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Novel Mannich Bases of Benzimidazole Derivatives: An Antibacterial Study of Environmental Bacterial Strains

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Abstract

A previous study was conducted on the synthesis and antibacterial evaluation of Mannich bases of 2-(thioalkyl)-1*H-methylbenzimidazole* derivatives. The results of this study showed that certain 2-(thioalkyl)-1*H*-methylbenzimidazole and 2-(thioalkyl)-methyl-1-(piperidin-1-ylmethyl)benzimidazole derivatives possess antibacterial activities against clinical strains, such as *Escherichia coli*, *Klebsiella pneumonia* (Gram-negative bacteria), *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Gram-positive bacteria). Following these favorable results, we extended the antibacterial evaluation of this series of molecules to environmental strains. The aim of this study was to assess the impact of the methyl-piperidine group fixed at position-1 of this new series of benzimidazoles on the antibacterial activity of environmental strains. In addition, we first evaluated the antibacterial activity of four 2-(thioalkyl)methylbenzimidazole derivatives and then that of five 2-(thioalkyl)-methyl-1-(piperidin-1-ylmethyl) benzimidazole derivatives. This study allowed us to show that compounds **1d** and **1e** could inhibit bacterial growth *in vitro* of the *Enterobacteria* P1 strain with inhibition diameters of 17.3 ± 0.6 mm and 10 ± 0.0 mm, respectively. However, addition of methyl-piperidinyl in this series showed that five (5) of 2-(thioalkyl)-methyl-1-(piperidin-1-ylmethyl) benzimidazole derivatives had an inhibitory effect on the *in vitro* growth of bacterial strains used except on *Enterobacteria* P2 with inhibition diameters between 10.0 ± 0.8 mm and 27.9 ± 1.4 mm. The introduction of the methyl-piperidinyl group at the 1-position

of 2-(thioalkyl)-1*H*-methylbenzimidazole derivatives greatly improved the antibacterial activity against environmental bacteria such as *Escherichia coli* A1, A2, A3, and A4 and two *Enterobacteria* P1.

Keywords

Methylbenzimidazole Derivatives, Inhibit Bacterial Growth, Mannich Base, *Enterobacteria*, *Escherichia coli*

1. Introduction

In recent decades, scientists have prioritized the fight against antimicrobial resistance because it affects most countries with varying levels of infection. These levels of variation are believed to be due to multiple reasons, including different rates of antibiotic use, hospital practices, income, and hygiene levels [1]. Indeed, the WHO states that more than 700,000 people die each year from these resistant infections and countless numbers of sick animals do not respond to the treatments administered. At the economic level, the same institution says that in just 10 years, more than 24 million people could fall into extreme poverty because of antimicrobial resistance [2]. Among resistant or multi-resistant bacteria, multi-drug-resistant enterobacteria, such as *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. Pneumoniae*), which are digestive tract bacteria responsible for a large number of infections, such as urinary tract infections [3], mild diarrhea, and other more severe forms such as hemorrhagic diarrhea. There is also methicillin-resistant staphylococcus aureus (MRSA), multiresistant tuberculous pyocyaneus bacilli, and *Acinetobacter baumannii*, which are bacteria that infect the lungs of people with cystic fibrosis [1]. Among the heterocyclic compounds with strong biological potential, benzimidazoles may be suitable. Indeed, this scaffold is present in a large number of drugs such as Thiabendazole, Mebendazole, Triclabendazole, Albendazole, and Oxibendazole and has a range of biological activities [4] including antibacterial [5], antimicrobial [6], anti-inflammatory [7], antiviral [8], anticancer [9], and anti-tuberculosis [10] activities. However, some studies have shown that Mannich bases associated with various heterocyclic compounds may be biologically effective [11] [12] [13]. They are found in the scaffolds of many commercial drugs, such as fluoxetine, an antidepressant, and trihexyphenidyl hydrochloride, which is used as an antispasmodic. Additionally, some benzimidazole compounds associated with Mannich bases have shown good biological activities [14] [15] [16].

Indeed, M. P. Vatsal *et al.* [17] synthesized benzimidazole N-Mannich bases with pyridine-3-amine and 5-methyl-pyridine-2-amine and evaluated them *in vitro* for their antibacterial, antimycotic, and antiprotozoal activities. These compounds were potent against *Leishamania mexicana* (*L. mexicana*), and *Trypanosoma cruzi* (*T. cruzi*) respectively with Inhibition Concentration 50 (IC_{50})

values of 0.25 and 1.02 mg/mL. Also, V. K. Sekar *et al.* [15] synthesized a series of Mannich bases of 2-substituted benzimidazole derivatives. The results of *in vitro* studies of these benzimidazole derivatives revealed that 3-(1-[(dimethylamino)methyl]-1*H*-benzimidazol-2-yl)-4-hydroxybenzene sulfonic acid and 3-(1-[(diethylamino)methyl]-1*H*-benzimidazol-2-yl)-4-hydroxybenzene sulfonic acid showed broad-spectrum antibacterial and antifungal activities. After a toxicity test on *Artemia salina*, these compounds were found to be non-toxic and completely cut the genomic DNA of the *E. coli* strain. They assumed that with appropriate molecular modifications, these compounds could be potent antimicrobial agents in the future. Our contribution to this research is to evaluate the antibacterial activity of a new series of base derivatives of Mannich benzimidazolés on environmental bacterial strains. We also demonstrated the impact of the 1-position methyl-piperidinyl group of the benzimidazole ring of 2-(thioalkyl)-methylbenzimidazole on antibacterial activity.

2. Material and Methods

2.1. Material

2.1.1. Microbial Strains

The microbial support consists of clinical strains of *Escherichia coli* (Gram-negative bacteria), called *E. coli* A1, *E. coli* A2, *E. coli* A3, and *E. coli* A4 and two *Enterobacteria* (P1 and P2). These strains were provided by the Unit of Antibiotics, Natural Substances, and Surveillance of Microorganisms in the Anti-infectious (ASSURMI) of the Bacteriology and Virology Department of the Pasteur Institute Côte d'Ivoire.

2.1.2. Chemicals

The synthetic compounds used were composed of four derivatives of 2-(thioalkyl)-methylbenzimidazole, namely 2-((methylthio)methyl)-1*H*-benzimidazole, 2-((isobutylthio)methyl)-1*H*-benzimidazole, 2-((butylthiomethyl)-1*H-benzimidazole*, 3-((1*H*-benzimidazol-2-yl)methyl) thio)ethyl propionate and five 2-mercapto benzimidazole-bearing Mannich bases such as 2-(methylthio)-1-(piperidine-1-yl)methyl)-1*H*-benzimidazole, 2-(isobutylthio)methyl)-1-(piperidine-1-yl)methyl)-1*H*-benzimidazole, 2-(ethylthio)-1-(piperidine-1-yl)methyl)-1*H*-benzimidazole, 2-(1-(piperidin-1-yl)methyl)-1*H*-benzimidazol-2-yl)methylthio) ethyl propionate, 3-((1-(piperidin-1-yl)methyl)-1*H*-benzimidazol-2-yl)methylthio)ethyl propionate [18].

2.2. Methods

2.2.1. Chemical Method

We recently published the synthesis and physicochemical characteristics of this new series of derivatives of 2-(thioalkyl)-1-methyl-(piperidin-1-ylmethyl) benzimidazole and their antibacterial activities on clinical resistant strains such as *P. aeruginosa*, *K. pneumoniae*, *E. coli*, and *S. aureus* [18]. The chemical structures of these molecules are listed in **Table 1** and **Table 2**, respectively.

Table 1. 2-(thioalkyl)methylbenzimidazole derivatives.

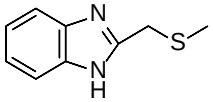
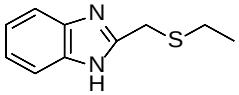
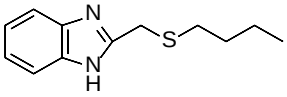
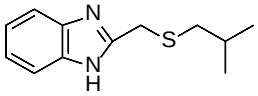
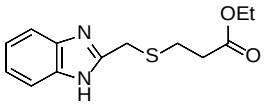
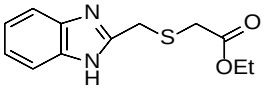
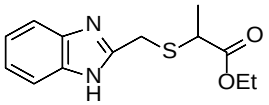
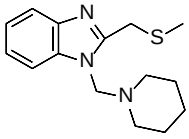
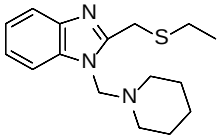
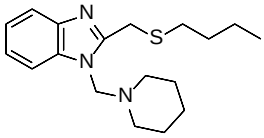
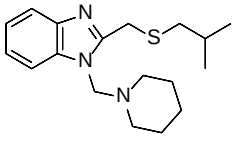
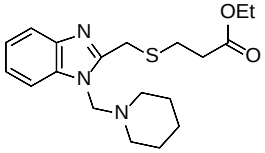
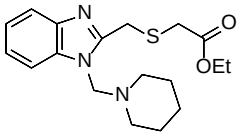
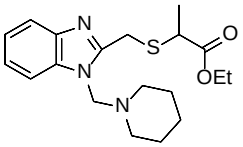
Compounds	Structure	Aspect	Melting point (˚C)	Yield (%)
1a		Yellow solid	148 - 150	96
1b		Yellow solid	132 - 134	62
1c		Yellow solid	144 - 146	57
1d		Yellow solid	126 - 128	68
1e		Yellow solid	104 - 106	25
1f		Yellow solid	68 - 70	96
1g		Yellow solid	100 - 102	38

Table 2. Chemical structure of 2-(thioalkyl)-methyl-1-(piperidin-1-ylmethyl) benzimidazole derivatives.

Compounds	Structure	Aspect	Yield (%)
2a		Yellow oil	56
2b		Yellow oil	62
2c		Yellow oil	51
2d		Yellow oil	51

Continued

2e		Yellow oil	65
2f		Yellow oil	63
2g		Yellow oil	67

2.2.2. Biological Method

1) Preparation of inoculum for solid medium testing

The inoculum was prepared from a young colony and incubated for 24 h. It was emulsified in 2 mL NaCl solution. Then, the optical density was adjusted to 0.5 MC Farland using a densimat. The sample volumes were 100 µL for *E. coli*, 1000 µL for *S. aureus*, and 10 µL for *P. aeruginosa*. This suspension was mixed with 10 mL of physiological water (NaCl 0.9%), and the bacterial inoculum was estimated to be 10^6 bacteria/mL.

The striae method was used to transplant the different preserved bacterial strains onto Muller-Hinton agar. They were then incubated in an oven at 37˚C for 18 - 24 h to obtain young and isolated colonies. These colonies were used to prepare bacterial inocula.

2) Preparation of the stock solution of chemicals

The substances were weighed and placed in test tubes to which a quantity of ethanol (70˚) was added. Different concentrations were tested.

3) Sensitivity test

a) Determination of areas of inhibition

The gel diffusion technique in cupules (wells) and the liquid macrodilution method were used to perform the tests [19] [20]. A 500 µg/mL concentration solution of the chemical was prepared. Petri dishes containing Muller-Hinton agar were seeded with the prepared inoculum using swabs. The cups were then dug by inserting the large tip of a Pasteur pipette into the agar and filled with 50 µL of the prepared chemical solution. The units were incubated at 37˚C for 24 h. The inhibition diameter around each cup was measured using a caliper. The effectiveness of the extracts was assessed according to the criteria described by Poncé *et al.* [21]. Thus, a substance is said to be ineffective if the inhibition diameter is less than 8 mm, whereas it is said to be effective if the diameter is between 9 and 14 mm. However, 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 for the selection of the most active extracts for the de-

termination of antibacterial parameters.

b) Preparation of the inoculum for liquid tests

A 24-hour bacterial colony was collected using a Pasteur pipette and emulsified in a test tube containing 10 mL of sterile Muller-Hinton broth. The mixture was incubated at 37˚C for 3 h. After incubation, a suspension of 0.3 mL of this pre-culture was taken and diluted in 10 mL of sterile Muller-Hinton broth and then homogenized.

Preparation of the concentration range

The concentration range was determined using the double-dilution method. To this end, a solution of retained chemicals (µg/mL) was prepared. A series of reason 2 dilutions were performed on this solution to obtain the concentration ranges.

c) Determination of antibacterial parameters

The determination of antibacterial parameters was carried out by dilution in a liquid medium according to the method used by Kouadio *et al.* [22]. Thus, in 10 experimental hemolysis tubes, 1 mL of each concentration range of chemical substances was brought into contact with 1 mL of the bacterial inoculum. The growth control tube received 1 mL of sterile distilled water in addition to the inoculum, whereas the sterility control group received only 2 mL of sterile Muller-Hinton broth (BMH). The tubes were then incubated for 24 h at 37˚C. After this incubation time, observations were made with the naked eye and the lowest concentration at which no bacterial growth was observed corresponded to the Minimum Inhibitory Concentration (MIC). The Minimum Bactericidal Concentration (MBC), it makes it possible to obtain 0.01% viable bacteria after 24 h of incubation at 37˚C. His determination begins with a number. This consisted of diluting the starting inoculum from 10^{-1} to 10^{-4} and seeding these different dilutions using a calibrated loop of 2 µL in 5 cm long striations, on a Muller-Hinton agar and then incubating for 24 h. These petri dishes were named A. After reading the MIC, the contents of tubes in which there was no visible growth were used to plant GMH on 5 cm streaks. This series of Petri dishes was named B. MBC was determined by comparing the bacterial growth of boxes A and B. Thus, the smallest tube concentration that had less than 0.01% viable bacteria compared with the initial inoculum was MBC. The MBC/MIC report clarified the modality of action of the substance [23]. As reported by Kamanzi *et al.* [24], extract is bactericidal when MBC is equal to MIC, it is bacteriostatic when its MBC is greater than its MIC or if the MBC/MIC ratio is higher than 4. When this ratio was equal to 32, the strain was considered tolerant.

d) Statistical analysis

The results were analyzed using Excel 2013 software for descriptive analyses. The results of the antibacterial tests are expressed as the mean standard deviation.

3. Results and Discussion

We first evaluated the antibacterial activity of four 2-(thioalkyl)methylbenzimi-

dazole derivatives by determining their inhibition diameters.

The results for these diameters are presented in Table 3.

The result of this antibacterial test performed on environmental strains indicates that among the derivatives derived from the synthesized benzimidazole S-alkylation (**1a**, **1d**, **1c**, and **1e**), compounds **1d** and **1e** inhibited the *in vitro* growth of the *Enterobacteria* P1 strain with inhibition diameters of 17.3 ± 0.6 mm and 10 ± 0.0 mm, respectively. Compounds **1a** and **1c** showed no inhibitory activity against any bacterial strains. Compounds with inhibitory activity against the *in vitro* growth of bacterial strains were selected to determine the antibacterial parameters (MBC and MIC), as shown in Table 4. Thus, the determination of the Minimum Inhibitory Concentration and the Minimum Bactericidal Concentration of these different compounds allowed us to calculate the MBC/MIC ratio of each compound. This allowed us to determine the modality of action of each compound. For this purpose, we can affirm that compounds **1d** and **1e** have bactericidal effects on the *Enterobacteria* strain P1.

To determine the influence of the 1-position methylpiperidinyl group of the 2-(thioalkyl)methylbenzimidazole derivatives, new antibacterial tests were performed on five derivatives of 2-(thioalkyl)-methyl-1-(piperidin-1-ylmethyl) benzimidazole by determining their inhibition diameters on environmental strains. Table 5 presents the results.

The five compounds tested had an inhibitory effect on the in vitro growth of the bacterial strains used except on enterobacteria P2 with inhibition diameters between 10.0 ± 0.8 mm and 27.9 ± 1.4 mm. The inhibitory effect (bacterial resistance) varied depending on the strain (Table 5). The absence of a zone of inhibition of the different compounds in the *Enterobacteria* P2 strain reflects the resistance of this strain to these compounds. The same was also observed for compound **2e** in *E. coli* A1. Based on the results obtained, we can therefore affirm that the addition of a methylpiperidine group in position-1 of the derivatives 2-(thioalkyl)methylbenzimidazole is favorable for antibacterial activity against environmentally resistant strains such as *E. coli* A2, *E. coli* A3, and *Enterobacteria* P1. However, this addition had no advantage over the *Enterobacterial* P2 strain. For this series of molecules, if we closely observe the antibacterial

Table 3. Diameters of inhibition zones of compounds derived from S-alkylation benzimidazole on environmental strains.

Environmental strains	Inhibition Diameters (mm)			
	1a	1d	1c	1e
E. coli A1	0.8 ± 0.1	5.3 ± 0.6	0.6 ± 0.2	0.5 ± 0.0
E. coli A2	0.7 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.0
E. coli A3	0.7 ± 0.1	7.2 ± 0.6	0.7 ± 0.2	0.5 ± 0.0
E. coli A4	0.7 ± 0.2	0.7 ± 0.1	0.6 ± 0.2	0.9 ± 0.0
Enterobacteria P1	0.6 ± 0.2	17.3 ± 0.6	0.8 ± 0.1	10.0 ± 0.0
Enterobacteria P2	0.8 ± 0.1	0.6 ± 0.2	0.7 ± 0.2	0.5 ± 0.1

Table 4. Mean values (mean ± SD of three tests) of antibacterial parameters of chemicals derived from S-alkylation benzimidazole on environmental strains.

Bacterial strains	**1e** (µg/mL)				**1d** (µg/mL)			
	MIC	MBC	MBC/MIC	Activity	MIC	MBC	MBC/MIC	Activity
EnterobactP1	1	2	2	Bactericidal	1	4	4	Bactericidal

Table 5. Mean diameter values of inhibition zones of benzimidazole 2-(thioalkyl)-methyl-1-(piperidin-1-ylmethyl) derivatives on environmental strains (averages ± SD).

Environmental strains	**Inhibition Diameters (mm)**				
	2a	**2b**	**2e**	**2d**	**2g**
E. coli A1	21.2 ± 0.8	23.0 ± 0.0	0.0 ± 0.0	18.3 ± 0.5	13.0 ±1.6
E. coli A2	27.9 ± 1.4	24.5 ± 2.5	21.2 ± 0.8	16.3 ± 0.2	22.6 ± 1.4
E. coli A3	17.2 ± 0.7	17.0 ± 0.2	12.3 ± 0.7	19.0 ± 0.0	22.6 ± 1.4
EnterobactP1	17.0 ± 1.3	15.3 ± 0.7	14.0 ± 0,8	19.0 ± 1.2	10.0 ± 0.8
EnterobactP2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

activity of the strain *E. coli* A1, we can claim that the length (carbon chain) substitutes linked to the sulfur atom could have an influence on the improvement of antibacterial activity both in the alkyl series and ester. Therefore, when the carbon chain was increased, the antibacterial activity of the molecule decreased. The sulfur atom was substituted for ethyl propionate, the methylpiperidyl group was added, and there was no improvement in the activity of the *E. coli* A1 strain.

4. Conclusion

Studies on this new series of benzimidazole molecules showed that compounds **1d** and **1e** had antibacterial activity against the *Enterobacteria* P1 strain. However, the addition of the methylpiperidin-1 group of the benzimidazole nucleus of this molecule series allowed us to confirm the excellent antibacterial activity of the 2-(thioalkyl)-methyl-1-(piperidin-1-ylmethyl) benzimidazole derivatives against all strains tested, except *Enterobacteria* P2. In addition, the substitution of the sulfur atom by ethyl propionate and the benzimidazole-1-ring position by the methylpiperidine group were not considered in the search for antibacterial compounds against these environmental strains. Based on the antibacterial activity of these strains, compounds **2a**, **2b**, **2d**, and **2g** could be used as antibacterial controls against environmental strains such as *E. coli* A1, *E. coli* A2, *E. coli* A3, and *Enterobacteria* P2. It would be interesting to conduct *in vivo* tests to study the toxicity of these compounds.

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Conflicts of Interest

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

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