Scientific Research Publishing

Advances in Biological Chemistry, 2021, 11, 165-177
https://www.scirp.org/journal/abc
ISSN Online: 2162-2191
ISSN Print: 2162-2183

Design, Synthesis and *in Vitro* Antibacterial Activity of 2-thiomethyl-benzimidazole Derivatives

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How to cite this paper: Evrard, A., Siomenan, C., Etienne, C.T., Daouda, T., Souleymane, C., Drissa, S. and Ané, A. (2021) Design, Synthesis and *in Vitro* Antibacterial Activity of 2-thiomethyl-benzimidazole Derivatives. *Advances in Biological Chemistry*, **11**, 165-177.
https://doi.org/10.4236/abc.2021.114012

Received: July 28, 2021
Accepted: August 24, 2021
Published: August 27, 2021

Abstract

A series of novel substituted benzimidazole **(7a - n)** derivatives were synthesized and characterized by ^{1}H, ^{13}C Nuclear Magnetic Resonance (NMR) spectra and High Resolution Mass Spectrometry (HRMS). The substitution was done in position -1 and -2 by appropriate groups. These compounds are obtained by N-alkylation reaction with thiomethyl-1*H*-benzimidazole intermediates **(5a - g)**. Design of intermediates **(5a - g)** was made by condensation reaction between 2-methylbenzimidazole thiourunium salt **(3)** and functionalized halides **(4)** in the presence of sodium hydroxide (NaOH). Among the twenty-one compounds synthesized, ten were evaluated for their antimicrobial activity on three bacterial strains namely: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. Only *E. coli* ATTC 25922 was susceptible to the synthesized derivatives **5g**, **7f** and **7h** with a significant antibacterial activity (CMI is between 250 and 500 µg/mL).

Keywords

Antibacterial Activity, Benzimidazole, 2-Methylbenzimidazole Thiouronium, Halide

1. Introduction

The benzimidazole scaffold is a significant pharmacophore with great interest due to its broad spectrum of biological activities [1] [2]. Its presence in a vast list of drugs such as etomidate, cimetidine, omeprazole, lansoprazole, azomycin,

flumazenil, thyroliberin, methimazole, pilocarpine and etomidate in played the role of pharmacophore or as a substituent group [3]. A large number of works done towards benzimidazole scaffold became patents for a majority of biological properties, ranging from antitumor [4], anti-inflammatory [5] [6], anticancer [7] [8] and antifungal activities [9] [10] [11]. Among the structural variations on this ring, those affecting 1, 2 and 5 positions were very important for their pharmacological effect. Indeed, the optimization of the biological properties depends on the nature of the substituents on these positions [12] [13]. Recent studies showed that presence of a thiol group in 2 position enhanced biological activities such as antimicrobial [14], inflammatory [15], antiviral [16], antibacterial [17], antioxidant [18] [19], anticancer [20] and anti-proliferative [21]. It should be noted that, despite the large therapeutic arsenal, there is still an efficiency limit effects proved by the increase in strains resistant to bacteria. In this case, the design and synthesis of new antibacterial agents based on the flexibility of the benzimidazole scaffold became essential. Therefore, in this article, we designed new thiomethylbenzimidazole derivatives by introducing diverse substituents on 1 and 2 positions. The originality of our work lied in the introduction of the methylene group between the C-2 carbon of benzimidazole and the sulfur atom. The *in vitro* antimicrobial evaluation of the obtained compounds was conducted as well. Moreover, a detailed analysis of the structure of compounds would provide an opportunity to understand the structure-activity relationship and to identify a more advantageous option. Obtained results may be used for purposeful search of chemotherapeutic agents among compounds finding promising objects for studies aimed at developing compounds with other types of pharmacological activity.

2. Materials and Methods

2.1. Materials

2.1.1. Materials of Chemistry

All reagent-grade chemicals were obtained from commercial suppliers and were used as received. Unless otherwise indicated, ^{1}H and ^{13}C (NMR) spectra were recorded on a Bruker Advance III spectrometer at ^{1}H (300 MHz), ^{13}C (75 MHz) or ^{1}H (400 MHz), ^{13}C (101 MHz) or ^{1}H (600 MHz), ^{13}C (400 MHz), respectively, in $CDCl_3$, DMSO-d6 and Acetone-d6 solutions. 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

Bacterial strains and *inoculum* preparation

The antibacterial tests were carried out on three bacterial mice: *E. coli* ATCC

25922, *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 all from the Bacteriology laboratory of the Pasteur Institute of Côte d'Ivoire and isolated from the gastric fluid of a patient hospitalized at the University Hospital Center of Cocody (Abidjan). The standardization of the bacterial *inoculun* was carried out on colonies of young culture in 0.9% NaCl and measurement of the optical density with DENSIMAT made it possible to obtain an *imoculum* corresponding to approximately 10 CFU/mL.

2.2. Methods

2.2.1. Methods of Synthesis

Synthesis of benzimidazole derivatives

Synthesis methods 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 2 hours. 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 brown crystals, yield = 92%, m.p = 192˚C.

General procedure for the synthesis of 2-((thioalkyl)methyl)-1*H*-benzimidazoles derivatives (5a - g)

To a solution of 2-methylbenzimidazole thiourunium chloride salt (1 eq, 2.61 mmol) in 10 mL of absolute ethanol was added 10 mL of sodium hydroxide solution. The mixture was stirred under reflux, and then an appropriate alkylating agent (1.2 eq, 3.14 mmol) was added. The reaction stayed like this for one more hour. 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: 7/3) to give compounds **5a - g**.

General procedure for the synthesis of N-Alkyl 2-((thioalkyl)méthyl)-1*H*-benzimidazoles derivatives (7a - n)

To a solution of 2-((thioAlkyl)methyl)-1*H*-benzimidazole (1 eq, 10 mmol) in 8 mL of DMF, potassium carbonate (6 eq, 60 mmol) was added and the mixture was stirred at 50˚C for 1 hour. Ethyl or benzyl chloride (4 eq, 40 mmol) was added and the mixture was stirred for 3 hours at 50˚C. The reaction mixture was cooled to room temperature and the organic phase was extracted with dichloromethane, dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue obtained was purified by silica column chromatography (hexane/ethyl acetate: 7/3) to give compounds **7a - n**.

Products characterizations

2-((methylthio)methyl)-1*H*-benzimidazole 5a

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** (400 MHz, Acetone-d_6)

δ 152.27 (C=N), 121.74 (CH-Ar), 28.32 (CH_2-S), 25.40 (S-CH_3). **HRMS (ESI)** Calc. for $C_9H_{10}N_2SNa$ ($M + Na^+$) = 201.0251 Found = 201.0254.

2-((isobutylthio)methyl)-1*H*-benzimidazole 5b

Yellow crystals, yield = 68%, m.p = 126˚C - 126˚C. **^{1}H NMR** (600 MHz, Acetone-d_6) δ 7.55 (t, J = 4.7 Hz, 2H, H-Ar), 7.18 (dd, J = 6.0, 3.1 Hz, 2H, H-Ar), 3.97 (s, 2H, CH_2S), 2.48 (d, J = 6.9 Hz, 2H, SCH_2), 1.80 (m, 1H, CH), 0.93 (d, J = 6.7 Hz, 6H, $2CH_3$). **^{13}C NMR** (400 MHz, Acetone-d_6) δ 152.21 (C=N), 121.71 (CH-Ar), 40.56 (CH), 28.01 (CH_2S), 21.23 (CH_3). **HRMS (ESI)** Calc. for $C_{12}H_{17}N_2S$ ($M + H^+$) = 221.1308 Found = 221.1313.

3-(((1*H*-benzimidazol-2-yl)methyl)thio)propanoate d'éthyle 5c

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** (400 MHz, Acetone-d_6) δ 171.23 (C=O), 151.99 (C=N), 121.82 (CH-Ar), 59.99 (OCH_2), 34.05 (CH_2CO), 28.60 (CH_2S), 26.62 (SCH_2), 13.61 (CH_3). **HRMS** (ESI) Calc. for $C_{15}H_{17}O_2N_2S$ ($M + H^+$) = 261.1054 Found = 261.1058.

2-((ethylthio)methyl)-1*H*-benzimidazole 5d

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). **^{13}C NMR** (400 MHz, Acetone-d_6) δ 152.29 (C=N), 121.76 (CH-Ar), 28.34 (CH_2S), 25.42 (SCH_2), 13.82 (CH_3). **HRMS** (ESI) Calc. for $C_{10}H_{13}N_2S$ ($M + H^+$) = 193.0929 Found = 193.0925.

2-((butylthio)methyl)-1*H*-benzimidazole 5e

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 (C=N), 142.25, 135.01 (Cq-Ar), 122.39, 121.81, 119.47, 109.27 (CH-Ar), 31.33 (CH_2S), 31.02 (SCH_2), 27.87 (CH_2), 21.76 (CH_2), 13.51 (CH_3). **HRMS (ESI)** Calc. for $C_{12}H_{16}N_2SNa$ ($M + Na^+$) = 243.2319 Found = 243.2315.

2-(((1*H*-benzimidazol-2-yl)méthyl)thio)acétate d'éthyle 5f

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** (400 MHz, Acetone-d_6) δ 169.66 (C=O), 151.28 (C=N), 121.83 (Cq-Ar), 60.78 (OCH_2), 32.97 (CH_2), 13.50 (CH_3). **HRMS (ESI)** Calc. for $C_{12}H_{15}N_2O_2S$ ($M + H^+$) = 251.0909 Found = 251.0913.

2-(((1*H*-benzimidazol-2-yl)méthyl)thio)propanoate d'éthyle 5g

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** (400 MHz, Acetone-d_6) δ 172.28 (C=O), 151.47 (C=N), 121.87 (C-Ar), 60.71 (OCH2), 41.03 (CH), 28.60 (CH_2S), 16.63 (CH_2), 13.48 (CH_3). **HRMS (ESI)** Calc. for $C_{15}H_{17}O_2N_2S$ ($M + H^+$) = 265.0909 Found = 265.0905.

N-éthyl-2-((methylthio)methyl)-1*H*-benzimidazole 7a

Yellow oil, yield = 62%. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.36 - 7.32 (m, 1H, H-Ar), 7.28 (d, *J* = 1.9 Hz, 1H, H-Ar), 7.24 (d, *J* = 1.8 Hz, 1H, H-Ar), 4.25 (q, *J* = 7.3 Hz, 2H, CH_2N), 3.92 (s, 2H, CH_2S), 2.13 (s, 3H, CH_3), 1.47 (t, *J* = 7.3 Hz, 3H, CH_3). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 150.09 (C=N), 142.08, 134.96 (Cq-Ar), 122.45, 121.85, 119.41, 109.28 (CH-Ar), 38.62 (CH_2N), 29.41 (CH_2S), 14.93 (CH_3S), 14.75 (CH_3CH_2). **HRMS (ESI)** Calc. for $C_{11}H_{15}N_2S$ ($M + H^+$) = 207.0949 Found = 207.0953.

N-éthyl-2-((méthyllthio)butyl)-1*H*-benzimidazole 7b

Yellow oil, yield = 67%. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.76 - 7.71 (m, 1H, H-Ar), 7.35 (ddd, *J* = 4.5, 2.3, 0.5 Hz, 1H, H-Ar), 7.28 (d, *J* = 1.9 Hz, 1H, H-Ar), 7.25 (d, *J* = 1.8 Hz, 1H, H-Ar), 4.27 (q, *J* = 7.3 Hz, 2H, CH_2N), 3.94 (s, 2H, CH_2S), 1.61 - 1.52 (m, 2H, CH_2), 1.48 (t, *J* = 7.3 Hz, 3H, CH_3), 1.41 - 1.26 (m, 2H, CH_2), 0.87 (t, *J* = 7.3 Hz, 3H, CH_3). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 150.57 (C2), 142.25 (C9), 135.01 (C4), 122.39 (C6), 121.81 (C7), 119.47 (C5), 109.27 (C8), 38.68 (CH_2N), 31.33 (CH_2S), 31.02 (CH_2S), 27.87 (CH_2), 21.76 (CH_2), 14.80 (CH_3), 13.51 (CH_3). **HRMS (ESI)** Calc. for $C_{14}H_{20}N_2SNa$ ($M + Na^+$) = 271.1439 Found = 271.1442.

N-éthyl-2-((méthylthio)éthyl)-1*H*-benzimidazole 7c

Yellow oil, yield = 79%. **^{1}H NMR** (300 MHz,) δ 7.87 - 7.62 (m, 1H, H-Ar), 7.37 - 7.33 (m, 1H, H-Ar), 7.28 (d, *J* = 2.0 Hz, 1H, H-Ar), 7.25 (d, *J* = 1.9 Hz, 1H, H-Ar), 4.27 (q, *J* = 7.3 Hz, 2H, CH_2N), 3.96 (s, 2H, CH_2S), 2.59 (q, *J* = 7.4 Hz, 2H, CH_2CH_3), 1.48 (t, *J* = 7.3 Hz, 3H, CH_3), 1.25 (t, *J* = 7.4 Hz, 3H, CH_3). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 150.59 (CN), 142.26, 135.03 (Cq-Ar), 122.46, 121.88, 119.52, 109.32 (CH-Ar), 38.74 (CH_2N), 27.55 (CH_2S), 25.64 (CH_2), 14.85 (CH_3), 14.28 (CH_3). **HRMS (ESI)** Calc. for $C_{16}H_{17}N_2S$ ($M + H^+$) = 221.1019 Found = 221.1021.

N-éthyl-2-((méthylthio)isobutyl)-1*H*-benzimidazole 7d

Red oil, yield = 75%. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.77 - 7.68 (m, 1H, H-Ar), 7.38 - 7.31 (m, 1H, H-Ar), 7.28 (d, *J* = 1.9 Hz, 1H, H-Ar), 7.25 (d, *J* = 1.9 Hz, 1H, H-Ar), 4.27 (q, *J* = 7.3 Hz, 2H, CH_2N), 3.94 (s, 2H, CH_2S), 2.47 (d, *J* = 6.8 Hz, 2H, CH_2), 1.79 (dt, *J* = 13.4, 6.7 Hz, 1H, CH), 1.48 (t, *J* = 7.3 Hz, 3H, CH_3), 0.93 (d, *J* = 6.6 Hz, 6H, $2CH_3$). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 150.62 (C=N), 142.26, 135.03 (Cq-Ar), 122.41, 121.82, 119.51, 109.30 (CH-Ar), 40.61 (CH_2N), 38.72 (CH_2S), 28.32 (CH_2), 28.10 (CH), 21.81 ($2CH_3$), 14.82 (CH_3).

HRMS (ESI) Calc. for $C_{14}H_{20}N_2SNa$ ($M + Na^+$) = 271.1310 Found = 271.1306

N-benzyl-2-((méthylthio)méthyl)-1*H*-benzimidazole 7e

Red oil, yield = 82%. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.79 (dt, *J* = 7.6, 1.3 Hz, 1H, H-Ar), 7.37 - 7.15 (m, 6H, H-Ar), 7.07 (dd, *J* = 7.3, 2.2 Hz, 2H, H-Ar), 5.47

(s, 2H, CH_2N), 3.84 (s, 2H, CH_2S), 2.13 (s, 3H, CH_3). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 150.55 (C=N), 141.91, 135.71, 135.57 (Cq-Ar), 128.74, 127.69, 126.10, 122.72, 122.03, 119.40, 109.55 (CH-Ar), 46.92 (CH_2N), 29.48 (CH_2S), 14.85 (CH_3). **HRMS (ESI)** Calc. for $C_{16}H_{17}N_2S$ ($M + H^+$) = 269.1019 Found = 269.1015.

N-benzyl-2-((méthylthio)éthyl)-1*H*-benzimidazole 7f

Yellow crystals, yield = 72%, m.p = 96˚C - 98˚C. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.80 (m, J = 7.5, 1.4 Hz, 1H, H-Ar), 7.39 - 7.18 (m, 6H, H-Ar), 7.15 - 7.03 (m, 2H, H-Ar), 5.52 (s, 2H, CH_2N), 3.91 (s, 2H, CH_2S), 2.61 (q, J = 7.4 Hz, 2H, CH_2), 1.26 (t, J = 7.4 Hz, 3H, CH_3). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 151.08 (C=N), 142.19, 135.86 (Cq-Ar), 135.76, 128.85, 127.79, 126.23, 122.76, 122.09, 119.58, 109.61 (CH-Ar), 47.08 (CH_2N), 27.78 (CH_2S), 25.60 (CH_2), 14.25 (CH_3). **HRMS (ESI)** Calc. for $C_{17}H_{18}N_2SNa$ ($M + Na^+$) = 306.1231 Found = 306.1235.

N-benzyl-2-((méthylthio)isobutyl)-1*H*-benzimidazole 7g

Yellow oil, yield = 69%. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.96 - 7.71 (m, 1H, H-Ar), 7.44 - 7.18 (m, 6H, H-Ar), 7.17 - 7.03 (m, 2H, H-Ar), 5.53 (s, 2H, CH_2N), 3.88 (s, 2H, CH_2S), 2.49 (d, J = 6.9 Hz, 2H, CH_2), 1.98 - 1.55 (m, 1H, CH), 0.96 (d, J = 6.6 Hz, 6H, $2CH_3$). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 151.15 (C=N), 142.17, 135.87 (Cq-Ar), 135.79, 128.87, 127.81, 126.28, 122.77, 122.09, 119.59, 109.63 (CH-Ar), 47.09 (CH_2N), 40.59 (CH_2S), 28.56 (CH_2), 28.10 (CH), 21.82 (2 CH_3). **HRMS (ESI)** Calc. for $C_{19}H_{22}N_2SNa$ ($M + Na^+$) = 333.1525 Found = 333.1522.

N-benzyl-2-((méthylthio)butyl)-1*H*-benzimidazole 7h

Yellow crystals, yield = 60%, m.p = 78˚C - 80˚C. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 7.62 (dd, J = 7.5, 1.6 Hz, 1H, H-Ar), 7.38 - 7.33 (m, 1H, H-Ar), 7.31 (dd, J = 8.3, 6.7 Hz, 2H, H-Ar), 7.28 - 7.22 (m, 1H, H-Ar), 7.16 (ddt, J = 7.0, 3.0, 1.8 Hz, 4H, H-Ar), 5.55 (s, 2H, CH_2N), 4.01 (s, 2H, CH_2S), 2.58 - 2.52 (m, 2H, CH_2), 1.52 - 1.43 (m, 2H, CH_2), 1.30 (dt, J = 14.8, 7.4 Hz, 2H, CH_2), 0.82 (t, J = 7.4 Hz, 3H, CH_3). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 152.06 (C=N), 142.59, 137.17 (Cq-Ar), 135.88, 129.06, 127.92, 127.15, 122.68, 122.09, 119.30, 110.99 (CH-Ar), 47.03 (CH_2N), 31.12 (CH_2S), 31.10 (SCH_2), 27.29 (CH_2), 21.69 (CH_2), 13.89 (CH_3). **HRMS (ESI)** Calc. for $C_{19}H_{22}N_2SNa$ ($M + Na^+$) = 333.1551 Found = 333.1552.

N-éthyl-2-(((1*H*-benzimidazol-2-yl)méthyl)thio)acétate d'éthyle 7i

Yellow oil, yield = 64%. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.86 - 7.67 (m, 1H, H-Ar), 7.41 - 7.34 (m, 1H, H-Ar), 7.34 - 7.20 (m, 2H, H-Ar), 4.29 (q, J = 7.3 Hz, 2H, OCH_2), 4.11 (s, 2H, SCH_2CO), 4.10 (q, J = 7.3 Hz, 2H, CH_2N), 3.39 (s, 2H, CH_2S), 1.50 (t, J = 7.3 Hz, 3H, CH_3), 1.23 (t, J = 7.1 Hz, 3H, CH_3). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 169.89 (C=O), 149.57 (C=N), 142.38, 135.05 (Cq-Ar), 122.68, 122.02, 119.69, 109.41 (CH-Ar), 61.46 (OCH_2), 38.77 (SCH_2C=O), 33.16 (CH_2N), 28.14 (CH_2S), 14.96 (CH_3), 14.01 (CH_3). **HRMS (ESI)** Calc. for $C_{14}H_{18}O_2N_2SNa$ ($M + Na^+$) = 301.1009 Found = 301.1011.

N-benzyl-2-(((1*H*-benzimidazol-2-yl)méthyl)thio)acétate d'éthyle 7j

Red oil, yield = 62%. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.90 - 7.72 (m, 1H, H-Ar), 7.51 - 7.22 (m, 6H, H-Ar), 7.22 - 6.98 (m, 2H, H-Ar), 5.52 (s, 2H, CH_2N), 4.10 (q, J = 7.3 Hz, 2H, OCH_2) 4.03 (s, 2H, SCH_2C=O), 3.39 (s, 2H, CH_2S), 1.20 (t, J = 7.1

Hz, 3H, CH_3). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 169.82 (C=O), 150.17 (C=N), 142.29, 135.93, 135.74 (Cq-Ar), 129.00, 127.97, 126.34, 123.06, 122.29, 119.76, 109.74 (CH-Ar), 61.49 (OCH_2), 47.19 (CH_2N), 33.21(SCH_2C=O), 28.47 (CH_2S), 14.00 (CH_3). **HRMS (ESI)** Calc. for $C_{19}H_{20}O_2N_2SNa$ ($M + Na^+$) = 363.1180 Found = 363.1178.

N-éthyl-2-(((1*H*-benzimidazol-2-yl)méthyl)thio)propanoate d'éthyle 7k

Red oil, yield = 60%. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.74 - 7.65 (m, 1H, H-Ar), 7.36 - 7.10 (m, 3H, H-Ar), 4.17 (q, J = 9 Hz, 2H, OCH_2), 4.14 - 4.00 (m, 4H, CH_2N, CH_2S), 3.57 (q, *J* = 7.2 Hz, 1H, CH), 1.46 - 1.35 (m, 6H, $2CH_3$), 1.17 (t, *J* = 7.1 Hz, 3H, CH_3). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 172.69 (C=O), 149.86 (C=N), 142.37, 134.97 (Cq-Ar), 122.69, 122.07, 119.59, 109.50 (CH-Ar), 61.31 (OCH_2), 41.34 (CH_2N), 38.82 (CH), 27.63 (CH_2S), 17.22 (CH_3), 14.97 (CH_3), 14.04 (CH_3). **HRMS (ESI)** Calc. for $C_{15}H_{21}O_2N_2S$ ($M + H^+$) = 293.1147 Found = 293.1150.

N-benzyl-2-(((1*H*-benzimidazol-2-yl)méthyl)thio)propanoate d'éthyle 7l

Red oil, yield = 63%. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.92 - 7.67 (m, 1H, H-Ar), 7.38 - 7.21 (m, 6H, H-Ar), 7.15 - 7.04 (m, 2H, H-Ar), 5.51 (d, *J* = 3.6 Hz, 2H, CH_2N), 4.08 (q, J = 12 Hz, 2H, OCH_2), 4.07 (d, J = 3.6 Hz, 1H, CH_2S), 4.06 (d, J= 3.6 Hz, 1H, CH_2S), 3.61 (q, *J* = 7.2 Hz, 1H, CH), 1.46 (d, *J* = 7.3 Hz, 3H, CH_3), 1.19 (t, *J* = 7.1 Hz, 3H, CH_3). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 172.64 (C=0), 150.41 (C=N), 142.31, 135.71, 128.97 (Cq-Ar), 127.94, 126.30, 123.01, 122.29, 119.77, 109.73 (CH-Ar), 61.32 (OCH_2), 47.21 (CH_2N), 41.39 (CH), 27.97 (CH_2S), 17.23 (CH_3), 14.00 (CH_3). **HRMS (ESI)** Calc. for $C_{20}H_{23}O_2N_2S$ ($M + H^+$) = 355.1341 Found = 355.1338.

N-éthyl-3-(((1*H*-benzimidazol-2-yl)méthyl)thio)propanoate d'éthyle 7m

Red oil, yield = 63%. **^{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), 4.10 (q, J = 7.3 Hz, 2H, CH_2N), 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), 1.50 (t, *J* = 7.3 Hz, 3H, CH_3), 1.19 (t, *J* = 7.1 Hz, 3H, CH_3). **^{13}C NMR** (400 MHz, Acetone-d_6) δ 171.23 (C=O), 151.99 (C=N), 142.33, 135.73 (Cq-Ar), 122.70, 122.10, 119.61, 109.52 (CH-Ar), 59.99 (OCH_2), 47.21 (CH_2N), 34.05 (CH_2CO), 28.60 (CH_2S), 26.62 (SCH_2), 13.61 (CH_3). **HRMS (ESI)** Calc. for $C_{15}H_{21}O_2N_2S$ ($M + H^+$) = 293.1279 Found = 293.1282.

N-benzyl-3-(((1*H*-benzimidazol-2-yl)méthyl)thio)propanoate d'éthyle 7n

Yellow crystals, yield = 61%, m.p = 134˚C - 136˚C. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.92 - 7.67 (m, 1H, H-Ar), 7.34 - 7.17 (m, 6H, H-Ar), 7.12 - 7.02 (m, 2H, H-Ar), 5.49 (d, *J* = 3.6 Hz, 2H, CH_2N), 4.04 (q, J = 7.1 Hz, 2H, OCH_2), 4.01 (s, 2H, CH_2S), 2.82 (t, *J* = 7.0 Hz, 2H, CH_2C=O), 2.58 (t, *J* = 7.0 Hz, 2H, SCH_2), 1.17 (t, *J* = 7.1 Hz, 3H, CH_3). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 172.61 (C=0), 150.38 (C=N), 142.27, 135.67, 128.95 (Cq-Ar), 127.91, 126.26, 123.01, 122.28, 119.78, 109.74 (CH-Ar), 61.30 (OCH_2), 47.23 (CH_2N), 34.06 (CH_2CO), 28.59 (CH_2S), 26.61 (SCH_2), 13.63 (CH_3). **HRMS (ESI)** Calc. for $C_{20}H_{23}O_2N_2S$ ($M + H^+$) = 355.1452 Found = 355.1448.

2.2.2. Biological Methods

Preparation of stock solutions

The synthetic compounds were dissolved in dimethyl sulfoxide (DMSO) with the aim to get a concentration of 1000 µg/mL. This stock solution was used for the antibacterial tests. At this concentration, DMSO has no effect on the growth of the bacteria tested (negative control). Stock solutions were sterilized at 121˚C/15minutes. Sterility tests showed no microbial contamination.

Preparation of agar diffusion test

First, the antibacterial activity of the compounds was evaluated by the diffusion method described by the National Committee for Clinical Laboraty Standards (NCCLS) [22] with some modifications. The previously prepared bacterial *inoculum* was inoculated by swabbing onto each plate of Mueller-Hinton agar. A volume of 80 µL of each compound was placed in the wells made using a Pasteur pipette and after incubation at 37˚C for 18 - 24 hours. The diameters (including wells) of the inhibition zones around the wells was measured (in mm) by using a caliper. The compound was named active if the diameter of inhibition is ≥8 mm. A Ciprofloxacin disc (5 µg) was used as a reference antibiotic (positive control). Each test was repeated three times.

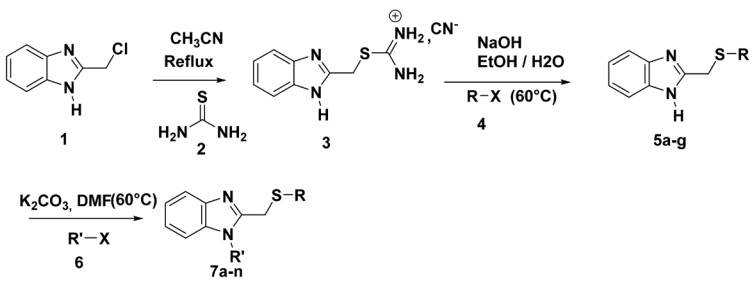
Methods to determine the Minimum Inhibitory Concentration (MIC)

The macro dilution technique in liquid medium reported by Okou *et al.* [23], with some modifications made it possible to determine the MIC. Double dilution series of each compound was distributed in 10 hemolysis tubes, followed by addition of bacterial *inoculum* at 10^6 CFU/mL. A final concentration range of 0.98 to 500 µg/mL was obtained. The whole was incubated at 37˚C for 18 - 24 hours. The MIC is defined as the lowest concentration for which there is no visible growth.

3. Results and Discussions

3.1. Chemistry

The synthesis of new benzimidazole derivatives **(7a - n)** was carried out by interaction between 2-(substitutedthio)methyl)-1*H*-benzimidazole **(5a - g)** with alkyl or benzyl halides (Scheme 1). These new derivatives were obtained using the method described by Lopes *et al.* [24]. This method consists to heat at 60˚C in dimethylformamide (DMF), the mixture of compounds (**5a - g)** and alkyl or benzyl halides **6** in the presence of potassium carbonate (K_2CO_3). The reaction



Scheme 1. Synthesis route of compounds 7a - n.

generated an amide ion which further reacts with the electrophile to give the N-alkylated benzimidazoles. Compounds **(7a - n)** were isolated and purified by silica column chromatography. About the compounds **(5a - g)** synthesis, they were carried out by nucleophilic substitution reaction (S-alkylation) between 2-methylbenzimidazole thiourunium chloride salt **(3)** and functionalized alkyl halides **(4)**. The reaction was set up in the presence of sodium hydroxide (NaOH) in a water-ethanol mixture for 1 hour. The benzimidazole thiouronium chloride salt **(3)** used to synthesize compounds **(5a - g)** was obtained *via* an intermediate reaction, by mixing compound **1** with thiourea **2** under reflux for 1 hour. Analysis of ^{1}H and ^{13}C NMR spectra of compounds **(5a - g)** showed the presence of peaks corresponding to different alkyl groups. In the NMR spectra of compounds **(7a - n)** we noted the disappearance of pyrrolic nitrogen proton around 12 ppm and the appearance of peaks corresponding to ethyl and benzyl groups. The presence of N-ethyl groups of compounds **(7a - n)** was characterized by the presence of a quadruplet around 4.2 ppm with a coupling constant of 7.3 Hz. We could speculate on the fact that, quadruplet corresponds to two protons directly linked to nitrogen atom. Formation of N-benzyl compounds was also confirmed by the presence of a singlet around 5.5 ppm corresponding to protons of methylene group (CH_2N). For compounds **7l** and **7n**, the two protons was observed in form of doublet. Structures of both compounds were also confirmed by the ^{13}C NMR spectrum.

3.2. Biology

3.2.1. Agar Diffusion Test

Among the synthesized benzimidazole derivatives, ten of them were *in vitro* evaluated by diffusion on agar method and macrodilution in liquid medium on *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853. The results of agar diffusion test are reported in Table 1.

Compounds **5c**, **5g**, **7f** and **7h** showed good antibacterial activity on *E. coli* ATTC 25922 with inhibition diameters in a range between 15 ± 0.04 mm and 18 ± 0.01 mm, meaning that they are very sensitive to this bacterial strain. They also showed good antibacterial activity against *S. aureus* ATCC 25923 with inhibition diameters ranging from 11 ± 0.10 to 18 ± 0.02 mm. On the other hand, they had no effect on *P. aeruginosa* ATCC 27853 at a concentration of 1000 µg/mL. Compounds **7i**, **7j**, **7m**, **7n**, **7k** and **7l**, did not reveal antibacterial activity on *E. coli* ATTC 25922 and *S. aureus* ATCC 25923 at concentration of 1000 µg/mL with inhibition diameters less than 8 mm. While, they showed an efficient antibacterial activity on *P. aeruginosa* ATCC 27853 with inhibition diameters between 12 ± 0.02 mm and 16 ± 0.04 mm.

3.2.2. Minimun Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (CMB) Determination

The MIC was determined only for the most active molecules observed during sensitivity tests with induction of a diameter in the zone of inhibition equal or greater than 15 mm [25]. The results of MIC and CMB are reported in Table 2.

Table 1. Zone of inhibition diameters in mm (mean ± standard deviation).

Compounds	Zone inhibition diameters (mm) ± SD		
	E coli ATTC 25922	*P. aeruginosa* ATCC 27853	*S. aureus* ATCC 25923
5c	15 ± 0.04	6 ± 0.00	15 ± 0.00
5g	18 ± 0.01	6 ± 0.00	18 ± 0.02
7f	16 ± 0.12	6 ± 0.00	12 ± 0.08
7h	16 ± 0.00	6 ± 0.00	11 ± 0.10
7i	6 ± 0.00	12 ± 0.02	6 ± 0.00
7j	6 ± 0.00	15 ± 0.80	6 ± 0.00
7m	6 ± 0.00	13 ± 0.06	6 ± 0.00
7n	6 ± 0.00	16 ± 0.04	6 ± 0.00
7k	6 ± 0.00	13 ± 0.04	6 ± 0.00
7l	6 ± 0.00	13 ± 0.00	6 ± 0.00
Ciprofloxacine (5 µg)	28 ± 0.01	31 ± 0.01	22 ± 0.04

Table 2. Minimum inhibitory concentration (MIC in µg/mL) and bactericidal concentration (CMB in µg/mL). NB: - is not determined.

Compounds	*E. coli* ATTC 25922		*P. aeruginosa* ATCC 27853		*S. aureus* ATCC 25923	
	CMI	*CMB*	*CMI*	*CMB*	*CMI*	*CMB*
5c	>500	-	-	-	>500	>500
5g	250	>500	-	-	250	>500
7f	500	>500	-	-	-	-
7h	500	>500	-	-	-	-
7i	-	-	-	-	-	-
7j	-	-	>500	>500	-	-
7m	-	-	-	-	-	-
7n	-	-	500	>500	-	-
7k	-	-	-	-	-	-
9l	-	-	-	-	-	-

Compounds **5g**, **7f** and **7h** showed significant antibacterial activity with MIC ranging from 250 to 500 µg/mL on *E. Coli* ATTC 25922. Among these three molecules, only **5g** inhibited efficiently *S. aureus* ATCC 25923 with a MIC = 250 µg/mL. As for **7n,** it exhibited good antibacterial activity on *P. aeruginosa* ATCC 27853 with a MIC = 500 µg/mL. All compounds determined with the CMB, data revealed that the values were greater than 500 µg/mL. Among them, **5g** presented the best Minimum Inhibitory Concentration (MIC = 250 µg/mL). **7k** and **7l** obtained respectively by introduction of an ethyl and benzyl group on pyrrolic nitrogen of compound **5g** were inactive. Therefore, we conclude that N-alkylation

was not improved the inhibitory activity of compound **5g**.

4. Conclusion

We have prepared a series of heterocyclic compounds **5a - g** and **7a - n** from 2-chloromethyl-1*H*-benzimidazole **1**. The antibacterial activity was evaluated *in vitro* for ten compounds against three bacterial mice: *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 using the diffusion on agar method and macrodilution in liquid medium and Ciprofloxacin as a reference. Some have shown good antibacterial activity on the three bacterial mice. The MIC was performed only for the most active compounds and some showed significant antibacterial activity on *E. Coli* ATTC 25922. Moreover among these compounds, **5g** exhibited the best minimum inhibitory concentration.

Acknowledgements

We wish to thank the laboratory (Laboratoire de Méthodologie et Synthèse de Produits Naturels) of the University of Quebec in Montreal (Canada) and the Laboratory LG2A of Jules Verne Picardic University (France) for providing us the chemical reagents and material for the spectroscopic analyzes.

Conflicts of Interest

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

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