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# Chemical Synthesis and Hemi-Synthesis of Novel Benzimidazole Derivatives Using Microwave-Assisted Process: Chemical Characterization, Bioactivities and Molecular Docking †

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**Abstract:** Benzimidazole derivatives represent a class of heterocyclic compounds that exhibit a wide range of pharmaceutical properties. The present study aimed to investigate the in-vitro antioxidant and antimicrobial activities of newly synthesized benzimidazole derivatives. Compound **1b** (2-(1H-1,3-benzodiazol-2-yl) phenol) was synthesized by reacting o-phenylenediamine (OPA) with chemical salicylaldehyde, while compounds **2b** (2-(2-[(1E)-2-phenylethenyl]-1H-1,3-benzodiazole) and **3b** (2-**[**(1E)-2,6-dimethylhepta-1,5-dien-1-yl**]**-1H-1,3-benzodiazole) were obtained through a hemi-synthesis process of, respectively, the cinnamon (cinnamaldehyde, 90.54%) and lemongrass (cis-citral, 43.9%) essential oils previously characterized by GC/MS. Compounds **4b** (2-phenyl-1H-benzimidazole) and **5b** (5-(1H-benzimidazol-2-yl)benzene-1,2,3-triol) were synthesized with a click chemistry method by reacting the OPA with benzoic acid and gallic acid directly in ethanol under microwave irradiation (MW) at 400 MHz. The structure/purity of the synthesized compounds was clarified by spectroscopy, ATR-FTIR and NMR $^1$H. Compounds **1b**–**5b** were screened for their antioxidant activity by using four complementary in-vitro assays: DPPH scavenging activity, ferric ion reducing power, β-carotene bleaching inhibition, and Thiobarbituric Acid Reactive Substance Assay (TBARS) formation inhibition. All the tested compounds showed antioxidant potential, with varying performance. Antimicrobial activity was investigated against American Type Culture Collection (ATCC) strains (three Gram- bacteria: *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*; one Gram+ bacteria: *Staphylococcus aureus*, and one yeast strain: *Candida albicans*) through the determination of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) by using the microdilution method and rapid colorimetric test of p-iodonitrotetrazolium chloride (INT). Compound **5b** exhibited the highest potential, especially against *S. aureus* (MIC = 0.156 mg·mL$^{-1}$) followed by *S. typhi* and *C. albicans* (MIC = 0.3125 mg·mL$^{-1}$) and then by *E. coli* and *P. aeruginosa*. Compound **1b** also showed great potential against *S. aureus* and *C. albicans* (MIC < 0.3125 mg·mL$^{-1}$), followed by *E. coli* and *S. typhi* (MIC = 0.3125 mg·mL$^{-1}$) and *P. aeruginosa* (MIC = 0.625 mg·mL$^{-1}$). Further molecular docking was conducted using AutoDock Vina 1.1.2 software on *S. aureus* thymidylate kinase (TMK) protein to highlight the structure–activity relationship of the potent molecules.

**Keywords:** benzimidazoles; synthesis; hemi-synthesis; microwave-assisted; NMR $^1$H; antioxidant activity; antimicrobial activity; in-silico docking

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## 1. Introduction

The search for novel antimicrobial compounds in clinical microbiology is prompted by the need to counteract the growing number of infectious diseases caused by multidrug-resistant strains (MDR: multidrug-resistant and TDR: totally drug-resistant) [1]. Bacterial resistance has dramatically reduced the effectiveness of the majority of treatments available today, and an increasing number of diseases have become more difficult to treat. Hence, there is an urgent need to develop new therapeutic agents and broad-spectrum pharmaceutical probes for clinical trials [2]. Natural biomolecules of vegetable origin, due to their chemical diversity, such as phenolic acids and chiral monoterpene aldehydes, offer unlimited possibilities for new drug discovery through organic synthesis patterns. These structurally assorted compounds may offer biological potentialities, such as the antioxidant [3,4] and the antimicrobial properties [5] slightly linked to their structure configuration. Moreover, they are good candidates for the hemi-synthesis of new bioactive agents targeting a particular biological activity or protein functionality. Benzimidazoles are heterocyclic compounds that represent, with their derivatives, an interesting class of molecules of great importance in medicinal chemistry, due to the broad diversity of biological properties that they may present (antibacterial, antiviral, antioxidant, anticancer, anti-inflammatory, etc.) [6]. Recently, in-silico docking has seen great advances in predicting the molecular interactions that hold a protein and ligand in the binding site, stimulating the progress of new drug development [7,8]. The docking of small molecules and the virtual screening of candidate compounds have become integral in the biomedical field and in drug design. Several software programs have been developed to provide a procedure to predict the interaction of small molecules with protein targets and incorporate flexibility within docking algorithms, such as the AutoDock 4.2 [9] and AutoDock vina 1.1.2 [10] programs.

In this study, we contributed to perform:

- A chemical synthesis and hemi-synthesis of new benzimidazole derivatives;
- A physicochemical characterization (purification and structural analysis) of the synthesized compounds by $^{1}$H NMR spectroscopy and FTIR;
- Evaluation of antioxidant and antimicrobial activities by in-vitro assays;
- Evaluation of docking scores of the synthesized compounds on 4QGH (PDB: Protein data base entry) protein of *Staphylococcus aureus* thymidylate kinase (TMK).

## 2. Methods

### *2.1. Plant Material and Extraction Procedure*

Lemongrass (*Cymbopogon citratus* L.) plant was collected from Tipaza province, Algeria, while cinnamon (*Cinnamomum verum* L.) bark strips were obtained from a local market in the downtown city of Tipaza. The extraction of essential oils was performed by the hydrodistillation method using a Clevenger-type apparatus [11]. Then, 100 g of vegetal material was steamed in 500 mL of boiled water for three hours. After evaporation, the EO was condensed and collected in a shaded bottle (vial) after elimination of water.

### *2.2. Essential Oil Analysis*

The components of the extracted essential oils were analyzed by gas chromatography/mass spectrometry (GC/MS) using a Hewlett Packard-6890 system, equipped with a HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) directly coupled to a selective mass detector, Hewllet Packard-5973. Helium was used as the carrier gas (1 mL/min). The analysis was performed using the following temperature program: 60–300 °C to 3 °C/min; without division for 1.50 min; with a sample volume of 2 μL of essential oil solution. The injector and detector temperatures were set at 240 °C. The ion source temperature was 180 °C, and mass spectra were obtained in electron ionization mass spectral (EIMS) at 70 eV electron energy. Identification of compounds was based on a comparison of the mass spectra of each peak with those recorded in the MS library (NIST02 and Wiley7) and comparing retention indices and mass spectra with literature data.

### *2.3. Synthesis Procedure*

#### 2.3.1. Synthesis/Hemi-Synthesis of Three Benzimidazole Aldehyde Derivatives

The amino derivative 1,2-phenylenediamine (OPA) was first diluted in ethanol (15 mL) to form an initial solution of reagent 1, and then the chemical salicylaldehyde (1 mmol of the corresponding aldehyde, calculated on the basis of its corresponding molecular mass (approximately 0.5 mL), was added to an ethanolic solution with a few drops of HCl 0.05%. For hemi-synthesis, the initial solution was mixed with EO 1 mmol of cinnamaldehyde (~90%) and citral (~44%), calculated on the basis of their percentage in the EO under constant agitation at room temperature, and a few drops of HCl 0.05% were added (Figure 1). The reaction was left for reflux (1 h) under an adapted microwave extractor at 400 MHz. The crystals of compounds **1b**, **2b** and **3b** were gradually formed in the reaction medium. The precipitates were filtered and washed with cold ethanol after the required time and then purified by thin-layer chromatography (TLC) and column chromatography.

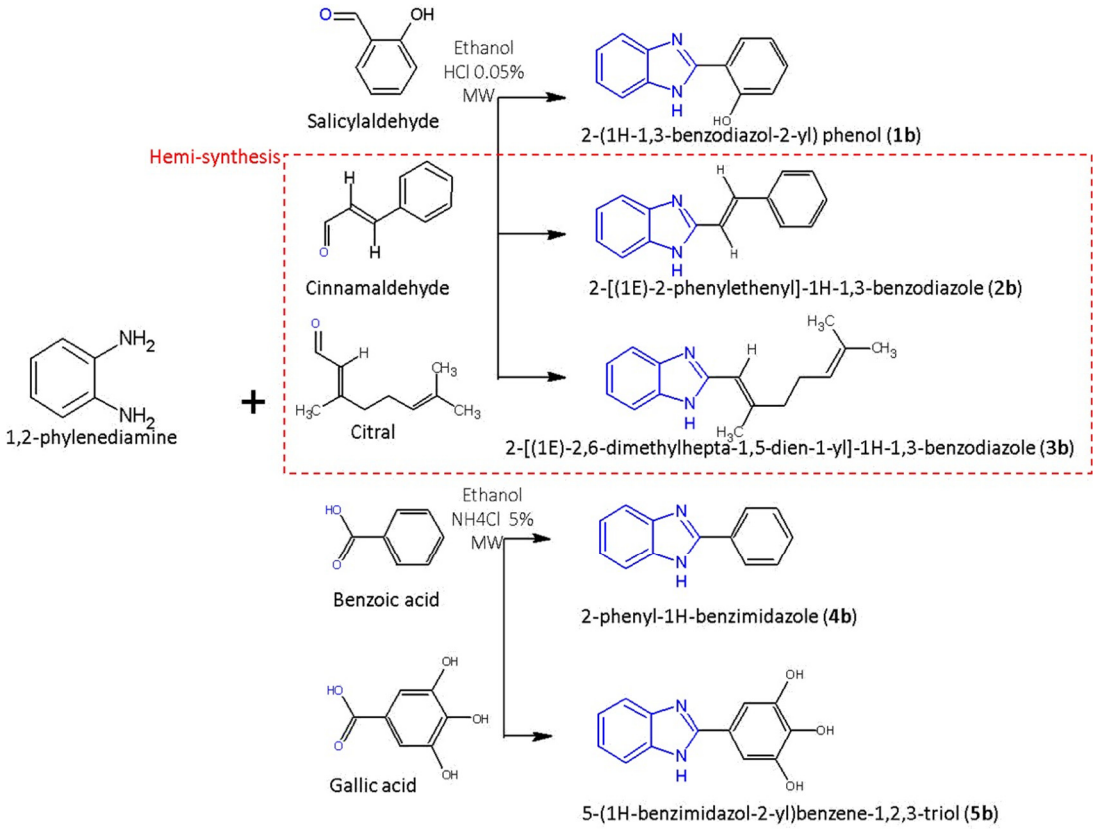


**Figure 1.** Synthesis procedure and chemical structure of synthesized molecules obtained by ChemSketch software.

#### 2.3.2. Synthesis of Benzimidazole Phenolic Acid Derivatives

The total chemical synthesis reaction was carried out by the condensation of OPA with the appropriate phenolic acids (0.03 mol). The reaction was initiated under microwave irradiation conditions at 400 MHz (Figure 1). Initially, the OPA (3.24 g, 0.03 mole) was dissolved in ethanol in the presence of a few drops of $NH_4Cl$ (5%) in a glass recipient (microwave synthesis reactor) of 30 mL at room temperature until completely dissolved to form solution 1. After this, each phenolic acid (in molar correspondence) was also dissolved in ethanol (30 mL) in the reactor and heated to 140 °C under 10 bars of internal pressure for 5 min. Then, it was cooled to room temperature; thus, solution 2 was formed. The two solutions were mixed and left to react under microwave irradiation at 400 MHz for 10 min to obtain a precipitate of 2-(4-phenyl substituted)-1H-benzimidazoles. After cooling at room temperature, the precipitated product was washed with cold dichloromethane or hexane, dried to room temperature, and recrystallized in ethanol.

### *2.4. Fractionating/Purification*

To determine the migration patterns of all the synthetized compounds, thin-layer chromatography (TLC) was performed on a thin plate of silica GF-254 with fluorescein developer deposited on a support and visualized under UV at 254/360 nm. The samples were diluted in ethanol and deposited on the bottom of the silica plate by spots. The plate was placed in a vessel containing the migration solvent, allowing the solvent to run to the top edge of the plate. The migration solvent was a mixture of hexane, dichloromethane and ethanol (2:6:2 *v*/*v*/*v*). After migration, the chromatography plate was then read directly under UV light; the spots appeared without having to resort to a developer. Afterward, column chromatography was performed to separate and purify the final products of the hemi-synthesized molecules since the reaction may have occurred also on other aldehydes of the EO mixture. The separation was carried out by gravity on silica particles of 70 to 200 nm, where the solvent flowed by drip. The eluent (mobile phase) used here was initially dichloromethane/hexane (50:50 *v*/*v*), which allowed the elution of the non-polar fraction, followed by ethanol and chloroform, which separated the fraction strongly retained by silica. The benzimidazole molecules produced were driven by the mobile phase, and they were recovered in 250 mL beakers to be dried under vacuum (45 °C).

### *2.5. Structural Analysis*

The structural analysis was first monitored by infrared spectroscopy (ATR-FTIR) to determine the functional groups of the purified molecules. The spectra were recorded in a few minutes without any limitation concerning the size of the studied molecule. FTIR spectroscopy in the $4\times10^3$ e 400 $cm^{-1}$ region was also used to measure trends and reaction patterns in real time, providing very specific information on the kinetics, mechanisms, reaction path and influence of variables on reaction performance. A $^1H$ NMR analysis was then performed on a Bruker 400 spectrometer (Bruker, Wissembourg, France, 400 MHz for 1H), in DMSO-$d_6$ as solvent. Chemical shifts (δ) were reported in ppm and coupling constants (*J*) in Hz and the internal standard was Tetramethylsilane (TMS). In a 5-mm-diameter NMR tube, 500 uL of sample (synthetic molecules or standard solution) was inserted and a capillary tube containing a solution of 3-trimethylsilyl-2,2,3,3-d4-propionic acid (TSP-d4) was dosed at 2.1 mmol proton/L. This solution served as a reference for chemical displacement. Depending on the concentration of the samples, 64 to 128 accumulations were made over a spectral width of 3200 Hz. Brüker's Topspin 2.6 NMR software was used for data processing. Structural drawing and naming attribution were monitored by Chemketch software and NMR spectra were processed by the MestRnova 14.2.0 software. Brüker's Topspin CMC 2.6 was used for structure authentication.

### *2.6. Bioactivities Properties Evaluation*

#### 2.6.1. Antioxidant Activity

The antioxidant activity of the synthesized benzimidazoles was evaluated using four complementary in-vitro tests: DPPH radical scavenging, reducing power (RP), inhibition of β-carotene bleaching/linoleate, and inhibition of lipid peroxidation in ovine brain cell homogenates (TBARS). The molecules were dissolved in ethanol with a well-known volume to set an initial concentration [C = 3 $mg{\cdot}mL^{-1}$] that was diluted at different concentrations until EC50 was determined (concentration providing 50% antioxidant activity or 0.5 absorbance in the reductive power; expressed in μg/mL) [12]. DPPH radical scavenging and RP activity was measured using an ELX800 microplate reader (Bio-Tek Instruments, Inc.; Winooski, VT, USA) at 515 and 690 nm, respectively, and calculated as the percentage of reagent discoloration. Inhibition of β-carotene bleaching was evaluated by the neutralization levels of linoleate-free radicals that avoided β-carotene bleaching. Lipid peroxidation inhibition in ovine brain homogenates was evaluated by the decrease in thiobarbitu-

ric acid reactive substances (TBARS); the color intensity of the malondialdehyde–thiobarbituric acid (MDA–TBA) was measured by its absorbance at 532 nm. Butylated hydroxytoluene (BHT) and Trolox were used as positive controls.

#### 2.6.2. Antimicrobial Activity

The synthesized compounds were tested for their antibacterial activity against ATCC strains (Manassas, VA 20108 USA). Three Gram- bacterial strains: *Escherichia coli* (ATCC® 10145), *Salmonella typhi* (ATCC® 19430), and *Pseudomonas aeruginosa* (GEP ATCC® 10145GFP™); one Gram- strain: *Staphylococcus aureus* (ATCC® 10832™); and one yeast strain: *Candida albicans* (ATCC® 90819™). The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) values were determined by a microdilution method and a rapid colorimetric test of p-iodonitrotetrazolium chloride (INT) [2]. Briefly, stock solutions of 100 mg·mL$^{-1}$ were prepared for each compound (**1b**–**5b**) in DMSO, and 100 µL of each stock solution was diluted in 400 µL of MHB (Mueller Hinton broth) or TSB (Tryptone Soy Broth) according to bacterial requirements (resulting in a solution of 20 mg·mL$^{-1}$). Subsequently, 10 µL inoculum (1.5 108 CFU/mL) of fresh bacterial cultures was added to all wells containing tested concentrations in the range of 20–0.156 mg·mL$^{-1}$. The microplate was then incubated at 37 °C for 24 h. The MIC of the sample was determined after addition of INT (0.2 mg·mL$^{-1}$, 20 µL) and incubation at 37 °C in an oven (Jouan, Berlin, Germany) for 30 min, where viable microorganisms reduced yellow dye to pink. MIC was defined as the lowest concentration of molecules that prevented this change and allowed complete inhibition of bacterial growth. To determine the minimal bactericidal concentrations (MBC), each negative well and positive control culture (10 µL) were sub-cultured into 96-well micro-plates containing culture medium and further incubated at 37 °C for 24 h. Gentamicine (10 Ug) and Ceftazidime (30 Ug) were used as positive controls for bacterial strains and Nystatine was used for the yeast strain.

### *2.7. Molecular Docking Study*

Molecular docking simulations were done using AutoDock Vina software on *S. aureus* thymidylate kinase (TMK) protein. A crystalized structure of TMK protein (PDB: 4QGH) was selected and obtained from the Protein Data Bank (http://www.rcsb.org/structure/4QGH, accessed on 3 March 2019). The protein was prepared for molecular docking by removing ligand heteroatoms and water molecules and by addition of polar hydrogens on AutoDock tools 1.5.7 software (ADT, The Scripps Research Institute, La Jolla, CA, USA). The ligands **1b** and **5b** were prepared for molecular docking simulation by setting the torsion tree and rotatable, nonrotatable bonds present in the ligand through AutoDock tools 1.5.7 software [13]. The binding scores of the receptor proteins were identified by Biovia DS visualizer. The molecular docking affinity of the receptors/ligands was validated based on the obtained binding energy (ΔG) and the predicted inhibition constant (Ki).

## 3. Results and Discussion

### *3.1. GC/MS Profiles of Essential Oils of Cinnamon and Lemongrass*

The GC/MS profile of cinnamon EO showed variations in the chemical constituents. The GC/MS chromatogram showed that the major compound found throughout the cinnamon oil was cinnamaldehyde at 90.54% (Table A1, Appendix A). Then, the remaining compounds were minor elements present in very small amounts. Other aldehydes such as benzaldehyde, hydrocinnamaldehyde, and 4-methoxycinnamaldehyde were also detected in very small amounts. However, the descending order of the major compounds present in cinnamon oil as a whole is indicated as follows: -cinnamaldehyde (90.54%) > coumarin (2.87%) > hydrocinnamaldehyde (0.92%). For lemongrass, the oil was dominated by monoterpene hydrocarbons (Table A2, Appendix A). This monoterpenic fraction

was characterized by a high percentage of cis-citral (43.53%), neral (34.87%), and β-Myrcene (4.55%). Other aldehyde components were identified, such as (R)-(+)-citronellal and trans-chrysanthemal, in very low concentrations.

### *3.2. Separation and Purification of Synthetic Products*

Qualitative analysis of the fractions obtained by thin-layer chromatography enabled the separation of the fractions and revealed a considerable number of constituents visualized under UV light at 254–360 nm, and the chromatographic profiles and column chromatography were used to separate/fractionate products. First, (2-(1H-1,3-benzodiazol-2-yl) phenol) was synthesized by reacting o-phenylenediamine (OPA) with chemical salicylaldehyde, while compounds **2b** (2-[(1E)-2-phenylethenyl]-1H-1,3-benzodiazole) and **3b** (2-[(1E)-2,6-dimethylhepta-1,5-dien-1-yl]-1H-1,3-benzodiazole) were obtained through a hemi-synthesis process of, respectively, the cinnamon (cinnamaldehyde, 90.54%) and lemongrass (citral, 43.9%) essential oils previously characterized by GC/MS. The reaction of OPA with benzoic and gallic acid gave the benzimidazolic compounds 2-phenyl-1H-benzimidazole (**4b**) and 5-(1H-benzimidazol-2-yl)benzene-1,2,3-triol (**5b**), respectively. In the synthesis reaction, the 1H-benzimidazole heterocycle substituted in position 2 was synthesized by reacting the diamine group of the OPA with the aldehyde function (-COH) of the corresponding aldehyde and the acid function (-COOH) of phenolic acid. These compounds were named using the ChemSketch 12.0 software.

### *3.3. Structural Analysis*

The infrared analysis performed by ATR-FTIR spectroscopy informs on functional groups and covalent bonding of the compound produced; it is based on the absorption of light by most of the molecules in the infrared region of the electromagnetic spectrum and by converting this absorption into molecular vibration. This absorption corresponds specifically to the bonds present in the molecule (N–H), (C–H), (C=H), (C–H) stretching and (C–C–C) out of plane bending. The spectra of the most obtained compounds showed significant absorbance rates estimated at around 90% absorbance between regions 3160 and 3469 $cm^{-1}$ and 75% absorbance between regions 1000 and 1500 $cm^{-1}$, with a specific C-N stretching band at 1368 $cm^{-1}$ in the benzimidazole ring. On the other hand, the RMN$^1$H analysis of the synthetized compounds revealed globally the presence of a 2H singlet at 8.1, 7.6 ppm, corresponding to the two pyrrolytic protons. We also noted the presence of a multiplet between 7.16 and 7.08 ppm, corresponding to the four aromatic protons. Additionally, two doublets were observed at 7.7 and 7.6 ppm, attributable to the four aromatic protons. The chemical shifts in the compounds are represented as follows:

*2-(1H-1,3-benzodiazol-2-yl)phenol* (**1b**): $^1$H NMR, (400 MHz, DMSO-$d_6$): δ 7.12 ppm (1H, ddd, *J* = 7.7, 7.5, 1.2 Hz), 7.23–7.33 ppm (2H, 7.28 (ddd, *J* = 8.1, 7.6, 1.6 Hz), 7.30 ppm (ddd, *J* = 8.3, 1.2, 0.4 Hz)), 7.41 ppm (1H, ddd, *J* = 7.7, 7.6, 1.2 Hz), 7.54 ppm (1H, ddd, *J* = 8.3, 7.6, 1.7 Hz), 7.72–7.79 ppm (2H, 7.75 (ddd, *J* = 7.7, 1.6, 0.5 Hz), 7.75 ppm (ddd, *J* = 7.7, 1.7, 0.4 Hz)), 7.93 ppm (1H, ddd, *J* = 8.1, 1.2, 0.5 Hz).

*2-[(1E)-2-phenylethenyl]-1H-1,3-benzodiazole* (**2b**): $^1$H NMR, (400 MHz, DMSO-$d_6$): δ 7.05 ppm (1H, ddd, *J* = 7.7, 7.6, 1.3 Hz), 7.10–7.22 ppm (2H, 7.17 (d, *J* = 14.0 Hz), 7.17 ppm (ddd, *J* = 8.1, 7.6, 1.4 Hz)), 7.26–7.46 ppm (6H, 7.36 (dddd, *J* = 8.0, 7.6, 1.5, 1.5 Hz), 7.42 ppm (d, *J* = 14.0 Hz), 7.41 ppm (dddd, *J* = 8.1, 1.8, 1.5, 0.5 Hz), 7.31 ppm (tdd, *J* = 8.0, 1.6, 0.5 Hz)), 7.61 ppm (1H, ddd, *J* = 8.1, 1.3, 0.4 Hz), 7.86 ppm (1H, ddd, *J* = 7.7, 1.4, 0.4 Hz).

*2-[(1E)-2,6-dimethylhepta-1,5-dien-1-yl]-1H-1,3-benzodiazole* (**3b**): $^1$H NMR, (400 MHz, DMSO-$d_6$): δ 1.53–1.54 ppm (6H, 1.54 (s), 1.54 (s)), 1.76 ppm (3H, s), 2.06–2.14 ppm (4H, 2.12 (t, *J* = 7.4 Hz), 2.10 ppm (td, *J* = 7.4, 7.2 Hz)), 5.26 ppm (1H, t, *J* = 7.2 Hz), 6.43 ppm (1H, s), 7.05 ppm (1H, ddd, *J* = 7.9, 7.6, 1.3 Hz), 7.21 ppm (1H, ddd, *J* = 8.1, 7.6, 1.5 Hz), 7.58 ppm (1H, ddd, *J* = 8.1, 1.3, 0.5 Hz), 7.71 ppm (1H, ddd, *J* = 7.9, 1.5, 0.5 Hz).

*2-phenyl-1H-benzimidazole* (**4b**): $^1$H NMR, (400 MHz, DMSO-$d_6$): δ 7.05 ppm (1H, ddd, *J* = 7.7, 7.6, 1.3 Hz), 7.12–7.22 ppm (2H, 7.17 (d, *J* = 14.0 Hz), 7.17 ppm (ddd, *J* = 8.1, 7.6, 1.4 Hz)), 7.26–7.46 ppm (6H, 7.36 (dddd, *J* = 8.0, 7.6, 1.5, 1.5 Hz), 7.42 ppm (d, *J* = 14.0 Hz), 7.41 ppm (dddd, *J* = 8.1, 1.8, 1.5, 0.5 Hz), 7.31 ppm (tdd, *J* = 8.0, 1.6, 0.5 Hz)), 7.61 ppm (1H, ddd, *J* = 8.1, 1.3, 0.4 Hz), 7.86 ppm (1H, ddd, *J* = 7.7, 1.4, 0.4 Hz).

*5-(1H-benzimidazol-2-yl)benzene-1,2,3-triol* (**5b**): $^1$H NMR, (400 MHz, DMSO-$d_6$): δ 7.06 ppm (1H, ddd, *J* = 8.1, 6.8, 1.4 Hz), 7.17 ppm (2H, d, *J* = 2.4 Hz), 7.42 ppm (1H, ddd, *J* = 8.0, 6.8, 1.3 Hz), 7.64 ppm (1H, ddd, *J* = 8.1, 1.3, 0.5 Hz), 7.87 ppm (1H, ddd, *J* = 8.0, 1.4, 0.5 Hz).

### *3.4. Bioactivity Properties*

#### 3.4.1. Antioxidant Activity

Due to the complexity of oxidation processes and the diverse nature of antioxidants, there is no universal method by which antioxidant activity can be measured quantitatively in a precise manner. Oftentimes, it is necessary to combine the responses of different and complementary tests to obtain an indication of the antioxidant capacity of the sample to be tested [14]. In the present study, the new benzimidazoles reported were screened for their antioxidant activity by using four in vitro assays: DPPH free radical scavenging, reducing power, β-carotene bleaching inhibition, and TBARS formation inhibition. The results are expressed in EC50 values (μg/mL), as summarized in Table 1. It is well known that reactive oxygen species (ROS), which can be superoxide radicals, hydroxyl and peroxyl, etc., are causes of oxidative stress, associated with various chronic diseases and DNA damage, leading to carcinogenesis [15]. The six molecules (**1b**–**5b**) showed antioxidant activity, with varying performance. Compounds **1b** and **5b** showed the highest activity, with significant EC50s <200 μg/mL, for DPPH and β-carotene. Compound **3b** showed the highest values for the DPPH test and iron reducing power, respectively, while compound **2b** gave relatively average results. Compound **1b** exhibited the highest potential, very similar to the synthetic antioxidant drug "Trolox" used as a standard for comparison, with EC50 = 53 μg/mL in comparison with Trolox, with an estimated value of 51 μg/mL for the DPPH test. The values obtained for the RP test show that these compounds had a high reducing iron potential, especially of compounds **1b** and **5b** (54 and 96 μg/mL). Compounds **2b** and **3b** gave also a good result (101 and 102 μg/mL). The degree of discoloration of β-carotene was measured by spectrophotometry and used as an estimation of antioxidant activity. Based on the results obtained, compound **5b** appears to be the best inhibitor of linoleic acid oxidation (EC50 = 94 μg/mL), followed by **5b** (132 μg/mL) and **2b** (181 μg/mL). The same observation was made regarding the antiperoxidal activity, where the compounds showed good lipid peroxidation inhibitory activity, for both molecule **5b** (101 μg/mL) and compounds **1b**/**3b** (EC50 = 134 μg/mL). The antioxidant response is herein considered in relation to chemical structure, which determines the redox behavior of the synthesized molecules. It was found that benzimidazole substituted in position 2, containing free hydroxyl groups, which are compounds **1b** and **5b**, displaying significant antioxidant activity, including free radical trapping power, iron ion reducing power, and lipid peroxidation inhibiting capacity, which is very remarkable. These are the most promising benzimidazoles for the development of antioxidant drugs.

**Table 1.** Antioxidant activity.

| Synthetized Molecule | Antioxidant Activity EC5O (μg/mL) | | | |
|---|---|---|---|---|
| | DPPH Test | Ferric Ion Reducing Power | β-Carotene | TBARS |
| **1b** | 53 ± 1 | 54 ± 4 | 192 ± 7 | 134 ± 2 |
| **2b** | 139 ± 4 | 101 ± 7 | 181 ± 5 | 156 ± 52 |
| **3b** | 220 ± 15 | 102 ± 22 | 220 ± 11 | 134 ± 2 |
| **4b** | 767 ± 6 | 544 ± 4 | 872 ± 37 | 1554 ± 25 |
| **5b** | 78 ± 5 | 96 ± 8 | 94 ± 3 | 101 ± 7 |
| **BHT** | 23 ± 3 | 30 ± 6 | 48 ± 5 | 76 ± 1 |
| **Trolox** | 51 ± 4 | 44 ± 4 | 63 ± 2 | 84 ± 6 |

3.4.2. Antimicrobial Activity

The search for new antimicrobial compounds in clinical microbiology is driven by the need to counteract the growing rate of infectious diseases caused by foodborne and/or multidrug-resistant strains (MDR: multidrug-resistant and TDR: totally drug-resistant). Currently, bacterial resistance is leading to a growing need for new and effective anti-infective materials to prevent and delay infections associated with implants and devices. The antibacterial activity of the synthetized molecules (**1b**–**5b**) has been tested against four bacterial ATCC strains and one yeast strain. The results are expressed in MIC and MBC values (mg·mL$^{-1}$), as represented in Table 2. Results clearly demonstrated different degrees of bacterial growth inhibition. Gram + bacterium *S. aureus* was more sensitive to the tested molecules, presenting MIC values ranging from 0.156 to 1.25 mg·mL$^{-1}$ compared with other strains. Compound **5b** was likely the most active compound, presenting an MIC value similar to the standard antibiotic Ceftazidime (MIC = 156 mg·mL$^{-1}$). According to the chemical characterization, the molecules with hydroxyl groups were the most active (compounds **1b** and **5b**). These molecules can be considered bactericidal and fungicidal. However, they can be used as antibiotics because of their ability to complex with soluble extracellular proteins and with bacterial cell walls, often resulting in inactivation and loss of function [16]. The antimicrobial activities of products containing hydroxyl groups may involve different modes of action, namely destabilization and permeability of the cytoplasmic membrane and inhibition of enzymes by oxidized products, possibly by reaction with sulfhydryl groups or by more non-specific interactions with proteins [17].

**Table 2.** Antibacterial activity.

| Synthetized Molecule | *E. coli* | | *S. aureus* | | *P. aeruginosa* | | *S. typhi* | | *C. albicans* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** |
| **1b** | 0.3125 | 2.5 | <0.3125 | 1.25 | 0.625 | 5 | 0.3125 | 5 | <0.3125 | 2.5 |
| **2b** | 0.3125 | 10 | 0.3125 | 5 | 1.25 | >10 | 0.3125 | 5 | 0.625 | 2.5 |
| **3b** | 0.3125 | 5 | 0.3125 | 2.5 | 2.5 | 5 | 0.3125 | 5 | 0.3125 | 5 |
| **4b** | 2.5 | 2.5 | 1.25 | 1.25 | >10 | >10 | 2.5 | >10 | 5 | >10 |
| **5b** | 0.625 | 2.5 | 0.156 | 0.625 | 2.5 | 5 | 0.3125 | 5 | 0.3125 | 5 |
| ***Antibiotics*** | | | | | | | | | | |
| **Gentamicine 10Ug** | <0.078 | | <0.078 | | 0.156 | | <0.156 | | nt | |
| **Ceftazidime 30Ug** | <0.156 | | 0.156 | | 0.156 | | <0.156 | | nt | |
| **Nystatine** | nt | | nt | | nt | | nt | | <0.078 | |

MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration, nt: not tested.

*3.5. Molecular Docking Results*

The thymidylate kinase is a key enzyme that is involved in DNA replication and repair mechanisms in most bacterial strains. It catalyzes the phosphorylation of deoxythymidine monophosphate (dTMP) to deoxythymidine diphosphate (dTDP). However, it is necessary to consider this protein in the search for new molecules that avoid the existing resistance mechanisms, as this thymidylate kinase is a new target of anti-*S. aureus* drugs [13]. The designed molecules **1b** and **5b** could inhibit the ligand-binding-induced receptor, as confirmed by virtual molecular docking using Autodock vina software. The docking scores of the binding affinity (ΔG) and inhibition constant (Ki) are presented in Table 3. The molecular docking could confirm the possible binding patterns that may occur with the synthesized compound against the thymidylate kinase protein. Autodock vina scores demonstrate that molecules **1b** and **5b** had significant ΔG values of −8.3 (Ki = 0.812 μm) and −9.4 Kcal/Mol (ki = 0.127 μm) with 4QGH binding sites, respectively (Table 3). This interaction affinity is due to the existence of potential H-bond donor and H-bond acceptor groups as well as the hydrophobic interactions with the docked molecules. The N−H bonds of the NH amine and the hydrogen atoms of the hydroxyl group OH may adopt

different binding positions, with the active pocket of the protein inducing the receptor inhibition being able to arrest the bacterial DNA replication and reparation mechanisms.

**Table 3.** Docking scores of **1b** and **5b** on PDB: 4QGH.

| Protein | Interacting Residue | Binding Energy, ΔG (Kcal/Mol) | Inhibition Constant, Ki (μm) |
|---|---|---|---|
| thymidylate kinase **TMK (4QGH)** | **1b** | **−8.3** | 0.812 |
| | **5b** | −9.4 | 0.127 |

## 4. Conclusions

Overall, the current study was designed to develop new bioactive drugs. Thus, a set of five new benzimidazole derivatives were synthetized by reacting o-phenylenediamine with several aldehydes and phenolic acids through chemical synthesis and hemi-synthesis using a quick microwave-assisted processes. Hemi-synthesis products were purified using column chromatography and the developed molecules were characterized by ATR-FTIR and NMR 1H spectroscopy. All synthetized compounds were screened for their antioxidant and antimicrobial activities using several invitro assays. Among the panel of new benzimidazole derivatives, compound 2-(1H-1,3-benzodiazol-2-yl) phenol (**1b**) and 5-(1H-benzimidazol-2-yl) benzene-1,2,3-triol (**5b**) showed significant potential. Hence, these compounds may serve as lead molecules to develop antimicrobial and antioxidant drugs. Additionally, compounds **1b** and **5b** (2-(1E)-2-phenylethenyl-1H-1,3-benzodiazole) were docked with *S. aureus* thymidylate kinase (TMK) protein (4QGH) using AutoDock Vina software to highlight the structure–activity relationship of these molecules. The results showed an excellent binding score. Further assays should be performed on cytotoxicity as well as in-vivo experimentation to validate their possible introduction into pharmaceutical trials.

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**Conflicts of Interest:** The authors declare that they have no conflicts of interest regarding this manuscript.

## Appendix A

**Table A1.** GC/MS composition of *Cinnamomun verum* L. bark strips.

| Rt (min) | Concentration % | Compound |
|---|---|---|
| 4.3 | 0.02 | Tetrachloroethylene |
| 8.6 | 0.04 | γ-Terpinene |
| 9.4 | 0.03 | Camphene |
| 10.6 | 0.25 | Benzaldehyde |
| 14.6 | 0.05 | D-Limonene |
| 23.1 | 0.05 | 1,2-Chromene |
| 24.6 | 0.92 | Hydrocinnamaldehyde |
| 25.3 | 0.07 | Phenyl 2-Propynyl Ether |
| 34.9 | 90.54 | Cinnamaldehyde |
| 38.2 | 0.61 | α-Cubebene |

| | | |
|---|---|---|
| 39.1 | 0.06 | Oxirane |
| 39.4 | 0.05 | α-Copaene |
| 39.8 | 0.07 | (+)-Sativene |
| 40.7 | 0.04 | (−)-Isosativene |
| 41.3 | 0.05 | β-Thujene |
| 43.2 | 0.03 | Benzenamine |
| 43.8 | 2.87 | Coumarin |
| 45.0 | 0.21 | Naphthalene |
| 46.1 | 0.05 | Amide Hydrocinnamique |
| 46.5 | 0.66 | α-Cadinene |
| 47.9 | 0.90 | δ-Cadinene |
| 48.5 | 0.19 | 1H-3a,7-Methanoazulene |
| 49.2 | 1.13 | 4-Methoxycinnamaldehyde |
| 53.4 | 0.08 | 2,4-Hexadiene |
| 55.9 | 0.36 | γ-Cadinene |

**Table A2.** GC/MS composition of *Cymbopogon citratus* L.

| Rt (Min) | % | Compound |
|---|---|---|
| 4.6 | 0.02 | Tridodecylamine |
| 7.8 | 0.08 | D-Limonene |
| 12.2 | 4.55 | β-Myrcene |
| 14.6 | 0.1 | α-Limonene |
| 15.4 | 0.31 | α-Pinene |
| 16.1 | 0.33 | β-Ocimene |
| 16.4 | 0.06 | Myrcenylacetat |
| 19.3 | 0.05 | Nortricyclene |
| 19.8 | 0.4 | Furan |
| 20.3 | 1.52 | L-Linalool |
| 21.3 | 0.06 | Fenchol |
| 22.5 | 0.23 | Cyclohexene |
| 23.3 | 0.44 | Trans-Chrysanthemal |
| 23.6 | 0.35 | (R)-(+)-Citronellal |
| 24.5 | 0.81 | Cyclopropene |
| 25.8 | 1.29 | 7-Methyl-1-Nonyne |
| 28.4 | 0.11 | O-Mentha-1(7),8-Dien-3-Ol |
| 30.5 | 34.87 | Neral |
| 32.8 | 43.88 | Cis-Citral |
| 33.7 | 0.32 | Geranial |
| 34.2 | 0.22 | Geranyl Vinyl Ether |
| 35.8 | 3.5 | Geraniol |
| 38.4 | 0.24 | Nerol |
| 39.5 | 3.37 | Nerol Acetate |
| 40.2 | 0.65 | Geranic Acid |
| 41.3 | 0.21 | β-Caryophyllene |
| 42.4 | 0.17 | α-Bergamotene |
| 50.1 | 0.06 | Neryl Acetate |
| 51.4 | 0.08 | β-Citronellal |
| 54.1 | 0.14 | Trans-β-Farnesene |
| 70.8 | 0.18 | Farnesyl |
| 72.5 | 0.06 | Trans-Caryophyllene |
| 75.3 | 0.11 | Cyclopropane Carboxamide |
| 76.7 | 0.2 | α-Trans-Sequicyclogeraniol |
| 78.3 | 0.31 | Farnesol |
| 79.6 | 0.14 | 3,7-Nonadien-2-Ol |
| 80.0 | 0.07 | Geranylacetone |

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