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Synthesis and effect of N-alkylation on antibacterial activity of 2-(Benzylthio) methyl-1*H*-benzimidazole derivatives

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Abstract

In pursuit the development of novel potent and selective antibacterial agents, we synthesized twelve (12) N-alkyl 2-benzylthiomethyl-1*H*-benzimidazole derivatives and evaluated their antibacterial activities. Their antibacterial profile was determined with minimal inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) against a small set of two (2) strains *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive). These compounds are produced by the condensation reaction of 2-benzylthiomethyl-1*H*-benzimidazole (5) with benzyl chloride or bromide (6) in the presence of potassium carbonate (K_2CO_3). The panel of twelve synthetized compounds (7a-l) were characterized by NMR ^1H, ^{13}C spectroscopy, and high-resolution mass spectrometry (HRMS). The results showed that compounds 7a, 7b, 7c, 7d, 7e, 7f, 7h, 7k, and 7l were potent against *Escherichia coli* and *Staphylococcus aureus*, with significant MICs values from 140 to 290 µg/mL. On *E. coli*, five (5) compounds 7b, 7f, 7i, 7k and 7l showed bactericidal effects within common an N-alkylation by R_3= phenyl, methyl, and CH_2OH on the benzimidazole scaffold and the benzylthiol substituted by R_2= Cl or CF_3. This is evidence or a probe of these chemical groups implementing the bactericidal activity.

Keywords: 2-Benzythiomethyl-1*H*-benzimidazole; Antibacterial activity; Bactericidal effect; Bacteriostatic effect; *Escherichia coli; Staphylococcus aureus*

1. Introduction

Heterocyclic compounds are a family of molecules that occupy a privileged position in medicinal chemistry. They are also particularly interesting and important in the development of new bioactive molecules in the pharmaceutical field [1-2]. Unfortunately, most of the bioactive molecules are ineffective against these bacteria. If the trend continues, we can expect an exponential increase in antibiotic multiresistance (AMR), which must be prevented or significantly reduced. The time between the development of drug resistance and the appearance of drug is still too long (almost ten years on average) [3]. The aim of researchers around the world is to reduce or close this gap and bring drugs to market quickly. The task is to decrease this gap time and many options coexist. Among the existing solutions, one challenge may be to inactivate the bacteria by modifying existing drugs. Or we use molecular docking to better identify lead compounds as drug candidates. The issue on that, it is daily getting harder, to find the best drug that, after synthesis, becomes a commercial drug with all the good bioactive parameters (functionality, toxicity, broad spectrum, and environmentally friendly). There is still an urgent need to develop alternative drugs to stop this resistance challenge via existing drugs.

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Within this family of heterocyclic compounds, benzimidazole derivatives are the most biological actives [4-9] interesting as they result from the juxtaposition of benzene and imidazole rings. They have been of particular interest to many researchers given their diverse biological activities including antimicrobial [10-12], antiviral [13, 14], anticancer [15-17], anti-inflammatory [18-20], and antioxidant [21]. Benzimidazole derivatives are also proven to be effective proton pump inhibitors [22], stage modulators [23], and antidiabetics [24, 25]. The benzimidazole moiety is present in commercial drugs such as Albendazole, Mebendazole and Thiabendazole used as anthelmintic [26]. Currently, the most important benzimidazole derivative is the natural N-ribosyl-dimethylbenzimidazole, which functions as a cobalt axis ligand in vitamin B12 [27]. N-alkylated and 2-substituted benzimidazole derivatives have shown strong activity in various pharmaceutical fields and are also used in lighting devices [28]. The aim of this work was to synthesize and determine the antibacterial activities of new N-alkyl/aryl 2-benzylthiomethyl-1*H*-benzimidazole from specific 2-benzylthiomethyl-1*H*-benzimidazole derivatives, although strains dependent on the derivatives could be studied in full detail in the perspectives.

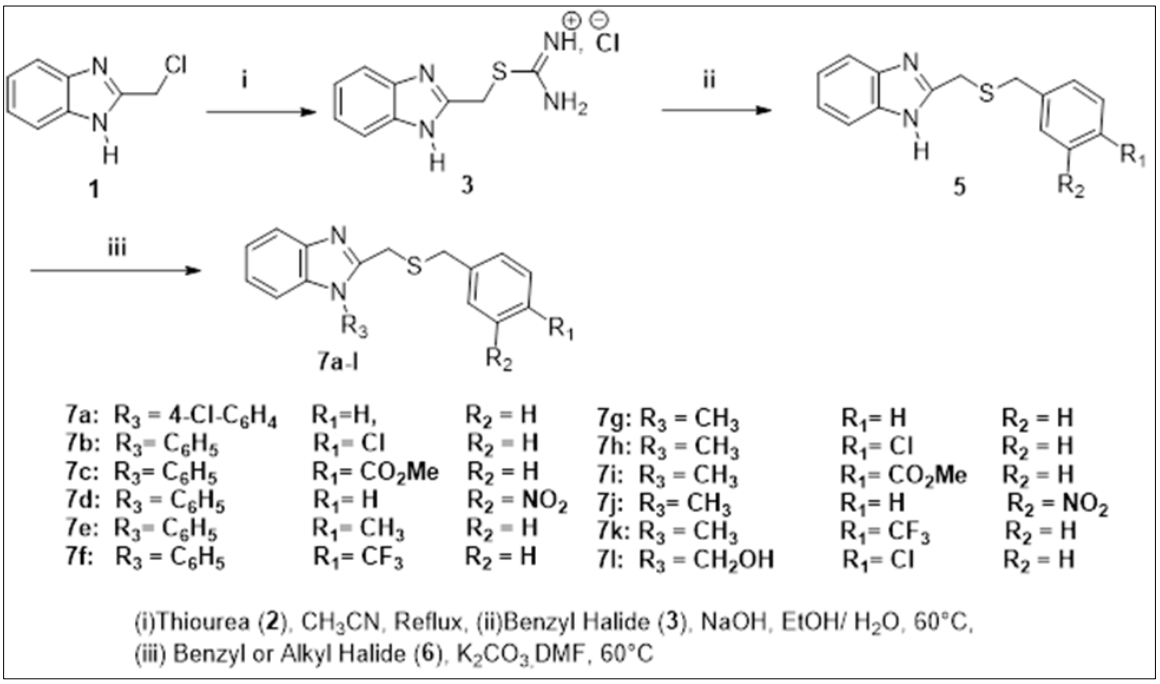

Figure 1 Synthesis of N-alkyl-2-benzylthiomethyl-1H-benzimidazole derivatives (**7a-l**)

2. Materials and Methods

2.1. Materials

2.1.1. Materials of Chemistry

Unless otherwise indicated, ^{1}H and ^{13}C NMR spectra were recorded at 400 and 101 MHz or 600 and 151 MHz, respectively, in $CDCl_3$, solution. For ^{1}H NMR assignments, the chemical shifts are reported in ppm on the δ scale. The following notation is used for the ^{1}H NMR spectral splitting patterns: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quadruplet), m (multiplet) and further qualified as app (apparent), br (broad) coupling constants, *J* are reported in Hz. HRMS were measured in the electrospray (ESI) mode on a LC-MSD TOF mass analyzer.

2.1.2. Biological Materials

The microbial support consisted of clinical strains of *E. coli* (Gram-negative bacteria) and *S. aureus* (Gram-positive bacteria) which are resistant to Piperacillin and Erythromycin, respectively. The strains were supplied by the Laboratory of Bacteriology-Virology of the Pasteur Institute in Côte d'Ivoire. These strains are all pathogenic and multi-resistant. *S. aureus* strains are resistant to Erythromycin, sensitive to Cefoxitin and Clindamycin. Those of *E. coli* are all resistant to Piperacillin/Tazobactam, sensitive to Ceftriaxone and Ticarcillin/Clavulanic Acid. The culture medium used was Mueller-Hinton broth (Oxoid) and Mueller-Hinton agar (Lab.Conda s.a). Dimethyl sulfoxide (DMSO) and distilled water were used as solvents for chemical solubilization.

2.2. Methods

2.2.1. Methods of Synthesis

Synthesis method of 2 methyl-1*H*-benzamidazole thiourunium chloride salt (3)

To a solution of 2-(chloromethyl)-1*H*-benzimidazole (1 eq, 57.2 mmol) in 50 mL of acetonitrile, thiourea (1 eq, 57.2 mmol) was added. The mixture was brought to reflux for 1.5h. After cooling to room temperature, a precipitate was formed, filtered, washed several times with ethyl acetate and then dried in the open air to afford yellow powder. Yield = 88%, HRMS (ESI): Calc for $C_9H_{12}ClN_4S$ (M+H) +: 243.1127, Found: 243.1132

Synthesis method of 2-((thiobenzyl) methyl)-1*H*-benzimidazole (5)

To a solution of 2-methylbenzimidazole thiourunium chloride salt (1 eq, 2.1 mmol) in 15 mL of absolute ethanol was added (2.5 eq, 0.35N) of sodium hydroxide solution. The mixture was stirred under reflux, then benzyl chloride derivative (1.2 eq, 2.52 mmol) was added. The reaction stayed like this for two more hours. After cooling to room temperature, the mixture was diluted in dichloromethane and washed several times with water. The organic phase was dried over anhydrous Na_2SO_4 and the solvent was evaporated *in vacuo*. The residue obtained after evaporation of solvent was purified by silica column chromatography (hexane / ethyl acetate: 85 / 15) to give compound 5.

General procedure for the synthesis of N-benzyl-2-(benzylthio) methyl benzimidazoles 7a-l

To a solution of 2-methylbenzimidazole thiourunium chloride salt (3) (1 eq, 2.1 mmol) in 15 mL of absolute ethanol was added (2.5 eq, 0.35N) of sodium hydroxide solution. The mixture was stirred under reflux, then benzyl chloride derivative (1.2 eq, 2.52 mmol) were added. The reaction stayed like this for two more hours. After cooling to room temperature, the mixture was diluted in dichloromethane and washed several times with water. The organic phase was dried over anhydrous Na_2SO_4 and the solvent was evaporated *in vacuo*. The crude was used *in situ* without purification, then added in a round bottom flask, dissolved and stirred in 5 mL of dimethylformamide (DMF). Furthermore (4.74 mmol, 6eq) potassium carbonate (K_2CO_3) were also added in to the flask. The reaction mixture was left under magnetic agitation at room temperature for 1 hour. After that benzyl halide (3.16 mmol, 4eq) was dropwise added and the mixture was heated to 70°C for 2 hours. At the end of the reaction, ice water was added and the formed precipitate was filtered, dried over $MgSO_4$ and purified by silica gel chromatography (Hexane/CH_2Cl_2 80:20) to give the compounds (7a-l) with yields between 50 and 80%.

((benzylthio)methyl) -1-(4-chlorobenzyl) -1*H*-benzoimidazole 7a

Khaki powder, Yield = 61%, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.85–7.78 (m, 1H, H_{Ar}), 7.37–7.16 (m, 10H, H_{Ar}), 7.01–6.93 (m, 2H, H_{Ar}), 5.36 (s, 2H, N-CH_2), 3.86 (s, 2H, CH_2), 3.80 (s, 2H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 150.64, 137.47, 135.55, 134.16, 133.87, 129.16, 129.06, 128.50, 127.77, 127.15, 123.23, 122.59, 119.74, 109.66, 46.65, 35.98, 27.56. HRMS(ESI): Calc for $C_{22}H_{20}ClN_2S$ (M+H) $^+$: 379.1656, Found: 379.1661

1-benzyl-2-(((4-chlorobenzyl) thio) methyl) -1*H*-benzimidazole 7b

Oil, Yield = 61%, ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.82 (dd, *J*=8.0, 3.2 Hz, 2H, H_{Ar}), 7.3 –7.19 (m, 9H, H_{Ar}), 7.04 (dd, *J* =7.0, 4.9 Hz, 2H, H_{Ar}), 5.42 (s, 2H, N-CH_2), 3.84 (s, 2H, CH_2), 3.75 (s, 2H, CH_2). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 150.54, 141.98, 136.14, 135.82, 135.69, 132.89, 130.41, 129.01, 128.57, 128.00, 126.33, 123.08, 122.44, 119.66, 109.75, 47.20, 35.07, 27.52. HRMS (ESI): Calc for $C_{22}H_{20}ClN_2S$ (M+H) $^+$: 379.1656, Found: 379.1661

Methyl 4-((((1-benzyl-1*H*-benzimidazol-2yl) methyl) thio) methyl) benzoate 3c

White powder, Yield = 75%, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.93–7.89 (m, 2H, H_{Ar}), 7.83–7.80 (m, 1H, H_{Ar}), 7.40 (d, *J* = 8.4 Hz, 2H, H_{Ar}), 7.35–7.21 (m, 6H, H_{Ar}), 7.07 – 7.01 (m, 2H, H_{Ar}), 5.42 (s, 2H, N-CH_2), 3.92 (s, 3H, CH_3-O), 3.88 (s, 2H, CH_2), 3.86 (s, 2H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 166.75, 150.35, 142.88, 135.53, 129.69, 129.05, 129.01, 128.09, 126.32, 123.36, 122.73, 119.43, 109.83, 52.05, 47.25, 35.57, and 27.48. HRMS (ESI): Calc for $C_{24}H_{23}N_2S$ (M+H)$^+$: 403.1143, Found: 403.1147

1-benzyl-2-(((3-nitrobenzyl)thio)methyl)-1*H*-benzimidazole 7d

Yellowish-orange powder, Yield = 75%, ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.17 (s, 1H, H_{Ar}), 7.95 (dd, *J* = 8.2, 1.4 Hz, 1H, H_{Ar}), 7.75 (dd, *J* = 6.8, 1.6 Hz, 1H, H_{Ar}), 7.67 (d, *J* = 7.6 Hz, 1H, H_{Ar}), 7.39–7.17 (m, 7H, H_{Ar}), 7.08–7.01 (m, 2H, H_{Ar}), 5.46 (s, 2H, N-CH_2), 3.88 (s, 2H, CH_2), 3.87 (s, 2H, CH_2). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 150.06, 148.08, 141.78, 139.82,

135.71, 135.57, 135.13, 129.05, 128.07, 126.29, 123.59, 123.28, 122.49, 121.84, 119.64, 109.68, 47.24, 35.00, and 27.85. HRMS (ESI): Calc for $C_{22}H_{20}N_3O_2S$ $(M+H)^+$: 390.1577, Found: 390.1581

1-benzyl-2-(((4-methylbenzyl)thio)methyl)-1*H*-benzimidazole 7e

White powder, Yield = 80%, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):7.84–7.81 (m, 1H, H_{Ar}), 7.35–7.20 (m, 8H, H_{Ar}), 7.04 (dd, *J* = 9.0, 5.7 Hz, 4H, H_{Ar}), 5.42 (s, 2H, N-CH_2), 3.89 (s, 2H, CH_2), 3.79 (s, 2H, CH_2), 2.30 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 150.79, 136.78, 135.56, 134.34, 129.15, 128.99, 128.93, 128.00, 126.39, 122.58, 119.44, 109.81, 47.26, 35.81, 27.52, 21,07. HRMS (ESI): Calc for $C_{23}H_{23}N_2S$ $(M+H)^+$: 359.2128, Found: 359.2132

1-benzyl-2-(((4-(trifluoromethyl) benzyl)thio) methyl)-1*H*-benzimidazole 7f

White powder, Yield = 85%, ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.83–7.80 (m, 1H, H_{Ar}), 7.48 (dd, *J* = 20.1, 8.2 Hz, 4H, H_{Ar}), 7.34–7.21 (m, 6H, H_{Ar}), 7.08 – 7.02 (m, 2H, H_{Ar}), 5.42 (s, 2H, N-CH_2), 3.84 (s, 2H, CH_2), 3.83 (s, 2H, CH_2). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 150.34, 141.82, 135.79, 135.61, 129.35, 129.03, 128.04, 126.31, 125.32, 123.22, 122.49, 119.65, 109.73, 47.18, 35.16, and 27.58. HRMS (ESI): Calc for $C_{23}H_{20}F_3N_2S$ $(M+H)^+$: 413.0821, Found: 413.0823

2-((benzylthio) methyl)-1-ethyl-1*H*-benzimidazole 7g

Oil, Yield = 82%, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.83–7.75 (m, 2H, H_{Ar}), 7.50–7.14 (m, 7H, H_{Ar}), 4.20 (q, *J* = 7.4 Hz, 2H, -N-CH_2), 3.97 (s, 2H, CH_2), 3.82 (s, 2H, CH_2), 1.44 (td, *J* = 7.4, 1.1 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 162.55, 150.26, 142.36, 137.53, 134.80, 130.64, 129.09, 128.92, 128.46, 127.09, 122.71, 122.40, 119.36, 110.04, 38.89, 36.03, 27.20, and 14.85. HRMS (ESI): Calc for $C_{17}H_{19}N_2S$ (M+H) +: 283.2527, Found: 283.2531

2-(((4-chlorobenzyl) thio) methyl)-1-ethyl-1*H*-benzimidazole 7h

White powder, Yield = 75%, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.81 – 7.73 (m, 1H, H_{Ar}), 7.40 – 7.18 (m, 7H, H_{Ar}), 4.19 (q, *J* = 7.4 Hz, 2H, -N-CH_2), 3.90 (s, 2H, CH_2), 3.74 (s, 2H, CH_2), 1.44 (t, *J* = 7.4 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 150.03, 142.11, 136.21, 135.01, 132.87, 130.47, 128.57, 122.78, 122.17, 119.59, 109.47, 38.82, 35.00, 27.24, and 14.92. HRMS (ESI): Calc for $C_{17}H_{18}ClN_2S$ $(M+H)^+$: 317.1125, Found: 317.1129

Benzoate 4-methyl (((1-ethyl-1*H*-benzimidazol-2-yl) methyl) thio) methyl 7i

Oil, Yield = 75%, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.18 (d, *J* = 8.9 Hz, 2H, H_{Ar}), 7.57 (d, *J* = 8.8 Hz, 2H, H_{Ar}), 7.41– 6.97 (m, 4H, H_{Ar}), 4.16 (q, *J* = 7.3 Hz, 2H, N-CH_2), 3.96 (s, 2H, CH_2), 3.91 (s, CH_3O-), 3.75 (s, 2H, CH_2), 1.44 (t, *J* = 7.3 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 167.35, 157.57, 143.27, 138.80, 136.13, 132.11, 129.60, 129.60, 128.47, 128.47, 123.11, 122.23, 119.67, 113.22, 52.08, 42.25, 35.57, 27.48, and 14.80. HRMS (ESI): Calc for $C_{19}H_{21}N_2O_2S$ (M+H) +: 341.1682, Found: 341.1687

3-(((nitrobenzyl) thio) methyl) -1*H*-benzimidazole 7j

Oil, Yield = 86%, ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.08 (d, *J* = 12.1 Hz, 1H, H_{Ar}), 7.93 (s, 1H, H_{Ar}), 7,87 (dd, *J* = 12.1, 7.5 Hz, 1H, H_{Ar}), 7.60 (d, *J* = 7.5 Hz, 1H, H_{Ar}), 7.34 –7.12 (m, 4H, H_{Ar}), 4.16 (q, *J* = 7.3 Hz, 2H, -N-CH_2), 3.86 (s, 2H, CH_2), 3.79 (s, 2H, CH_2), 1.37 (t, *J* = 7.3 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 149.55, 148.00, 142.01, 139.92, 129.06, 123.59, 122.76, 122.06, 121.75, 119.51, 109.42, 38.71, 34.83, 27.43, and 14.86. HRMS (ESI): Calc for $C_{17}H_{18}N_3O_2S$ (M+H) +: 328. 2133, Found: 328.2138

1-ethyl-2-(((4-(trifluoromethyl) benzyl) thio) methyl)-1*H*-benzimidazole 7k

Oil, Yield = 80%, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.82–7.73 (m, 1H, H_{Ar}), 7.50 (dd, *J* = 20.7, 8.2 Hz, 4H, H_{Ar}), 7.37–7.25 (m, 3H, H_{Ar}), 4.20 (q, *J* = 7.3 Hz, 2H, N-CH_2,), 3.90 (s, 2H, CH_2), 3.81 (s, 2H, CH_2), 1.44 (t, *J* = 7.3 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 149.84, 142.31, 141.93, 135.07, 129.42, 125.34, 125.30, 122.78, 122.13, 119.67, 109.44, 38.78, 35.06, 27.32, and 14.90. HRMS (ESI): Calc for $C_{18}H_{18}F_3N_2S$ $(M+H)^+$: 351.2128, Found: 351.2132

2-(2-(((4-chlorobenzyl) thio) methyl)-1*H*-benzimidazol-1-yl) ethan-1-ol 7l

Yellow powder, Yield = 59%, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.97-7.65 (m, 2H, H_{Ar}), 7.55 (d, *J* = 7.7 Hz, 2H, H_{Ar}), 7.41–7.10 (m, 4H, H_{Ar}), 4.30 (t, *J* = 5.1 Hz, 2H, N-CH_2), 3.97 (t, *J* = 5.1 Hz, 2H, CH_2-O), 3.84 (s, 2H, CH_2), 3.64 (s, 2H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 162.50, 155.68, 151.18, 141.12, 136.07, 135.04, 132.77, 130.34, 128.52, 123.03, 122.46, 118.97, 109.64, 60.56, 46.50, 34.96, and 27.18. HRMS (ESI): Calc for $C_{17}H_{18}ClN_2OS$ (M+H) +: 333.1125, Found: 333.1131

2.2.2. Biological Methods

The liquid microdilution method was used to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). A colony isolated from an 18 hours' bacterial culture was collected and homogenized in 10 mL of 0.9% NaCl and incubated for 3 hours at 37°C. From the bacterial suspension, 0.1 mL was added to 10 mL of 0.9 NaCl solution. This prepared bacterial suspension constituted the starting bacterial inoculum. To do this, the bacterial inoculum was homogenized and diluted from 10 to 10 until dilution 10^{-4}. Four successive dilutions were obtained from 10-1 to 10-4. The initial bacterial inoculum and the 4 successive dilutions were inoculated with a 2 µL calibrated loop in Muller-Hinton agar boxes with 5 cm long streaks. This preparation represents Box (A) that will help to determine the MBC. Antibacterial testing was conducted using the liquid microdilution method [29]. Final concentrations ranging from 100 to 3.12 mg/mL were achieved. In a series of 5 test tubes, a growth control tube and a sterility control tube, a volume of one milliliter of an extract known concentration from the concentration range was added to the test tubes. The growth control tube received 0.5 mL of sterile distilled water while all test tubes received 0.5 mL of bacterial inoculum. The sterility control tube received 1 mL of 0.9% NaCl solution. The tubes were incubated for 24 hours at 37 °C. The MIC is the lowest concentration of extract for which no bacterial growth is observed. The contents of the tubes in which there was no visible growth were used to seed the Muller-Hinton agar on 5 cm ridges using a 2 µL calibrated loop. This Petri dish is called B. Analysis of the results after 24 hours of incubation allowed to calculate the CMB which corresponds to the lowest concentration that kills 99.99% of the bacteria in culture.

3. Results and Discussion

3.1. Chemistry

The synthesis of novel 2-benzylthiomethyl-1*H*-benzimidazole derivatives (7a-l) was carried out in three reactions steps. First, by the interaction between 2-chlorobenzimidazole (1) and thiourea (2) in CH_3CN under reflux. We obtained the 2-methylbenzimidazolethiouronium chloride salt (3) which reacts with different benzyl halides (4) to obtain the 2-benzylthiomethyl-1*H*-benzimidazole derivatives (5). To obtain the best yield, crude derivative of 2-benzylthiomethyl-1*H*-benzimidazole (5) react *in situ* with benzyl chloride or ethyl bromide (6) in the presence of potassium carbonate (K_2CO_3) in hot dimethylformamide (DMF) to give compound (7). This last step for position-1 modification of the 2-benzylthiomethyl-1*H*-benzimidazole derivative, is performed by introduction of benzyl or alkyl group following a N-alkylation reaction described by Elaridi et *al*. [30] (Figure 1). All compounds obtained were characterized by the ^1H, ^{13}C NMR spectroscopy and HRMS.

On the ^1H NMR spectra of the 7a-f compounds, protons of the methylene group bound to the nitrogen atom (N-CH_2) of 2-benzylthiomethyl-1*H*-benzimidazole were observed as singlet around 5.35 ppm. The ^{13}C NMR spectra of the compounds (7a-f) revealed the presence of a peak around 45 ppm assignable to the carbon of this same methylene group directly bound to the nitrogen atom (CH_2-N).

On the ^1H NMR spectrum of compounds (7g-k), the protons of the nitrogen-bonded methylene group (N-CH_2) appear as quadruplets around 4.20 ppm with a coupling constant (J = 7.3Hz). We also note the presence of triplets around 1.44 ppm with a coupling constant (J = 7.3Hz) corresponding to the three protons of methyl group (CH_3) directly bound to the group (-CH_2-N). The ^{13}C NMR spectrum shows two peaks representing the compound formation probe (7g-k). One of these is about 38 ppm due to the carbon of the methylene group attached to the nitrogen atom (CH_2-N) and the other one in the vicinity of 14 ppm indicating the presence of methyl group carbon (CH_3). Both signals confirm the binding of the ethyl group to the nitrogen at the position-1 of the 2-benzylthiomethyl-1*H*-benzimidazole derivatives.

The ^1H NMR spectral analysis of compound (**7l**) indicated a triplet signal at 4.30 ppm with a coupling constant (J= 5.1Hz) corresponding to the protons of the nitrogen-bound methylene group (CH_2-N). Another triplet at 3.97 ppm with a coupling constant (J = 5.1 Hz) was also observed and assigned to the two methylene protons group bound to the hydroxy (CH_2-OH). This coupling constant confirms the argument that presence of the two methylene (CH_2-N and CH_2-OH) and are linked to each other. The ^{13}C NMR spectrum shows the presence of methylene carbons bound to nitrogen and hydroxyl function by two peaks, one occurring at 60.56 ppm assignable to (CH_2-N) and the second one at 46.56 ppm corresponding to the carbon (CH_2-OH).

3.2. Biology

The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and ratio (MBC/MIC) of each compound tested using the liquid microdilution method were reported in the Table 1 below:

Table 1 Bactericidal and Bacteriostatic effects of obtained compounds 7a-l on bacteria strains *E. coli* and *S. aureus* determined by MIC and MBC values

Compounds	*S. aureus* 2275C2021				*E. coli* 2279C2021			
	MIC (µg/mL)	MBC (µg/mL)	MBC/ MIC	Effect	MIC (µg/mL)	MBC (µg/mL)	MBC/ MIC	Effect
7a	280	570	2	bc	-	-	-	-
7b	200	830	4	bs	200	830	4	bs
7c	140	580	4	bs	-	-	-	-
7d	280	570	2	bc	-	-	-	-
7e	150	630	4	bs	-	-	-	-
7f	140	580	4	bs	290	580	2	bc
7h	210	860	4	bs	-	-	-	-
7i	-	-	-	-	200	810	4	bs
7k	290	580	2	bc	140	580	4	bs
7l	210	840	4	bs	210	840	4	bs

Non-determined. bc means bactericidal, bs means bacteriostatic

On *S. aureus* 2275C2021, compounds 7a, 7b, 7c, 7d, 7e, 7f, 7h, 7k, and 7l showed significant antibacterial activity with MIC ranging from 140 to 290 µg/mL. Of these nine compounds, the ratios (MBC/MIC) showed that compounds 7a, 7d, and 7k exhibited bactericidal potency. Compounds 7b, 7c, 7e, 7f, 7g, and 7l showed bacteriostatic effects. On the bacterial strain *E. coli* 2279C2021, five compounds, namely 7b, 7f, 7i, 7k and 7l, showed a significant antibacterial effect with MIC ranging from 140 to 290 µg/mL. The reports (MBC/MIC) showed that only compound 3f showed bactericidal potency, while the other compounds 7b, 7i, 7k, and 7l showed bacteriostatic potency. In general, N-alkylation improved antibacterial activity on *S. aureus* and *E. coli*. The MIC values for 2-benzylthiomethyl-1*H*-benzimidazole derivatives range from 140 to 320 µg/mL for *S. aureus* and 140 to 400 µg/mL for *E. coli*. When the benzyl group was introduced at the -1 position of 2-benzylthiomethyl-1*H*-benzimidazole derivatives, compounds 7a, 7b, 7c, 7d, 7e, and 7f were active on *S. aureus* and two (02) were active on *E. coli* (7b and 7f). N-benzylation was found to improve the antibacterial activity on *S. aureus* more than on *E. coli*.

4. Conclusion

In this work, the synthesis of twelve (12) new N-alkyl 2-benzylthiomethyl-1*H*-benzimidazole derivatives (7a-l) was performed starting from the corresponding 2-benzylthiomethyl-1*H*-benzimidazole and various halogenated benzyl or alkyl. The performance yield of the products was between 50 and 86%. All compounds have been characterized and confirmed by NMR spectroscopic analysis (^{1}H, ^{13}C NMR) and high-resolution mass spectroscopy (HRMS). Ten (10) of the compounds described were subjected to antibacterial test in two strains of *E. coli* and *S. aureus.* Compounds 7a, 7d and 7k showed bactericidal activity on *S. aureus*, while compounds 7b, 7c, 7e, 7f, 7g, and 7l also showed bacteriostatic activity. On the other hand, derivative 7f showed bactericidal activity and 7b, 7i, 7k and 7l derivatives showed bacteriostatic activity against *E. coli* bacterial strain. From this, we can summarize the antibacterial activity enhanced by N-alkylation on *S. aureus* and *E. coli*. However, the presence of an electron-withdrawing group (EWG) such as chloride, nitro, or trifluoromethyl in S-benzyl plus N-alkylation implements in a better way the antibactericidal activity on *S. aureus* compared to *E. coli*.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest regarding the publication of this paper.

Author Contributions

P.A A. performed the syntheses. S.C. and S.C participated in the formulation and direction of the project. S.C, F.B conceptualized the synthesis and A.E purification method. F.K.K conduct the biological experiments. S.C, S.C., and P.A.A wrote the article. A.A supervised the project. All authors have read and accepted the published the manuscript version.

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