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

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Abstract: Benzimidazoles 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 benzimidazoles derivatives. Compound 1b, (2-(1H-1,3-benzodiazol-2-yl) phenol) was synthesized by reacting o-phenylenediamine (OPA) with chemical salicylaldehyde, while compound 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 hemi-synthesis process of respectively the cinnamon (cinnamaldehyde, 90.54%), and lemongrass (cis-citral, 43.9%) essential oils previously characterized by GCMS. Compounds 4b, (2-phenyl-1H-benzimidazole) and 5b, (5-(1H-benzimidazol-2-yl)benzene-1,2,3-triol) were synthesized with click chemistry method by reacting the OPA with Benzoic acid and gallic acid directly in ethanol under microwave irradiation MW 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 TBARS formation inhibition. All the tested compounds showed antioxidant potential with different performances. Antimicrobial activity was investigated against ATCC strains (three Gram-bacteria: *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*, one Gram+ bacteria: *Staphylococcus aureus*, and one yeast stain *Candida albicans*) through the determination of MIC and 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 *S. typhi* and *C. albicans* (MIC = 0.3125 mg·mL^{-1}), then *E.coli* and *P. aeruginosa*. Compound 1b also showed a 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}). A further molecular docking was proceeded using AutoDock Vina 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

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: Multi-Drug-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, it's a crucial point to develop new therapeutic agents and broad-spectrum pharmaceutical probes for clinical trials [2]. The natural biomolecules of vegetable origin due to their chemical diversity such as the phenolic acids and chiral monoterpene aldehydes, offer unlimited possibilities for new drug discovery through organic synthesis patterns. Those 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 a good candidate 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 large diversity of biological properties that they may present (antibacterial, antiviral, antioxidant, anticancer, anti-inflammatory…etc.) [6]. Recently, in-silico docking has made great strides in predicting the molecular interactions that hold a protein and ligand in the binding site stimulating the progress of new drugs development [7,8]. The docking of small molecules and the virtual screening of candidate compounds have become an integral part in the biomedical field and drug design. Several software 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: AutoDock and AutoDock vina 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 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 of Tipaza downtown city. The extraction of essential oils was performed by hydrodistillation method using a Clevenger type apparatus [9]. 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 Oils 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 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 °C–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 EIMS at 70 eV electron energy. Identification of compounds was based on a comparison of 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 Aldehydes Derivatives

The amino derivative 1,2-phenylenediamine (OPA) is first diluted in ethanol (15 mL) to form an initial solution of reagent 1, and then the chemical salicylaldehyde (1 mmol of corresponding aldehyde, calculated on the basis of its corresponding molecular mass, approximately 0.5 mL) is added to an ethanolic solution with 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 few drops of HCl 0.05% was added (Figure 1). The reaction was left for reflux (1 h) under adapted microwave extractor 400 MHz. The crystals of compound 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.

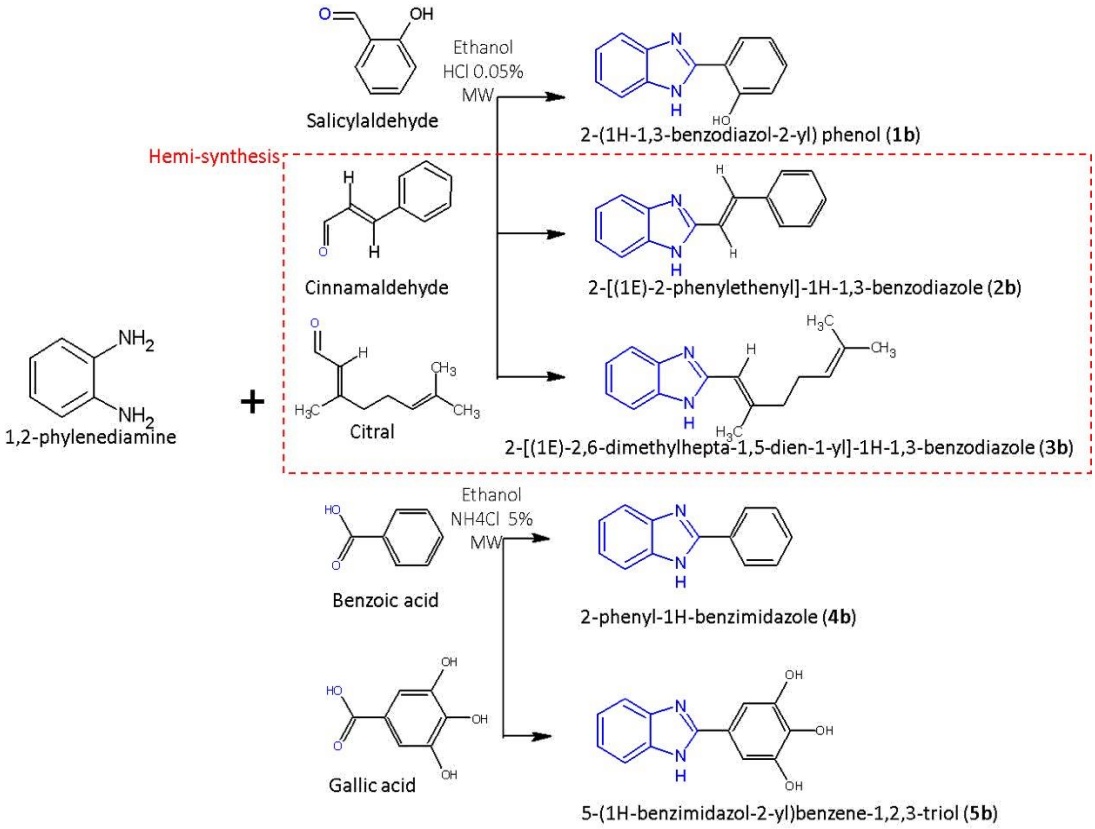

Figure 1. Synthesis procedure and Chemical structure of synthesized molecules obtained by Chemsketch software.

2.3.2. Synthesis of Benzimidazole Phenolic Acids Derivatives

The total chemical synthesis reaction is carried out by the condensation of OPA with the appropriate phenolic acids (0.03 mol). The reaction is 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, each phenolic acid (in molar correspondence) was also dissolved in an ethanol (30 mL) in the reactor and heated to 140 °C under 10 bars of internal pressure for 5 min. Therefore, it was cooled to room temperature, thus solution 2 is formed; The two solutions are mixed and let 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 pattern of all the synthetized compounds, a 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 simples 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 is then read directly under UV light, the spots appear without having to resort to a developer. Afterward, a column chromatography was performed to separate and purify the final products of the hemi-synthesized molecules since the reaction may occur also on other aldehydes of the EO mixture. The separation is carried out by gravity on silica particles of 70 to 200 nm where the solvent flows by drip. The eluent (mobile phase) used here initially the dichloromethane/hexane (50:50 *v*/*v*) which allows the elution of the non-polar fraction followed by ethanol and chloroform, which separates the fraction strongly retained by silica. The benzimidazole molecules produced are driven by the mobile phase, and they are 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 are recorded in a few minutes without any limitation concerning the size of the studied molecule. FTIR spectroscopy in the 4000e400 cm1 region was also used to measure trends and reaction patterns in real time, providing very specific information on kinetics, the mechanism, the reaction path and the influence of variables on reaction performance. A ^{1}H NMR Analysis were then performed on a Bruker 400 spectrometer (Bruker, Wissembourg, France, 400 MHz for 1H), in DMSO-d6 as solvent. Chemical shifts (δ) were reported in ppm and coupling constants (*J*) in Hz and the internal standard was TMS. In a 5 mm diameter NMR tube, 500 uL of sample (synthetic molecules or standard solution) is inserted and a capillary tube containing a solution of TSP-d4 dosed at 2.1 mmol proton/L. This solution serves as a reference for chemical displacements (6 'H = 0.00 ppm). Depending on the concentration of the samples, *64 to* 128 accumulations were made over a spectral width of 3200 Hz. The use of Brüker's Topspin 2.6 NMR software was used for data processing. Structural drawing and naming attribution were monitored by Chemketch software and NMR sectra was processed by MestRnova program. Brüker's Topspin CMC 2.6 was used for structure authentification.

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 cells homogenates (TBARS). The molecules are dissolved in ethanol with a well-known volume to set an initial concentration [C= 3 mg·mL^{-1}] that is diluted at different concentrations until EC50 is determined (Concentration providing 50% antioxidant activity or 0.5 absorbance in the reductive power; Expressed in μg/mL) [10]. 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 a percentage of reagents discoloration. Inhibition of β-carotene bleaching was evaluated by the neutralization levels of linoleate free radicals that avoids β-carotene bleaching. Lipid peroxidation inhibition in ovine brain homogenates was evaluated by the decrease in thiobarbituric acid reactive substances (TBARS); the color intensity of the malondialdehyde-thiobarbituric acid (MDA-TBA) was measured by its absorbance at 532 nm. BHT and Trolox was used as positive controls.

2.6.2. Antimicrobial Activity

The synthesized compounds were tested for their antibacterial activity against 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™). MIC and MBC values were determined by a micro-dilution method and a rapid colorimetric test of p-iodonitrotetrazolium chloride (INT) [11]. 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 (Trypton Soya 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 were added to all wells containing tested concentrations in the range of 20–0.156 mg·mL^{-1}. The microplate is 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 the 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). 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 ligand 1b and 5b, was prepared for molecular docking simulation by setting the torsion tree and rotatable, nonrotatable bonds present in the ligand through AutoDock tools software [12], The binding scores of the receptor proteins is identified by Biovia DS visualizer. The molecular docking affinity of the receptors/ligand is validated basing on the obtained binding energy (ΔG) and the predicted inhibition constant (Ki).

3. Results and Discussion

3.1. GCMS Profiles of Essential Oils of Cinnamon and Lemongrass

The GCMS profile of cinnamon EO showed variations in the chemical constituents. The GCMS chromatogram showed that the major compound found throughout the cinnamon oil was cinnamaldehyde at 90.54% (Table A1). Then the remaining compounds are 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 whole cinnamon oil is indicated as follows: -cinnamaldehyde (90.54%) > coumarin (2.87%) > hydrocinnamaldehyde (0.92%). For lemongrass, the oil was dominated by monoterpene hydrocarbons (Table A2). 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 the (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 is used to separate/fractionate products. (2-(1H-1,3-benzodiazol-2-yl) phenol) was synthesized by reacting o-phenylenediamine (OPA) with chemical salicylaldehyde, while compound 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 hemi-synthesis process of respectively the cinnamon (cinnamaldehyde, 90.54 %), and lemongrass (citral, 43.9%) essential oils previously characterized by GCMS. The reaction of OPA with benzoic and gallic acids gives 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 are named by the ChemSketch 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 spectrum of the most obtained compounds showed a significant absorbance rates estimated at about 90% absorbance between region 3160 and 3469 cm^{-1} and 75% absorbance between region 1000 and 1500 cm^{-1}, with a specific C-N stretching band at 1368 cm^{-1} of the benzimidazole ring. In the other hand, the RMN^{1}H analysis of the synthetized compounds reveals globally the presence of a 2H singlet at 8.1, 7.6 ppm corresponding to the two pyrrolytic protons. We also note the presence of a multiplet between 7.16 and 7.08 ppm corresponding to the 4 aromatic protons. Also, two doublets are observed at 7.7 and 7.6 ppm attributable to the four aromatic protons. The chemical shifts of the compounds are represented as follow:

2-(1H-1,3-benzodiazol-2-yl) phenol (1b): ^{1}H NMR, (400 MHz, DMSO-d6): 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-d6): 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-d6): 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-d6): 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-d6): 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. Bioactivities 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 have an indication on the antioxidant capacity of the sample to be tested [13]. In the present study, the new benzimidazoles reported were screened for their antioxidant activity by using four in vitro assays: DPPH free radicals scavenging, reducing power, β-carotene bleaching inhibition and TBARS formation inhibition. The results are expressed in EC50values (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 chronical diseases and DNA damages leading to carcinogenesis [14]. The six molecules (1b–5b) showed antioxidant activity with different performances. 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 closely similar to the synthetic antioxidant drug "Trolox" used as standard of comparison. with EC 50 = 53 μg/mL in comparison with the Trolox which represents the standard with an estimated value of 51 μg/mL for the DPPH test. The values obtained for the RP test show that those compounds had a high reducing iron potential especially of the compound 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 is 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 found with the antiperoxidal activity where the compounds showed a good lipid peroxidation inhibitory activity either for molecule 5b (101 μg/mL) and compounds 1b/3b (EC50 = 134 μg/mL). The antioxidant response in herein considered in relation to chemical structure that determine the redox behavior of the synthesized molecules, it is found that benzimidazole substituted in position 2 containing a free hydroxyl groups which are compounds 1b and 5b have a significant antioxidant activity, including free radical trapping power, iron ion reducing power and lipid peroxidation inhibiting capacity, which are very remarkable. Those 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 food borne and/or multi-drug resistant

strains (MDR: Multi-Drug-Resistant and TDR: Totally drug-resistant). Currently, the 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 4 bacterial ATCC strains and one yeast strain. The results are expressed in MIC and MBC values ($mg{\cdot}mL^{-1}$) as represented in the Table 2. Results clearly demonstrated different degrees of bacteria growth inhibition. Gram-positive bacteria *S. aureus* was more sensitive to the tested molecules presenting MIC values ranging from 0.156 to 1.25 $mg{\cdot}mL^{-1}$ comparing with other strains. Compound 5b was likely the most active compound by presenting MIC value similar to the standard antibiotic Ceftazidime (MIC = 156 $mg{\cdot}mL^{-1}$). According to the chemical characterization, the molecules with hydroxyl groups were the most active (compound 1b and 5b). These molecules can be qualified as 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 [15]. 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 [16].

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	

nt: not tested.

3.5. Molecular Docking Results

The thymidylate kinase is a key enzyme that is involved in DNA replication and reparation mechanisms in most bacterial strains. It catalyzes phosphorylation of deoxythymidine monophosphate (dTMP) to deoxythymidine diphosphate (dTDP). However, it is necessary to interest to this protein for the search for new molecules that avoid the existing resistance mechanisms, for this thymidylate kinase as a new target of anti *S. aureus* drug [13]. The designed molecules 1b and 5b could inhibit the ligand binding-induced receptor confirmed by a 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 makes clear that 1b and 5b molecules have a 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 position with the active pocket of the protein inducing the receptor inhibition being able to arrest the bacterial DNA replication and reparation mechanisms.

Table 3. Dicking scores of 1b and 5b on 4QGH.

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

4. Conclusions

Overall, the current study is designed to develop new bioactive drugs. Thus, a set of five new benzimidazoles 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 by using several invitro assays. Among all the panel of the new benzimidazoles 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, compound 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 a great binding score. Further assays should be performed on cytotoxicity as well as in-vivo experimentation to validate their possible introduction to the 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. GCMS 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. GCMS 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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