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Influence of N-methyl piperidine on Antibacterial Activity of 2-(thioalkyl)-1*H*-methylbenzimidazole Derivatives

Coulibaly Souleymane[1], Coulibali Siomenan[1*], Ablo Evrard[1], Coulibaly Bakary[2], Camara Tchambaga Etienne[1] and Adjou Ané[1]

[1] Laboratoire de Constitution et Réaction de la Matière, UFR Sciences des Structures de la Matière et Technologie, Université Félix Houphouët Boigny de Cocody, 22 BP 582 Abidjan 22, Côte d'Ivoire.

[2] Laboratoire d'Agrovalorisation, Département Biochimie-Microbiologie, UFR Jean Lorougnon Guédé de Daloa, BP 150 Daloa.

[*]**Corresponding author**: Coulibali Siomenan, Laboratoire de Constitution et Réaction de la Matière, UFR Sciences des Structures de la Matière et Technologie, Université Félix Houphouët Boigny de Cocody, 22 BP 582 Abidjan 22, Côte d'Ivoire E-mail: bsiomenan@yahoo.fr

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Abstract

Antibiotic resistance becomes a public health issue nowadays. The overcoming and settlement of this ongoing crisis could be to find novel, potent, and selective antimicrobial agents. For that we synthesized 2-(thioalkyl)-*1H*-methylbenzimidazole derivatives (3a-g) by S-alkylation, and added piperidine via Mannich reaction to yield 2-(thioalkyl)-methyl-1-(piperidin-1-ylmethyl) benzimidazole derivatives (4a-g). The antimicrobial profiles of both derivatives were first estimated by their inhibition diameters and determined with Minimal Inhibitory Concentrations (MICs) against a small set of bacteria clinical strains of *Escherichia coli, Klebsiella pneumoniae* (Gram-negative bacteria), *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Gram-positive bacteria). The panel of synthesized compounds (3a-g) and (4a-g) were characterized by NMR ^{1}H, ^{13}C spectroscopy, and high-resolution mass spectrometry (HRMS). The results revealed that compounds 3a and 3b, were both bactericidal on *P. aeruginosa* and *E. coli* with a great MIC value of 1µg/mL. Then the addition of piperidine show promising inhibition diameter but did not implement the bactericidal activity in terms of MIC on P. aeruginosa.

***Keywords:** Antibacterial activity, Bactericidal effect, 2-(thioalkyl)-1H-methylbenzimidazole, Piperidine, Pseudomonas aeruginosa.*

Introduction

Infectious diseases are diseases caused by pathogens such as fungi, bacteria, parasites, viruses, prions, etc. These diseases are becoming more and more widespread because of the resistance effect of certain pathogens observed during the administration of certain antibiotics. For this purpose, according to statistics reported by the World Health Organization in 2022, bacterial-resistant

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infections are associated with nearly 4.95 million deaths each year. Of these deaths, 1.27 million are directly attributed to antimicrobial resistance [1]. This large number of deaths each year makes antibiotic resistance a public health problem. To find solutions to this problem, it would therefore be urgent to invest in the research of new molecules of therapeutic interest. To do so, it would be important to make structural changes to the basic scaffold of old antibiotics or to design new antibiotics from organic compounds with possible biological activity. In this study, we opted for the benzimidazole scaffold to look for bioactivity.

Benzimidazole is a heterocyclic compound that has been the subject of several chemical and biological studies. Indeed, work carried out around this scaffold has made benzimidazole, an important heterocyclic compound in medicinal chemistry [2]. It should be noted that the benzimidazole core and its derivatives possess important biological activities. Among all of them, we can cite antibacterial activities [3][4], anti-tuberculosis [5][6], antifungal [7][8], anticancer [9] [10], antiviral [11] [12], anthelmintic [13], anti-inflammatory [14]. Thus, this biological profile of benzimidazole derivatives has made this heterocycle a privileged core in the search for new molecules capable to fight against infectious diseases. On the other hand, Mannich reactions have been used as a synthesis tool for the preparation of many commercial drugs. This is the case with Fluoxetine, an antidepressant agent, Ranitidine, an H2 receptor antagonist, Triprolidine, an H1 receptor antagonist, and Trihexyphenidyl hydrochloride, an antispasmodic. Mannich reactions, when applied to the benzimidazole core, lead to certain Mannich base derivatives of benzimidazole having several biological activities such as antibacterial activities [15] [16], antifungal [17], anti-inflammatory [18], anticancer [19], anti-tuberculosis [20], antiviral [21], etc. The concept of N-Mannich base prodrug may be useful or improve the dissolution behavior of poorly soluble drugs in an effort of improving oral bioavailability of various NH-acidic compounds (phenytoin, sorbitol, acetazolamide, chlorothiazide, chlorzoxazone, and allopurinol). Moreover, N-Mannich bases with piperidine were found to possess markedly greater up to a factor of two thousand, intrinsic dissolution rates in comparison with the parent compounds [22]. As a result, Mannich bases of benzimidazole have attracted significant attention in the development of new pharmacologically interesting compounds.

In this work, we first design benzimidazole derivatives substituted in position-2 by thioalkyl groups while inserting a methylene group between the carbon in the 2-position of benzimidazole and the sulfur atom. Then a second time, these benzimidazole derivatives were combined with the piperidine group via Mannich reaction to obtain new compounds with antibacterial activity.

Materials and Methods

Materials of Chemistry

All reagents and solvents were purchased from Sigma Aldrich and used without further purification unless otherwise noted. All anhydrous solvents, reagent grade solvents for chromatography, and starting materials were purchased at the highest commercial quality from either Aldrich Chemical or Fisher Scientific. The reactions were monitored by Thin Layer Chromatography (TLC) on precoated Merck 60 F_{254} silica gel plates and visualized using UV-Lamp (6 W, 254 nm, and/or 365 nm) or $KMnO_4$ solution followed by heating. Unless otherwise indicated, ^1H and ^{13}C NMR spectra were recorded either on a Bruker Avance at 300, 600, and 75, 151 MHz. The spectra were internally referenced to the residual proton solvent signal. Residual solvent peaks were taken as reference ($CDCl_3$: 7.26 ppm, Acetone-d_6: 2.05 ppm, DMSO-d_6: 2.50 ppm) at room temperature. For ^1H NMR assignments, the chemical shifts are given in ppm on the δ scale. Multiplicities are described as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), and further qualified as app (apparent), br (broad signal) coupling constants, J is reported in Hz. HRMS were measured

in the Electrospray (ESI) mode on an LC-MSD TOF mass analyzer. Solid compound melting points were measured using a Köfler bench.

Materials of Biology

The microbial medium consists of clinical strains of *Klebsiella pneumoniae* (Gram positive bacteria) *S. aureus* (Gram positive bacteria) and *E. coli* (Gram negative bacteria), *P. aeruginosa* (Gram negative bacteria). These strains were supplied by the l'Unité des Antibiotiques, des Substances Naturelles et de la Surveillance des Microorganisms aux Anti-infectious (ASSURMI) du Département de Bactériologie et virologie de l'Institut Pasteur de Cote d'Ivoire.

Methods

Synthesis Methods of benzimidazoles derivatives

2-Methylbenzimidazole thiourunium chloride salt synthesis method: Thiourea (57.2 mmol) was added to a solution of 2(chloromethyl)-1H-benzimidazole (57.2 mmol) in 50 mL of acetonitrile. The mixture was carried under reflux for 2 h. The reaction was monitored by TLC. At the end of the reaction, a precipitate was formed, then hot-filtered and washed several times with ethyl acetate. The product was air-dried and gave brown crystals, yield=92%, m.p=192°C-194°C

General procedure for the synthesis of 2-thioalkyl methyl benzimidazoles derivatives (3a-g): To a solution of 2methylbenzimidazole thiourunium chloride salt (2.61 mmol) in 10 mL of ethanol, 10 mL of aqueous NaOH solution was added. The mixture was agitated until the reflux was reached. Then the alkylating agent (1.2 eq, 3.14 mmol) was added and left under reflux for 1 h. Monitoring by TLC, in the end, the reaction mixture was cooled to room temperature. The aqueous layer was extracted with dichloromethane, dried over $MgSO_4$, and evaporated in vacuum. The product obtained was purified by silica gel chromatography using eluent hexane/ ethyl acetate (66/33).

2-((methylthio)methyl)-1*H*-benzimidazole (3a): Yellow crystals, yield=96%, m.p=148°C-150°C. ^{1}H NMR (600 MHz, Acetone-d_6) δ 7.55 (dd, *J*=6.0, 3.2 Hz, 2H, H_{Ar}), 7.18 (dd, *J*=6.0, 3.2 Hz, 2H, H_{Ar}), 4.00 (s, 2H, CH_2S), 2.57 (s, 3H, SCH_3). ^{13}C NMR (151 MHz, Acetone-d_6) δ 152.27, 121.74, 28.32, 25.40. HRMS (ESI) Calc. for $C_9H_{10}N_2SNa$ (M^+Na^+) =201.0251 Found=201.0254

2-((isobutylthio)methyl)-1*H*-benzimidazole (3b): Yellow powder, m.p=258°C-260°C; yield=80%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.25 (s, 1H, NH), 9.34 (d, *J*=6.8 Hz, 1H, H_{Ar}), 8.67 (s, 1H, CH=N), 7.89–7.65 (m, 4H, H_{Ar}), 7.56 – 7.41 (m, 3H, H_{Ar}), 7.29 (td, *J*=7.0, 1.2 Hz, 1H, H_{Ar}). ^{13}C NMR (75 MHz, DMSO-d_6) δ 150.57, 148.37, 146.75, 134.85, 134.55, 130.40, 129.34, 129.03, 127.47, 116.01, 115.37. HRMS (ESI) Calc. for $C_{14}H_{12}N_5O_2$ $[M^+H]^+$=282.1881 Found=282.1883

Propanoic acid, 3-((1*H*-benzimidazol-2-yl) methyl) thio)propanoate (3c): Yellow crystals, yield=25%, m.p=104°C-106°C. ^{1}H NMR (600 MHz, Acetone-d_6) δ 7.56 (dd, *J*=6.0, 3.2 Hz, 2H, H_{Ar}), 7.19 (dd, *J*=6.0, 3.2 Hz, 2H, H_{Ar}), 4.08 (q, *J*=7.1 Hz, 2H, OCH_2), 4.04 (s, 2H, CH_2S), 2.85 (t, *J*=7.2 Hz, 2H, CH_2C=O), 2.62 (t, *J*=7.2 Hz, 2H, SCH_2), 1.19 (t, *J*=7.1 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, Acetone-d_6) δ 171.23, 151.99, 121.82, 59.99, 34.05, 28.60, 26.62, 13.61. HRMS (ESI) Calc. for $C_{15}H_{17}O_2N_2S$ (M^+H^+) =261.1054 Found=261.1058

2-((ethylthio)methyl)-1*H*-benzimidazole (3d): Yellow crystals, yield=62%, m.p=132°C-136°C. ^{1}H NMR (600 MHz, Acetone-d_6) δ 7.56 (dd, *J*=6.0, 3.2 Hz, 2H, H_{Ar}), 7.19 (dd, *J*=6.0, 3.2 Hz, 2H, H_{Ar}), 4.01 (s, 2H, CH_2S), 2.59 (q, *J*=7.4 Hz, 2H, SCH_2), 1.21 (t, *J*=7.4 Hz, 3H, CH_3). 13C NMR (151 MHz, Acetone-d6) δ 152.29, 121.76, 28.34, 25.42, 13.82. HRMS (ESI) Calc. for $C_{10}H_{13}N_2S$ (M^+H^+)=193.0929 Found=193.0925

2-((butylthiomethyl)-1*H*-benzimadazole (3e): Yellow crystals, yield=57%, m.p=144°C-146°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (t, *J*=4.7 Hz, 2H, H_{Ar}), 7.15 (dd, *J*=6.0, 3.1 Hz, 2H, H_{Ar}), 3.95 (s, 2H, CH_2S), 2.44 (t, *J*=6.9 Hz, 2H, SCH_2), 1.61-1,52 (m, 2H, CH_2), 1,41-1,26 (m, 2H, CH_2) 0.90 (t, *J*=6.7 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.57, 142.25, 135.01, 122.39, 121.81, 119.47, 109.27, 31.33, 31.02, 27.87, 21.76, 13.51. HRMS (ESI) Calc. for C12H16N2SNa (M^+Na^+)=243.2319 Found=243.2315.

2-((1*H*-benzimidazol-2-yl)methyl)thio)ethyl acetate (3f): Yellow crystals, yield=96%, m.p=68°C-72°C. ^{1}H NMR (600 MHz, Acetone-d_6) δ 7.56 (dd, *J*=6.0, 3.2 Hz, 2H, H_{Ar}), 7.19 (dd, *J*=6.0, 3.1 Hz, 2H, H_{Ar}), 4.13 (s, 2H, SCH_2C=O), 4.10 (q, *J*=7.1 Hz, 2H, OCH_2), 3.45 (s, 2H, CH_2S), 1.21 (t, *J*=7.1 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, Acetone-d_6) δ 169.66, 151.28, 121.83, 60.78, 32.97, 13.50. HRMS (ESI) Calc. for $C_{12}H_{15}N_2O_2S$ (M^+H^+)=251.0909 Found=251.0913

2-((1*H*-benzimidazol-2-yl)methyl)thio)ethyl propanoate (3g): Yellow crystals, yield=38%, m.p=100°C-102°C. ^{1}H NMR (600 MHz, Acetone-d_6) δ 7.60 (dt, *J*=6.7, 3.3 Hz, 2H, H_{Ar}), 7.23 (dd, *J*=6.1, 3.1 Hz, 2H, H_{Ar}), 4.26 (d, *J*=14.7 Hz, 1H, CH_2S), 4.17 (d, *J*=14.7 Hz, 1H, CH_2S), 4.12 (q, *J*=7.1 Hz, 2H, OCH_2), 3.72 (q, *J*=7.2 Hz, 1H, CH), 1.42 (d, *J*=7.2 Hz, 3H, CH_3), 1.23 (t, *J*=7.1 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, Acetone-d_6) δ 172.28, 151.47, 121.87, 60.71, 41.03, 28.60, 16.63, 13.48. HRMS (ESI) Calc. for $C_{15}H_{17}O_2N_2S$ (M^+H^+)=265.0909 Found=265.0905.

General procedure for the synthesis of 2-(thioalkyl)-methyl-1-(piperidin-1-ylmethyl) benzimidazole derivatives (4a-g*)*: In a 100 mL flask containing 15 mL of ethanol, 11.2 mmol of 2-(thioalkyl) methyl-1H-benzimidazole derivatives (4a-g), 11.2 mmol of piperidine, and 33.6 mmol (3 eq) of formaldehyde were added. Then the mixture was refluxed for 2h. In the end, the reaction medium was allowed to cool down and extracted with ethyl acetate. The organic layer was dried with Na_2SO_4 and evaporated under vacuum to remove ethyl acetate. The oil was purified by silica gel chromatography using hexane/ethyl acetate as eluent (80/20).

2-((methylthio)methyl)-1-(piperidin-1-yl)methyl)-1*H*-benzimidazole (4a): Yellow oil. yield=56%, ^{1}H NMR (300 MHz, Acetone-d_6) δ(ppm) 7,65–7,50 (m, 2H; H_{Ar}), 7.28–7.09 (m, 2H; H_{Ar}), 4.86 (s, 2H; N-CH_2-N), 4.06 (s, 2H; S-CH_2), 2.49 (t, *J*=5.2 Hz, 4H_{pip}), 2.11 (s, 3H; CH_3), 1.57 – 1.25 (m, 6H, H_{pip}). ^{13}C NMR (75 MHz, Acetone-d_6) δ(ppm) 151.76, 142.47, 136.61, 121.53, 119.06, 110.36, 65.76, 51.62, 25.55, 24.13, 14.46. HRMS (ESI) Calc $[M^+Na]^+$ $C_{15}H_{21}N_3NaS$=298.4127 Found= 298.4130.

2-(isobutylthio)methyl)-1-(piperidin-1-yl)methyl)-1*H*-benzimidazole (4b): Yellow oil, yield=51% , ^{1}H NMR (300 MHz, Acetone-d_6) δ(ppm) 7.56- 7.48 (m, 3H, H_{Ar}), 7.18- 7.04 (m, 2H, H_{Ar}), 4.88 (s, 2H, N-CH_2-N), 4.01 (d, *J*=41.4 Hz, 2H, S-CH_2), 2.54– 2.44 (m, 4H, H_{pip}), 1.84 – 1.70 (m, 1H), 1.59 – 1.34 (m, 4H, H_{pip}), 1.29 (d, *J*=3.2 Hz, 2H, H_{pip}), 0.91 (d, *J*=6.7 Hz, 6H). ^{13}C NMR (75 MHz, Acetone-d_6) δ(ppm) 151.98, 122.25, 121.52, 119.06, 110.33, 65.79, 51.65, 40.24, 25.54, 24.13, 21.25. HRMS (ESI): Calc $[M^+H]^+$ $C_{18}H_{28}N_3S$=318.5012 Found=318.5016

3-((1-(piperidin-1-yl)methyl)-1*H*-benzimidazol-2-yl)methylthio)ethyl propanoate (4c): Yellow oil, yield=65%, ^{1}H NMR (300 MHz, Acetone-d_6) δ(ppm) 7.66 – 7.49 (m, 2H, H_{Ar}), 7.29 – 7.11 (m, 2H, H_{Ar}), 4.86 (s, 2H, N-CH_2-N), 4.15 (s, 2H, S-CH_2), 4.08 (q, *J*=7.1 Hz, 2H, CH_2-CH_3), 2.84 (t, *J*=7.1 Hz, 2H, CH_2-CH_2), 2.66 – 2.59 (m, 2H, CH_2), 2.49 (t, *J*=5.2 Hz, 4H, H_{pip}), 1.57 – 1.35 (m, 6H, H_{pip}), 1.19 (t, *J*=7.1 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, Acetone-d_6) δ(ppm) 171.25, 151.81, 142.45, 136.57, 122.37, 121.61, 119.12, 110.38, 65.81, 60.00, 51.63, 34.11, 26.45, 25.54, 24.13, 13.70. HRMS (ESI): Calc $[M^+Na]^+$ $C_{19}H_{27}N_3NaO_2S$=384.4950 Found=384.4953

2-(ethylthio)methyl)-1-(piperidin-1-yl)methyl)-1*H*-benzimidazole (4d): Yellow oil, yield=62%, ^{1}H NMR (300 MHz, Acetone-d_6) δ(ppm) 7.64 – 7.49 (m, 2H, H_{Ar}), 7.20 – 7.14 (m, 2H, H_{Ar}), 4.87 (s, 2H, N-CH_2-N), 4.10 (d, *J*=6.6 Hz, 1H, S-CH_2), 2.57 (q, *J*=7.3 Hz, 2H, CH_2), 2.49 (t, *J*=5.1 Hz, 4H, H_{pip}), 1.57 – 1.26 (m, 6H, H_{pip}), 1.20 (td, J=7.4 ; 1.7 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, Acetone-d_6) δ(ppm) 152.05, 122.28, 119.04, 110.34, 65.79, 51.64, 27.18, 25.54, 24.12, 13.93. HRMS (ESI): Calc[M+H] + $C_{16}H_{24}N_3S$=290.4431 Found=290.4437

2-(butylthio)methyl)-1-(piperidin-1-yl)methyl)-1*H*-benzimidazole (4e): Orange oil, yield=51%, ^{1}H NMR (300 MHz, Acetone-d_6) δ(ppm) 7.64 – 7.51 (m, 2H, H_{Ar}), 7.28 – 7.11 (m, 2H, H_{Ar}), 4.88 (s, 2H, N-CH_2-N), 4.10 (s, 2H, S-CH_2), 2.58 (t, *J*=7.3 Hz, 2H, CH_2), 2.50 (t, *J*=5.2 Hz, 4H, H_{pip}), 1.63 – 1.25 (m, 10H), 0.85 (t, *J*=7.3 Hz, 3H, CH3). ^{13}C NMR (75 MHz, Acetone-d_6) δ(ppm) 151.98, 142.50, 136.60, 122.23, 121.50, 119.07, 110.30, 65.78, 51.64, 31.16, 30.93, 27.59, 25.55, 24.13, 21.58, 13.04. HRMS (ESI): Calc $[M^+Na]^+$ $C_{18}H_{27}N_3NaS$=340.4824 Found=340.4821

2-((1-(piperidin-1-yl)methyl)-1*H*-benzimidazol-2-yl)methylthio)ethyl acetate (4f): Yellow oil, yield=63%, ^{1}H NMR (300 MHz, Acetone-d_6) δ(ppm) 7.66 – 7.51 (m, 2H, H_{Ar}), 7.30 – 7.11 (m, 2H, H_{Ar}), 4.87 (s, 2H, N-CH_2-N), 4.22 (s, 2H, S-CH_2), 4.05 (q, *J*=7.1 Hz, 2H, CH_2-CH_3), 3.47 (s, 2H. CH_2-CO), 2.51 (t, *J*=5.2 Hz, 6H), 1.61 – 1.25 (m, 4H), 1.23 – 1.15 (t, *J*=7.1 Hz 3H, CH_3). ^{13}C NMR (75 MHz, Acetone-d_6) δ(ppm) 169.67, 151.47, 142.54, 136.54, 122.36, 121.57, 119.14, 110.35, 65.77, 60.74, 51.58, 32.82, 27.96, 25.53, 24.12, 13.56. HRMS (ESI) : Calc $[M^+Na]^+$ $C_{18}H_{25}N_3NaO_2S$=370.4614 Found=370.4618

2-(1-(piperidin-1-yl)methyl)-1*H*-benzimidazol-2-yl)methylthio)ethyl propanoate (4g): Orange oil, yield=67%, ^{1}H NMR (600 MHz, Acetone-d_6) δ 7.60 (dt, *J*=6.7, 3.3 Hz, 2H, H_{Ar}), 7.23 (dd, *J*=6.1, 3.1 Hz, 2H, H_{Ar}), 4.26 (d, *J*=14.7 Hz, 1H, CH_2S), 4.17 (d, *J*=14.7 Hz, 1H, CH_2S), 4.12 (q, *J*=7.1 Hz, 2H, OCH_2), 3.72 (q, *J*=7.2 Hz, 1H, CH), 2,49 (t, *J*=5,2 Hz, 4H, H_{pip}), 1.42 (d, *J*=7.2 Hz, 3H, CH_3), 1,57 – 1,35 (m, 6H, H_{pip}), 1.23 (t, *J*=7.1 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, Acetone-d_6) δ 172.28, 151.47, 121.87, 65,81, 60.71, 41.03, 34,11, 28.60, 26.45, 25.54, 24.13, 16.63, 13.48. HRMS (ESI): Calc $[M^+Na]^+$ $C_{19}H_{27}N_3NaO_2S$=384.3523 Found=384.3519

Biological methods

Preparation of inoculum for solid-state testing

The inoculum was prepared from a 24 h young colony. It was emulsified in 2 mL of NaCl solution. Then the optical density was adjusted to 0.5 MC Farland using a densimat. The collected volume is 100 µL for *E. coli*, 1000 µL for *S. aureus*, and 10 µL for P. aeruginosa, and 10 µL *Klebsiella pneumoniae*. This suspension was mixed in 10 mL of physiological water (0.9% NaCl), thus constituting the bacterial inoculum estimated at 106 bacteria/mL. The various retained bacterial strains were transplanted by the Muller-Hinton Agar Streak Method. Their incubation was done in the oven at 37°C for 18 to 24 h to obtain young and isolated colonies. These colonies were used to prepare the bacterial inoculum.

Preparation of the stock chemical solution

The substances were weighed and placed in the test tubes to which a quantity of 70°C ethanol was added. Different concentrations were obtained.

Sensitivity test

Identification of inhibition zones: The capsule diffusion technique in wells and the liquid micro-dilution method were used to perform the tests [23-24]. A 500 µg/mL concentration solution of the chemical was prepared. Petri dishes containing Muller-Hinton agar were seeded by swabbing with the prepared inoculum. Then, cups were dug by pressing the big tip of a Pasteur pipette into the agar and filled with 50 µL of the prepared chemical solution. The unit was incubated at 37°C for 24 hours. After this time, the inhibition diameter around each cup was measured using a caliper. The effectiveness of the extracts was assessed according to the criterion of Ponce et al. [25]. Thus, a substance is said to be ineffective if the diameter of inhibition is less than 8 mm whereas it is said to be effective if the diameter is between 9 and 14 mm. On the other hand, it is considered very effective when the diameter is between 15 and 19 mm and extremely effective if the diameter is greater than 20 mm. This test allowed the selection of the most active extracts for the determination of antibacterial parameters.

Preparation of the inoculum for liquid tests: A 24 h bacterial colony was collected using a Pasteur pipette and emulsified in a test tube containing 10 mL of sterile Muller-Hinton Broth (MHB). The mixture was incubated at 37°C for 3 h. After this incubation, a 0.3 mL suspension of this pre-culture was removed and diluted in 10 mL of sterile MHB and homogenized

Preparation of the concentration range: The concentration range was obtained by the double dilution method. To do this, a concentration solution in µg/mL of the chemicals retained was prepared. A series of 2-reason dilutions were carried out from this solution to obtain ranges of concentrations.

Determination of antibacterial parameters: Antibacterial parameters were determined by liquid dilution using the method used by Kouadio et al [26]. Thus, in 10 experimental hemolysis tubes, 1 mL of each chemical concentration range was brought into contact with 1 mL of bacterial inoculum. The growth control received 1 mL of sterile distilled water in addition to the inoculum while the sterility control received only 2 mL of sterile MHB. The tubes were incubated for 24 hours at 37°C. After this incubation time, a naked eye observation was made and the lowest concentration for which no bacterial growth was observed in the naked eye was the Minimum Inhibitory Concentration (MIC). As for the Bactericidal Minimum Concentration (BMC), it allows to obtain, after 24 h of incubation at 37°C, 0.01% of viable bacteria. His determination began with enumeration. This consisted of diluting the starting inoculum from 10-1 to 10-4 and seeding these various dilutions with a 2 µL calibrated loop in 5 cm long ridges on a Muller-Hinton agar and incubating for 24 h. These Petri dishes have been named A. After reading the MIC, the contents of the tubes in which there was no visible growth were used to seed MHB on 5 cm ridges.

This series of Petri dishes are named B. The BMC was determined by comparing the bacterial growth of boxes A and B. Thus, the lowest tube concentration that has less than 0.01% viable bacteria compared to the initial inoculum is BMC. The BMC/MIC report clarified the modality of action of the substance [27]. According to Kamanzi et al. [28], the extract is bactericidal when its BMC is equal to its MIC or if the BMC/MIC ratio is less than or equal to 4. It is said to be bacteriostatic when its BMC is greater than its MIC or if the BMC/MIC ratio is greater than 4. When this ratio is equal to 32, the strain is said to be tolerant.

Statistical analysis: The results were analyzed by the excel 2013 software for the descriptive analyses. The antibacterial test results obtained were expressed as a standard deviation average [29].

Results and Discussion

Chemistry

The synthesis of Mannich bases derivatives of benzimidazole (**4a-g**) was achieved in three reactive steps. The reaction sequence begins by obtaining 2-methyl benzimidazole thiouronium chloride salt (1) as previously described by Ablo et al. [29]. This compound was condensed with alkyl halides or functionalized halides (**2a-g**) to give the compounds 2-(thioalkyl)-methyl-1H-benzimidazole (**3a-g**) with yields of between 25% and 96% by nucleophilic substitution reaction (S-alkylation). The 2-(thioalkyl)-methyl-1(piperidin-1-ylmethyl)benzimidazole (**4a-g**) derivatives were obtained by reacting the 2-(thioalkyl)-methyl-1H-benzimidazole (**3a-g**) derivatives with formaldehyde and piperidine under reflux of ethanol for one hour. Seven derivatives of 2-(thioalkyl)-methyl)-1-(piperidin-1-ylmethyl)benzimidazole **(4a-g)** are obtained with yields between 63% and 78% (**FIG 1**).

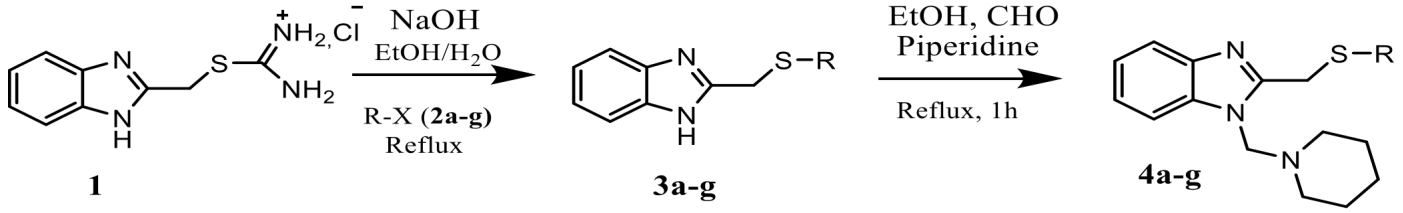

FIG. 1：**Synthesis route of 2-(thioalkyl)-methyl)-1-(piperidin-1-ylmethyl)benzimidazole derivatives (4a-g).**

Analysis of the ^{1}H and ^{13}C NMR spectra of the compounds **(4a-g)** shows the presence of peaks corresponding to the different alkyl groups. The NMR spectra of the compounds **(4a-g)** obtained show the disappearance of the pyrrolic nitrogen proton around 12 ppm and the appearance of peaks corresponding to the piperidine group. Analysis of the NMR spectra of the proton of these compounds reveals the presence of a singlet signal in the vicinity of 4.86 ppm corresponding to the two protons of the methylene group ($-CH_2-$) bound to the nitrogen atom and piperidine nucleus. We also observe two groups of signals on the spectrum, one between 2.41 and 2.60 ppm in the form of a multiplet corresponding to four protons and the other between 1.27 and 1.61 ppm in the form of a multiplet and integrating for six protons. These chemical displacements confirm the presence of the piperidine nucleus.

The ^{13}C NMR spectra analysis confirms this interpretation by the presence of a peak between 63 and 70 ppm corresponding to the methylene carbon bound to the two nitrogen atoms ($-N-CH_2-N$). The two carbons of the methylene groups bound to piperidine nitrogen are observed at around 51 ppm for all compounds. The other three carbons of piperidine are observed between 23 ppm and 28 ppm.

Biological

The antibacterial activity of the 2-thioalkyl methyl benzimidazole derivatives synthesized was achieved on *P. aeruginosa, K. pneumoniae, E. coli,* and *S. aureus* by the capsule diffusion technique in wells and the liquid macro-dilution method. The inhibition diameters of the 3a, b, c, and 3e compounds have been described in **TABLE 1** below:

TABLE 1**: Inhibition Diameters (average ± sd) zones of chemicals derived from S-alkylation benzimidazole on clinical strains**

Clinical Strains	Inhibition Diameters (mm)			
	3a	**3b**	**3c**	**3e**
P. aeruginosa	13.0 ± 0.4	12.7 ± 1.7	10.3 ± 0.9	0.7 ± 0.2
K. pneumoniae	6.7 ± 1.2	11.5 ± 0.4	0.7 ± 0.2	0.6 ± 0.1
E. coli	6.3 ± 1.0	17.7 ± 1.0	4.0 ± 1.2	0.7 ± 0.2
S. aureus	4.0 ± 2.1	8.0 ± 0.8	6.3 ± 1.2	0.5 ± 0.2

According to the criterion of Ponce et al [25], the compound 2-((methylthio)methyl)-1H-benzimidazole (**3a**) was the best promising bactericidal for all the tested strains from sensitive to *S.aureus*, *P. Aeruginosa*, and *K. pneumoniae* to very sensitive to *E. coli* with inhibition diameters ranging from 8 to almost 18 mm. Thus, 2-((methylthio)methyl)-1H-benzimidazole (**3a**), 2((isobutylthio)methyl)-1H-benzimidazole (**3b**), Propanoic acid, 3-((1H-benzimidazol-2-yl)methyl)thio)propanoate (**3c**) are the derivatives which could generate the best activity against P. aeruginosa while they are sensitive to this strain (inhibition diameter 10 to 13 mm). The 2-((butylthio)methyl)-1H-benzimidazole (**3e**) is non-sensitive on all strains. As compound **3e** showed no activity on the different bacterial strains. Thus, this one was not used for the determination of antibacterial parameters later. Apart from *P. Aeruginosa*, compounds **3a, 3b** are non-sensitive to the three others *K. pneumoniae, E. coli,* and *S. aureus*. For that, we went further than the inhibition diameter as antibacterial parameters to explore in detail two criteria (MIC and BMC) on only two strains *P. Aeruginosa* on which all of them were active, and *E. coli* upon which **3b** was very sensitive, in **TABLE 2** below:

TABLE 2**: Antibacterial parameters (MIC and BMC) of chemicals derived from S-alkylation benzimidazole on clinical strains.**

Clinical Strains	**3a** (µg/mL)				**3b** (µg/mL)			
	MIC	BMC	BMC/MIC	Effect	MIC	BMC	BMC/MIC	Effect
P.aeruginosa	1	2	2	bc	-	-	-	-
E. coli	-	-	-	-	1	4	4	bc

-Non-determined. bc means bactericidal, bs means bacteriostatic

The 2-((methylthio)methyl)-1H-benzimidazole **3a** was bactericidal on *P. aeruginosa* and 2-((isobutylthio)methyl)-1H-benzimidazole **3b** showed bactericidal behavior on *E. coli*. These results are per with the behavior observed with the inhibition diameters.

After knowing the role and antibacterial activity of benzimidazole derivatives designed by substitution in position-2 by thioalkyl groups while inserting a methylene group between the carbon in the 2-position of benzimidazole and the sulfur atom. The aim was to explore the role for the second time of these benzimidazole derivatives combined with the piperidine group via the Mannich reaction. The addition of piperidine yield on compounds 4. We explore their activity on the last same three strains. The inhibition diameters of the 2-(thioalkyl)-methyl-1-(piperidin-1-ylmethyl) benzimidazole derivatives **4a, c, f, e**, and **4g** compounds have been described in **TABLE 3** below:

TABLE 3**: Inhibition diameters zones of Mannich bases on clinical strains (average ± sd)**

Clinical Strains	**Inhibition Diameters (mm)**				
	4a	**4c**	**4f**	**4e**	**4g**
P. aeruginosa	20.0 ± 0.8	12.2 ± 0.6	14.7 ± 1.7	18.2 ± 0.6	17.0 ± 0.0
K. Pneumoniae	22.0 ± 1.6	24.3 ± 0.5	22.3 ± 1.9	13.2 ± 1.1	10.2 ± 0.3
E. coli	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0
S. aureus	15.5 ± 0.4	25.0 ± 0.8	21.0 ± 0.0	17.0 ± 0.4	18.2 ± 0.3

The addition of piperidine by Mannich reaction decreased completely the inhibition diameters observed before for *E. coli*. The trend observed is still the same against *P. aeruginosa* but surprisingly for 4e in comparison with the parent molecules **3e** which became 25fold times very sensible (effective) from 0.7 ± 0.2 mm to 18.2 ± 0.6 mm. This showing, the influence of piperidine on the increasing of inhibition diameters on *P. aeruginosa*. This is even more visible in the case of *S.aureus* where an even greater value is observed. The inhibition diameter is changed from 4- to 30-fold times greater (34-fold even for compound **4e** vs **3e**). This allows us to explore both parameters, the minimum inhibitory concentration (MIC), bactericidal minimum concentration (BMC), and ratio (BMC/MIC) of each compound tested using the liquid macrodilution method were reported in **TABLE 4** below:

TABLE 4**: Average values (n=3 ± SD) of antibacterial parameters (MIC and BMC) for 4a, c, f, and g on the clinical strain P. aeruginosa**

	P. Aeruginosa			
Compounds	**MIC (µg/mL)**	**BMC (µg/mL)**	**BMC/ MIC**	**Effect**
4a	45	90	2	bc
4c	26.87	26.87	1	bc
4f	22.5	45	2	bc
4g	26.87	26.87	1	bc

On *P. Aeruginosa*, four compounds including **4a, 4c, 4f**, and **4g** showed significant antibacterial activity with MIC ranging from 22.5 to 45 µg/mL and BMC ranging from 26.87 to 90 µg/mL. All of them showed bactericidal potency. Compounds **4c, 4f**, and **4g** are the most potent two-fold compared to compound 4a in terms of MIC. In terms of MICs, the addition of piperidine did not increase the efficiency or the potency of antibacterial activity looking at the MIC or the rate BMC/MIC but the presence of piperidine and ester group joined to the Sulphur (S-R), more the potency increases. The results show that the trend observed in inhibitions diameters correlated with the bactericidal effect.

Conclusion

The work carried out has made it possible to confirm the antibacterial activity of 2-thioalkyl methyl benzimidazoles (**3**) and 2(thioalkyl)-methyl-1-(piperidin-1-ylmethyl) benzimidazole (**4**) derivatives on different strains of *S. aureus* but more on *P. aeruginosa.* The CMI and BMC determination revealed a bactericidal effect on *E. coli* and *P. aeruginosa* bacterial strains. In addition, major groups of chemical compounds piperidine plus an ester group as alkyl to the thiol function in position 2, probably responsible for this activity, have been highlighted and are better. Given these results, this 2-((1-(piperidin-1-yl)methyl)-1*H*-benzimidazol-2yl)methylthio)ester could hope in the relief of microbial diseases, a real threat to public health. It is therefore possible to go further with pharmacological studies on *P. aeruginosa*, confirm the results obtained *in vivo* tests, and conduct in-vitro toxicity.

REFERENCES

1. Organisation mondiale de la Santé, « Appel urgent à une meilleure utilisation des vaccins existants et à la mise au point de nouveaux vaccins pour lutter contre la résistance aux antimicrobiens », communiqués de presse, Genève, 12 juillet 2022.
2. Sharma S, Gangal S, Rauf A. Convenient one-pot synthesis of novel 2-substituted benzimidazoles, tetrahydrobenzimidazoles and imidazoles and evaluation of their *in vitro* antibacterial and antifungal activities. European Journal of Medicinal Chemistry. 2009;44(4):1751-7.
3. Tahlan S, Kumar S, Narasimhan B. Antimicrobial potential of *1H*-benzo [d] imidazole scaffold: a review. BMC chemistry. 2019;13(1):1-27.
4. Redayan MA, Ali WB, Mohammed AM. Synthesis, Characterization and Antibacterial Evaluation of some Novel Benzimidazole Derivatives Containing 1, 3, 4-thiadiazole moiety. Oriental Journal of Chemistry. 2017;33(6):3138.
5. Pieroni M, Tipparaju SK, Lun S, et al. Pyrido [1, 2-a] benzimidazole-based agents active against tuberculosis (TB), multidrug-resistant (MDR) TB and extensively drug-resistant (XDR) TB. ChemMedChem. 2011;6(2):334-42.
6. Lilienkampf A, Pieroni M, Wan B, et al. Rational design of 5-phenyl-3-isoxazolecarboxylic acid ethyl esters as growth inhibitors of Mycobacterium tuberculosis. A potent and selective series for further drug development. Journal of medicinal chemistry. 2010;53(2):678-88.
7. Woolley DW. Some biological effects produced by benzimidazole and their reversal by purines. Journal of Biological Chemistry. 1944;152(2):225-32.
8. Mishra L, Singh VK, Dubey NK, et al. Synthesis and fungicidal activity of some 5-membered heterocyclic derivatives containing benzimidazoles. Bioscience, biotechnology, and biochemistry. 1993;57(6):989-91.
9. Cheong JE, Zaffagni M, Chung I, et al. Synthesis and anticancer activity of novel water soluble benzimidazole carbamates. European journal of medicinal chemistry. 2018 20;144:372-85.
10. Tahlan S, Ramasamy K, Lim SM, et al. 4-(2-(*1H*-Benzo [d] imidazol-2-ylthio) acetamido)-N-(substituted phenyl) benzamides: design, synthesis and biological evaluation. BMC chemistry. 2019;13(1):1-6.
11. Tonelli M, Paglietti G, Boido V, et al. Antiviral activity of benzimidazole derivatives. I. Antiviral activity of 1-substituted-2-[(benzotriazol-1/2-yl) methyl] benzimidazoles. Chemistry & Biodiversity. 2008;5(11):2386-401.
12. Sharma D, Narasimhan B, Kumar P, et.al . Synthesis, antimicrobial and antiviral activity of substituted benzimidazoles. Journal of enzyme inhibition and medicinal chemistry. 2009;24(5):1161-8.
13. Mavrova AT, Anichina KK, Vuchev DI, et al. Antihelminthic activity of some newly synthesized 5 (6)-(un) substituted-*1H*-benzimidazol-2-ylthioacetylpiperazine derivatives. European journal of medicinal chemistry. 2006;41(12):1412-20..
14. Sharma R, Bali A, Chaudhari BB. Synthesis of methanesulphonamido-benzimidazole derivatives as gastro-sparing antiinflammatory agents with antioxidant effect. Bioorganic & Medicinal Chemistry Letters. 2017;27(13):3007-13.
15. El-masry AH, Fahmy HH, Ali Abdelwahed SH. Synthesis and antimicrobial activity of some new benzimidazole derivatives. Molecules. 2000;5(12):1429-38.

16. Vijayaraghavan S, Somani RR, Shirodkar PY, et al. Microwave assisted synthesis and antimicrobial activity of some newer mannich bases. International Journal of PharmTech Research. 2009;1(3):811-5.
17. Kalirajan R, Chitra M, Jubie S, et al. Synthesis and biological evaluation of Mannich bases of 2-substituted Benzimidazoles. Asian Journal of Chemistry. 2009;21(7):5207.
18. Mariappan G, Bhuyan NR, Kumar P, et al. Synthesis and biological evaluation of Mannich bases of benzimidazole derivatives.
19. Taher AT, Khalil NA, Ahmed EM. Synthesis of novel isatin-thiazoline and isatin-benzimidazole conjugates as anti-breast cancer agents. Archives of pharmacal research. 2011;34(10):1615-21.
20. Xu Z, Zhang S, Gao C, et al. Isatin hybrids and their anti-tuberculosis activity. Chinese Chemical Letters. 2017 Feb 1;28(2):159-67.
21. Shingare MS, Mane DV, Shinde DB, Thore SN, Bhawsar SB. Synthesis of Mannich bases of benzimidazole as possible antiviral agents. Asian Journal of Chemistry. 1996;8:225-8.
22. Lakshmi VS. Biological activity of Mannich bases. Indian journal of pharmaceutical sciences. 2003;65(3):213
23. Dosso M, Faye-Kette H. Contrôle de qualité de l'antibiogramme en pratique courante: Expérience du laboratoire de bactériologie de l'Institut Pasteur de Côte d'Ivoire. Bactériolo int, n spécial. 2000;53.
24. Koné MW. Potentiel des plantes médicinales de Côte-d'Ivoire dans le contrôle des haemonchoses chez les ovins. Université de Cocody Abidjan UFR Biosciences Thèse de Doctorat Unique en Biologie végétale, Spécialité: Phytochimie. 2005;224.
25. Ponce AG, Fritz R, Del Valle C, et al. Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. LWT-Food Science and Technology. 2003;36(7):679-84.
26. Kouadio NJ, Guessennd NK, Kone MW, et al. Evaluation de l'activité des feuilles de Mallotus oppositifolius (Geisel.) Müll.-Arg (Euphorbiaceae) sur des bactéries multirésistantes et criblage phytochimique. International Journal of Biological and Chemical Sciences. 2015;9(3):1252-62.
27. Fauchère JL, Avril JL. Bactériologie générale et médicale. Ellipses; 2002.
28. Kamanzi A. Plantes médicinales de Côte d'Ivoire: investigations phytochimiques guidées par des essais biologiques. Doctorat d'Etat ès-Sciences en biologie végétale. UFR Biosciences, Université de Cocody, Abidjan. 2002.
29. Evrard A, Siomenan C, Etienne CT, et al. Design, Synthesis and *in vitro* Antibacterial Activity of 2-thiomethyl-benzimidazole Derivatives. Advances in Biological Chemistry. 2021;11(4):165-77.