δ: 9.80 (d, 1H, *J* = 5.8 Hz, H_5), 7.86 (d, 1H, *J* = 16 Hz, CH=C$\underline{H}$), 7.70 (m, 1H, H_8), 7.64 (s, 1H, Cl-Ph-3), 7.59 (m, 1H, H_7), 7.54 (d, 1H, *J* = 16 Hz, C$\underline{H}$=CH), 7.42 (m, 1H, diCl-Ph-5), 7.31 (m, 1H, diCl-Ph-6); 7.24 (m, 1H, H_6), 2.76 (s, 3H, C$\underline{H}_3$). ^{13}C NMR (75MHz, DMSO-d_6) δ: 179.3 (C=O), 152.8 (C_{8a}), 146.6 (C_2), 139.9 (CH=CH), 137.3 (diCl-Ph-2), 135.8 (diCl-Ph-4), 133.9 (C_5), 133.0 (diCl-Ph-1), 132.8 (diCl-Ph-3), 128.4 (C_7), 128.0 (diCl-Ph-5), 127.8 (diCl-Ph-6), 120.9 (CH=CH),

120.0 (C_8), 116.4 (C_6), 115.1 (C_3), 18.1 ($\underline{C}H_3$). MS: for $C_{17}H_{12}Cl_2N_2O$ calcd. 330.03 (M^+), found 330.03 Recrystallization from hexane/DCM (3:1).

3-(4-bromophenyl)-1-(2- methylimidazo[1,2-*a*]pyridin-3-yl)prop-2-en-1-one (5i)

Yellow pale solid, m.p.: 207-209°C, yield 84%. ^{1}H NMR (300MHz, DMSO-d_6) δ: 9.68 (d, 1H, *J* = 6.9 Hz, H_5); 7.70 (d, 2H, *J* = 6.5 Hz, Br-Ph-3,5); 7.68 (d, 1H, *J* = 15.3 Hz, CH=C$\underline{H}$), 7.64 (d, 2H, *J* = 6.5 Hz, Br-Ph-2,6), 7.58 (d, 1H, *J* = 15.3 Hz, C$\underline{H}$=CH), 7.42 (m, 1H, H_8), 7.28 (m, 1H, H_7); 7.22 (m, 1H, H_6); 2.73 (s, 3H, C$\underline{H}_3$). ^{13}C NMR (75MHz, DMSO-d_6) δ: 179.0 ($\underline{C}$=O), 152.6 (C_{8a}), 146.7 (C_2), 139.8 (CH=$\underline{C}$H), 137.3 (Br-Ph-4), 132.8 (C_8), 131.1 (Br-Ph-1), 130.5 (Br-Ph-3,5), 128.4 (C_7), 127.4 (Br-Ph-2,6), 121.9 ($\underline{C}$H=CH), 120.1 (C_8), 116.5 (C_6), 115.1 (C_3), 18.1 ($\underline{C}H_3$). MS: for $C_{17}H_{13}BrN_2O$ calcd. 340.02 (M^+), found 342.02 Recrystallization from ethanol.

3-(4-fluorophenyl)-1-(2- methylimidazo[1,2-*a*]pyridin -3-yl)prop-2-en-1-one (5j)

Yellow solid, m.p.: 192-194°C, yield 75%. ^{1}H NMR (300MHz, DMSO-d_6) δ: 9.68 (d, 1H, *J* = 6.9 Hz, H_5), 7.70 (d, 2H, *J* = 6.5 Hz, F-Ph-3,5), 7.68 (d, 1H, *J* = 15.3 Hz, CH=C$\underline{H}$), 7.42 (d, 2H, *J* = 6.5 Hz, F-Ph-2,6), 7.58 (d, 1H, *J* = 15.3 Hz, C$\underline{H}$=CH), 7.42 (m, 1H, H_8), 7.28 (1H, m, H_7), 7.22 (m, 1H, H_6), 2.73 (s, 3H, C$\underline{H}_3$). ^{13}C NMR (75MHz, DMSO-d_6) δ: 180.0 ($\underline{C}$=O), 157.4 (F-Ph-4), 152.6 (C_{8a}), 146.7 (C_2), 139.9 (CH=$\underline{C}$H), 132.8 (C_5), 131.1 (F-Ph-1), 128.4 (C_7), 128.3 (F-Ph-2,6), 121.9 ($\underline{C}$H=CH), 120.6 (F-Ph-3,5), 120.1 (C_8), 116.5 (C_6), 115.1 (C_3), 18.1 ($\underline{C}H_3$). MS: for $C_{17}H_{13}FN_2O$ calcd. 280.10 (M^++1), found 281.10. Recrystallization from hexane/DCM (3:1).

3-(4-diméthylaminophenyl)-1-(2-méthylimidazopyridin-3-yl)prop-2-en-1-one (5k)

Yellow solid, m.p.: 197-200°C, yield 30%. ^{1}H NMR (300MHz, DMSO-d_6) δ: 9.66 (d, 1H, *J* = 4.5 Hz, H_5); 7.78 (d, 1H, *J* = 16 Hz, CH=C$\underline{H}$); 7.52 – 7.69 (m, 4H, $(NCH_3)_2$Ph-2,6 ; H_6 and H_8); 7.45 (d, 1H, *J* = 16 Hz, C$\underline{H}$=CH); 7.08-7.2 (m, 3H, $(NCH_3)_2$Ph-3,5); 7.28 (1H, m, H_7); 7.22 (m, 1H, H_6), 2.85 (s, 3H, C$\underline{H}_3$), 2.26 (s, 6H, $(NC\underline{H}_3)_2$).
^{13}C NMR (75MHz, DMSO-d_6) δ: 179.4 ($\underline{C}$=O), 152.0 (C_{8a}), 146.5 (($NCH_3)_2$Ph-4), 143.6 (C_2), 141.5 (CH=$\underline{C}$H), 132.0 (C_5), 129.5 ($NCH_3)_2$Ph-1), 125.4 (C_7), 125.3 ($NCH_3)_2$Ph-3,5), 122.9 ($NCH_3)_2$Ph-2,6), 121.7 ($\underline{C}$H=CH), 120.3 (C_6), 114.7 (C_8), 115.1 (C_3), 40.2 ($N\underline{C}H_3)_2$, 18.1 ($\underline{C}H_3$). MS: for $C_{19}H_{19}N_3O$ calcd. 305.15 (M^++1), found 306.16. Precipitation from water.

2.2. Antibacterial assay

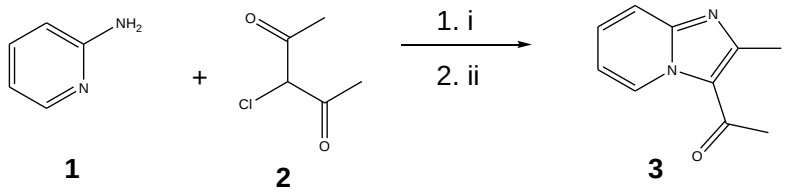
All the chemicals used were of analytical grade. High purity water was employed. N,N-dimethylformamide (DMF) (≥98), was purchased from Aldrich. All chemical reagents were used as received. Muller-Hinton agar plates were used for antibiogram test to establish the sensitivity profile of clinical strains to antibiotics namely Azithromycin, Cefotaxim and Ciprofloxacin, used as control antimicrobial agents. The *in vitro* antibacterial activities of the synthesized compounds were screened against various bacteria, including multidrug-resistant clinical isolates. The standard microorganisms used were obtained from the American Type Culture Collection (ATCC) including Gram-positive (*Staphylococcus aureus* ATCC 25923) and Gram-negative (*Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922) bacteria. Besides, all clinical isolates strains were procured from the national public health laboratory, Abidjan, Côte d'Ivoire. All bacterial strains were cultured in the Brain Heart Broth (BHB) and then on nutrient agar and finally on chromogenic media. Compounds (**5a–k**) were screened for their antimicrobial activities against *S. aureus*, *P. aeruginosa*, and E. coli. The starting inoculums of about 10^6 germs / mL for Gram-negative bacilli and 10^7 germs/ mL for Gram-positive Cocci were used. As all the tested chalcones and standard antibiotics were dissolved in DMF (8 mg/mL), the concentration ranges of chalcones were prepared in ten test hemolysis tubes by using the liquid serial dilutions method. For each tested compound, the first dilution used by diluting in sterile Mueller Hinton broth to have a final concentration from 1/40 (0.5% v/v DMF) since this concentration was found to be the under-inhibitory concentration of DMF on ATCC strains and clinical strains used in this study. Therefore, mother's solutions of chalcone were diluted by serial 2-fold dilution to obtain the final concentration ranging from 0.390 to 200 mg/mL. Then, 50 µL of diluted solutions were mixed with 950µL of sterile Mueller-Hinton broth (MHB). The activity was expressed by the minimal inhibitory concentration (MIC). According to the Antibiogram Committee of the French Society for Microbiology (CA-SFM), the latter is defined as being the lowest concentration of the compound that inhibits the visible growth of bacteria [9]. Here its determination was made from the spectrophotometrically measurement of the turbidity induced by the growth of the

studied germs by optical density determination (OD_{600}). The MIC will therefore be the lowest concentration at which there is no turbidity. For each drug, the lowest concentration of substances that leaves at most 0.01% of surviving germs from the initial inoculum, was also considered as the minimum bactericidal concentration (MBC). MBC was obtained by seeding out the contents of tubes that displayed no visible growth of bacteria, onto Mueller-Hinton agar plates and incubating at 37°C for 24 h. All experiments were performed twice by using DMF as negative control. The compounds were classified as bacteriostatic when the MBC/MIC ratio was ≥ 8 and bactericidal when the MBC/MIC ratio is ≤ 4 [10].

3. Results and discussion

3.1. Chemistry

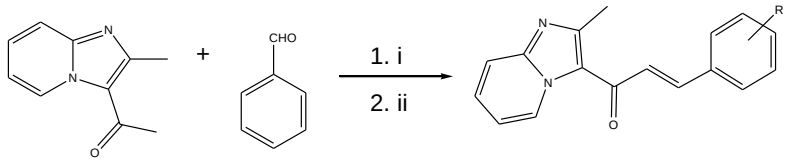
The synthesis of the target compounds **5a–k**, and his intermediate (**3**) is depicted in **schemes 1** and **2**. Key acetyle derivative (**3**) was obtained by reacting 2-aminopyridine (**1**) with 3-chloro-pentan-2,4-dione (**2**) in refluxing ethanol with a yield of 53%. Subsequent Claisen–Schmidt condensation with various substituted benzaldehydes (**4**) at room temperature afforded α,β-unsaturated ketone **5a-k** in good yield.



1 **2** **3**

Reagents and conditions : (i) EtOH, reflux, 6h ; (ii) NH_4OH diluted

*Scheme 1: Synthesis of 3-acetyl-2-methylimidazo[1,2-a]pyridine **(3)***



3 **4** **5a - k**

Reagents and media : (i) NaOH \ EtOH, ambient temperature, 5h; ii) AcOH 30 %

*Scheme 2: Synthesis of imidazo[1,2-a]pyridinyl-chalcones (**5a-k**)*

The spectral data of the synthesized compounds were investigated. The ^{1}H NMR spectra of ketone **3** showed two singlet signals at 2.65 and 2.51ppm corresponding to methylenic protons at position 2 of the imidazo[1,2-*a*]pyridine ring and to methylenic protons of acetyl group at position 3 of the same heterocycle, respectively. For α,β-unsaturated ketone **5a** , the ethylenic protons responded as doublet at δ 7.8 and doublet at δ 7.5 with coupling constant J = 15.6 which indicates trans geometric isomerism. With the exception of proton at position 5 of the imidazo[1,2-*a*]pyridine ring, aromatic protons either from imidazo[1,2-*a*]pyridine or from the benzene ring were elucidated at 7.1–7.8 ppm. In addition, for chalcone **5k**, methylene protons of $(NCH_3)_2$ group, were magnetically equivalent and displayed as singulet signal at δ 2.3. For ^{13}C NMR, the carbonyl belonging to propenone chain of **5a–k** was elucidated around 178–180 ppm.

3.2. Antibacterial activity

An antibiogram by the diffusion method in agar medium was carried out beforehand in order to determine the sensitivity profile of clinical strains to the three antibiotics (Azithromycin, Cefotaxim and Ciprofloxacin) used as references (Table 1).

Table 1: Antibacterial profile of clinical strains

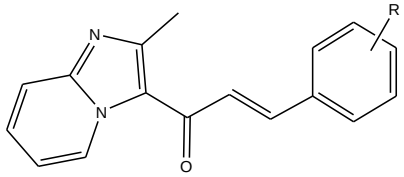
Antibiotics	**Clinical strains of bacteria**		
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Escherichia coli*
Cefotaxim (CTX)			R
Ciprofloxacin (CIP)		R	R
Erythromycin (E)	I		

R: Resistant; S: Sensitive; I: Intermediate

The antibiogram showed that the clinical strains used in experiments are for the most part resistant to the antibiotics tested. Being resistant to two antimicrobial drugs (Ciprofloxacin and Cefotaxim), the clinical strain of *Escherichia coli* was a multidrug-resistant (MDR) bacterium.

The results of antibacterial activities of the synthesized compounds **5a-k** and control drugs against these clinical bacteria and reference strains namely *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922, are given in **Table 2**.

Table 2: Minimum inhibition concentration (MIC) of compounds (**5a-k**) into µg/mL



5a - k

Compds	**R**	**Gram-positive strains**		**Gram-negative strains**			
		S. aureus		***P. aeruginosa***		***E. coli***	
		ATCC 25923	**Clinical strain**	**ATCC 27853**	**Clinical strain**	**ATCC 25922**	**Clinical strain**
5a	H	> 200	> 200	> 200	> 200	> 200	> 200
5b	4-CH_3	> 200	> 200	> 200	> 200	> 200	> 200
5c	2-OH	25	50	50	50	> 200	> 200
5d	3,4-diOCH_3	25	100	50	50	100	100
5e	4-NO_2	> 200	> 200	100	100	> 200	> 200
5f	4-Cl	> 200	> 200	> 200	> 200	> 200	> 200
5g	2,6-diCl	100	> 200	> 200	> 200	> 200	> 200
5h	2,4-diCl	3.125	6.25	100	> 200	> 200	> 200
5i	4-Br	25	50	50	50	100	> 200
5j	4-F	100	> 200	25	50	25	50
5k	4-$N(CH_3)_2$	> 200	> 200	25	100	> 200	> 200
DMF		-	-	-	-	-	-
AZM		25	50				
CIP				6.25	100		
CTX						3.125	200

AZM: Azithromycin

The determination of the MIC enabled to select **5a**, **5b**, **5f**, as being inactive molecules against all tested bacteria with a MIC greater than 200 µg/mL, the highest concentration tested. Moreover, observations on reference species revealed that **5c**, **5d**, **5e**, **5g**, **5h**, **5i**, **5j**, **5k**, displayed good activities against all microbial species, with a MIC ranging from 3.125 µg/mL to 100 µg / mL. In this group **5d**, **5i** and **5j** particularly distinguished themselves with a broad activity against all Gram-positive and Gram-negative tested germs. Besides, while the activity was generally moderate against different bacterial species, **5h** with a MIC of 3.125 µg / mL, exhibited remarkable activity against *S. aureus*. Likewise, **5e** and **5k** were selectively active on *P. aeruginosa*. Interestingly, **5c**, just like **5h**, was

specifically active against Gram-positive bacterium and non-fermentative Gram-negative bacterium (*P. aeruginosa*). **5g** was the only one with a narrow activity limited to *S. aureus*.

In case of clinical strains, antibacterial screening revealed that:

- **5c**, **5h** and **5i** were showed effective against of *S. aureus*;
- **5e, 5k**, posseded a specific inhibitory activity on the *P. aeruginosa* clinical strain (MIC = 100 µg /mL);
- with MIC ranging from 50 to 100 µg/mL, chalcones **5d** and **5j** exerted potent activity against MDR strain of *E. coli*, better than that of Cefotaxim (200 µg / mL) on this clinical strain. These 2 chalcones are remarkable, since they exhibited activities against resistant strain of *P. aeruginosa* as well, and even clinical strain of *S. aureus* for **5d**.

The results of the determination of minimum bactericidal concentration (MBCs) of the seven best compounds namely **5c, 5d, 5e, 5h, 5i, 5j** and **5k** selected for their MIC lower than 200 µg/mL is shown in **Table 3**.

Table 3: Minimum bactericidal concentration (MBC) of selected compounds into µg/mL

Compds	**Gram-positive strains**				**Gram-negative strains**							
	S. aureus				*P. aeruginosa*				*E. coli*			
	ATCC 25923		Clinical strain		ATCC 27853		Clinical strain		ATCC 25922		Clinical strain	
	MBC	MBC /MIC	MBC	MBC /MIC	MBC	MBC /MIC	MBC	MBC /MIC	MBC	MBC /MIC	MBC	MBC /MIC
5c	50	2	200	4	200	4	200	4				
5d	100	4	200	2	200	4	100	2				
5e					100	1						
5h	6.25	2	6.25	1								
5i	50	2	100	2	200	4	200	4				
5j					100	4	200	4	100	4	200	4
5k					100	4	200	2				

Calculation of the MBC / MIC ratio of the chalcones tested gave values between 1 and 4. Thus, imidazo[1,2-*a*]pyridinyl-chalcones had a bactericidal power on all the strains whether clinical or not.

At the end of the antibacterial screening, interesting trends in the structure-activity relationships (SAR) were observed that can be summarized as follows:

(i) Toward *S. aureus*, taking into consideration that phenyl derivative 5a, didn't give any antibacterial activity, the presence of substituents like OH (**5c**), 3,4-diOCH_3 (**5d**), F (**5j**), 2,4-diCl (**5h**), 2,6-diCl (**5g**), Br (**5i**) enabled to exert considerable activity. Indeed, with the exception of compounds **5g** and **5j**, all compounds previously cited, displayed activity stronger than or equipotent compared to standard drug, Azithromycin. The best results were obtained with 2,4-diCl derivative of imidazo[1,2-*a*]pyridinyl-chalcone, which turned out to be 8-fold more active than Azithromycin against reference strain and clinical strain of *S.aureus* with MICs ranging from 3.125 to 6.25 µg/mL.

(ii) Four chalcones, namely hydroxyl derivative (**5c**), dimethoxy derivative (**5d**), brominated derivative (**5i**) and fluorinated derivative (**5j**), displayed more effective activity on clinical strain of *P. aeruginosa* than those Ciprofloxacin, reference antibiotic. These compounds demonstrated moderate bactericidal activities against ATCC strain of *P. aeruginosa* with MICs in the range of 25 –100 µg/mL, but it is still remarkable since this bacterium is more and more often responsible for nosocomial infections.

(iii) The active compounds against reference strains of *E. coli* were 3,4-diOCH_3 derivative (**5d**), brominated derivative (**5i**) and fluorinated derivative (**5j**) that showed antimicrobial activity at a concentrations ranging from 100 to 25 µg /mL. However, against clinical strain of *E. coli* only two of them (**5d** and **5j)** keep an efficient effect. Among them, the most effective candidate was fluorinated derivative (**5j**). With MIC at 50 µg /mL, this compound was effectively, 4-fold more active on this MDR strain of E. coli compared to Cefotaxim.

(iv) The presence of electron-donating groups type methoxy in position 3 and 4 on phenyl ring of compound **5d** (MIC: 25-100 µg/mL against *S. aureus*; 50 µg/mL against *P. aeruginosa*; 100 µg/mL against *E. coli*) enabled to

deploy broad spectrum bactericidal activity against both Gram-positive and Gram-negative bacteria, including the drug-resistant species.

(v) The concomitant presence of a chlorine-type halogen atom at the 2- and 6-positions of the phenyl ring of the **5g** compound resulted in a narrow, specifically active antibacterial spectrum at a high concentration against the reference strain of the Gram-positive bacterium (*S aureus*). Surprisingly, the change in the position of the chlorine at the 2- and 4-position of the **5h** phenyl ring led to a very potent molecule effective at much lower concentrations than did Azithromycin, with respective MICs of 3.125 and 6.25 µg / mL against reference and clinical strains of *S aureus*.

(vi) In general, the comparison of inhibition among the different compounds suggests that the presence of a electron-withdrawing group (NO_2) or of bulkier group ($N(CH_3)_2$) in para position on the phenyl moiety (**5e** and **5k**) led to low antibacterial activities against the examined bacterial strains as well as a shrinking of the spectrum of action to *P. aeruginosa*. In contrast,, an electron-donating substituent as OMe (**5d**), at the same position, strongly broad the bactericidal spectrum of activity.

4. Conclusion

The antibacterial tests carried out on the synthesized compound suggest that imidazo[1,2-*a*]pyridinyl-chalcone derivatives has a bactericidal effect against reference and clinical strains of Gram-positive (*Staphylococcus aureus)* and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. The antibacterial activity of some tested compounds was similar or higher than the activity of commonly used reference drugs such as Azithromycin, Cefotaxim and Ciprofloxacin. Structure activity relationship studies revealed influence of substitution pattern at phenyl ring on spectrum antibacterial derivatives of this series. Since Gram-negative bacteria are more resistant to antimicrobial agents than Gram-positive bacteria due to their different morphological features, lead compounds active against resistant strain of *Pseudomonas aeruginosa* (**5c**,**5d**, **5e**,**5i**, **5j** and **5k**) or toward MDR strain of *Escherichia coli* (**5d** and **5j**) identified in this work, are extremely relevant for designing new antimicrobials for medical use.

Conflict of Interest

The authors declare no conflict of interest as regards this work.

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