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Research Article

Synthesis, Antibacterial Evaluation, and QSAR of Caffeic Acid Derivatives

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The present study evaluates the antibacterial effects of a set of 16 synthesized caffeic acid ester derivatives against strains of *Staphylococcus aureus* and *Escherichia coli*, as well as discusses their structure-activity relationship (SAR). The antibacterial assays were performed using microdilution techniques in 96-well microplates to determine minimal inhibitory concentration (MIC). The results revealed that five of the compounds present strong to optimum antibacterial effect. Of the sixteen ester derivatives evaluated, the products with alkyl side chains, as propyl caffeate (**3**), butyl caffeate (**6**), and pentyl caffeate (**7**), presented the best antibacterial activity with MIC values of around 0.20 μM against *Escherichia coli* and only butyl caffeate (**6**) showing the same MIC against *Staphylococcus aureus*. For products with aryl substituents, the best MIC results against the tested strain of *Escherichia coli* were 0.23 μM for (di-(4-chlorobenzyl)) caffeate (**13**) and 0.29 μM for diphenylmethyl caffeate (**10**) and all were less active against the *Staphylococcus aureus* strain. Preliminary quantitative structure-activity relationship (QSAR) analyses confirmed that certain structural characteristics, such as a median linear carbon chain and the presence of electron withdrawal substituents at the *para* position of the aromatic ring, help potentiate antibacterial activity.

1. Introduction

Bacterial diseases are a global health problem and are prevalent in developing and low-income countries [1, 2], causing 13 to 17 million deaths annually, equivalent to 25% of global deaths and 45% for developing countries [3]. Due to the bacterial resistance of *Staphylococcus aureus* to methicillin and vancomycin [4] and *Escherichia coli* to β-lactams [5], there is an urgent need for discovery and development of new classes of antimicrobial compounds to treat bacterial infections. Methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for high hospital infection rates [6, 7]. These infections range from simple acnes, boils, and abscesses to more severe forms such as pneumonia, meningitis, endocarditis, and septicemia [8, 9]. β-Lactams are not always fully effective against strains of MRSA [10]. Vancomycin is an alternative; however, there are already case reports of intermediate [11] and full resistance to the drug [12].

Escherichia coli is active in the human intestinal tract [13]; it participates in vitamin K_2 synthesis and in defense against pathogenic bacteria [14]. *Escherichia coli* can cause severe food poisoning and more severe conditions such as peritonitis [15], meningitis, and urinary infections [16]. In *E. coli* strains, production of extended-spectrum β-lactamases (ESBL) [17] hydrolyzes most penicillins, cephalosporins, and monobactams (aztreonam), the cephamycins and carbapenems being among the few exceptions [18]. *E. coli* strains are resistant to aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol, and sulfamethoxazole-trimethoprim [19]. In the most serious infections involving ESBL, carbapenems are indicated [20], but there are already reports in the literature of resistance to carbapenems [21, 22].

Many studies have investigated the bioactivity of caffeic acid derivatives finding that some of these analogues possess remarkable activities, with potential as anti-inflammatory [23–27], antiviral [28], antiatherosclerotic [29], anti-HIV [30], antioxidant [31, 32], immunomodulatory [33], antitumor [34–37], neuroprotective [38], antifungal [39], and antibacterial agents [40]. Caffeic acid phenethyl ester (CAPE) or phenethyl caffeate is considered one of the most important derivatives of caffeic acid and has several various biological activities, such as antibacterial action [41–43]. It is found in propolis extract and occurs in many plants [41]. Due to the problems associated with treatment of bacterial infections, such as drug-resistant strains, various studies have been conducted to discover new and safer antibacterial agents. In the present study, synthesis and antibacterial activity evaluations of 16 caffeic acid derivatives against two representative strains *Staphylococcus aureus* (ATCC-25619) and *Escherichia coli* (ATCC-2536) were performed.

2. Chemicals

All of the chemical products used during synthesis were from Sigma-Aldrich. ^{1}H-NMR (200 and 50 MHz) and ^{13}C-NMR (500 and 125 MHz) spectra were, respectively, recorded on Varian Mercury and Varian-RMN-System spectrometers. Chemical shifts (δ) are expressed in parts per million (ppm) using TMS as an internal standard. Spin multiplicities are given as s (singlet), brs (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), quint (quintet), sext (sextet), sept (septet), and m (multiplet). High-resolution mass spectrometry was carried out using an Ultraflex II TOF/TOF spectrometer with a high performance solid state laser ($\lambda = 355$ nm) and a reflector using the MALDI (matrix-assisted laser desorption ionization) technique. Column adsorption chromatography (CC) was performed on silica gel (Merck 60, 230–400 mesh); analytical TLC was performed on precoated silica gel plates (Merck 60 F_{254}). FTIR spectra were recorded on a Bruker FTIR spectrometer, Vertex 70 model, using KBr pellets.

3. Materials and Methods

3.1. Synthesis of Compounds **1**–**8** *for Fischer's Esterification.* A mixture of caffeic acid (0.25 g, 1.39 mmol) and alcohol (50 ml) was heated under reflux in the presence of sulfuric acid (0.4 ml) until completion of the reaction (5–21 hours) and verified by a single spot in TLC. The alcohol was then removed under reduced pressure, and the solution was diluted with 20 ml of water. The product was extracted with ethyl acetate (15 ml). The organic phase was neutralized successively with 5% sodium bicarbonate and water, dried over anhydrous sodium sulfate, and filtered. After evaporation under reduced pressure, this phase yielded the ester derivatives [44].

3.2. Reaction with Alkyl and Aryl Halides for Compounds **9**–**14**

3.2.1. Synthesis of Compounds **9** *and* **10**. Caffeic acid (0.2 g, 1.11 mmol) in acetone (13.5 ml) was heated under reflux in the presence of triethylamine (0.6 ml) and halide (1.14 mmol) until complete reaction (44–120 hours), which was verified by a single spot in TLC. The solvent was then removed under reduced pressure, and the solution was diluted with 20 ml of water. The product was extracted with ethyl acetate (20 ml). The organic phase was treated with water, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified on a silica gel column (eluent: hexane-ethyl acetate and an increasing polarity gradient) [45, 46].

3.2.2. Synthesis of Compounds **11**–**14**. A mixture of caffeic acid (0.2 g, 1.11 mmol) and potassium carbonate (0.17 g, 1.22 mmol) was dissolved in 5.5 mL of dimethylformamide in the presence of halide (1.11 mmol). The mixture was stirred at room temperature, until complete reaction (27–50 hours), which was verified by a single spot in TLC. The solvent was then removed under reduced pressure, and the solution was diluted with 15 ml of water. The product was extracted with ethyl acetate (10 ml). The organic phase was neutralized successively with 5% sodium bicarbonate (10 ml), washed with brine (10 ml), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by using a chromatographic column with hexane and ethyl acetate eluents in an increasing polarity to isolate the ester derivatives [47].

3.2.3. Synthesis of Compounds **15** *and* **16** *for the Mitsunobu Reaction.* A mixture of caffeic acid (0.1 g, 0.55 mmol) and alcohol (0.55 mmol) was dissolved in 1.85 ml tetrahydrofuran. The reaction mixture was stirred under magnetic stirring at 0°C for about 30 minutes. Afterwards, diisopropyl azodicarboxylate (0.11 ml, 0.55 mmol) and triphenylphosphine (0.15 g, 0.55 mmol) were added as esterification agents, with continuous stirring at room temperature for about 46–50 hours, which was verified by a single spot in TLC. The solvent was then removed under reduced pressure, and the solution was diluted with 10 ml of water. The product was then extracted with ethyl acetate (10 ml). The organic phase was neutralized successively with saturated sodium bicarbonate, washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The residue was then purified on a silica gel column (eluent: hexane-ethyl acetate, 7 : 3) [48].

(1) Methoxyethyl Caffeate (**5**). Brown liquid, 61.64% yield; IR υmax (KBr, cm^{-1}): 3319, 3071, 2945, 1689, 1600 and 1442, 1272 and 1176; ^{1}H-NMR (DMSO-d_6, 200 MHz): δH 3.27 (3H; s), 3.56 (2H; t; $J = 4.8$ Hz), 4.22 (2H; t; $J = 4.8$ Hz), 6.28 (1H; d; $J = 16.0$ Hz), 6.76 (1H; d; $J = 8.0$ Hz), 7.01 (1H; dd; $J = 8.0$ Hz, 2.0 Hz), 7.06 (1H; d; $J = 2.0$ Hz), 7.47 (1H; d; $J = 16.0$ Hz); ^{13}C-NMR (DMSO-d_6, 50 MHz): δC 58.2, 63.0, 69.9, 113.8, 114.9, 115.8, 121.6, 125.5, 145.5, 145.6, 148.5, and 166.7 [49]; HRMS (MALDI) calculated for $C_{12}H_{14}O_5$ $[M+H]^+$: 239.0919, found 239.1599.

(2) Diphenylmethyl Caffeate (**10**). Brown liquid, 27.29% yield; IR υmax (KBr, cm^{-1}): 3370, 3059, 2970, 1702, 1606 and

1448, 1271 and 1149; ^{1}H-NMR (DMSO-d_6, 200 MHz): δH 6.43 (1H; d; J = 16.0 Hz), 6.77 (1H; d; J = 8.0 Hz), 6.90 (1H; s), 7.06 (1H; dd; J = 8.0 Hz, 2.0 Hz), 7.10 (1H; d; J = 2.0 Hz), 7.47–7.28 (10H; m), 7.59 (1H; d; J = 16.0 Hz), [44]; ^{13}C-NMR (DMSO-d_6, 50 MHz): δC 76.1, 113.7, 115.0 115.8, 121.7, 125.5, 126.6, 127.8, 128.6, 140.8, 145.6, 146.0, 148.6, and 165.6 [50]; HRMS (MALDI) calculated for $C_{22}H_{18}O_4$ $[M+Na]^+$: 369.1103, found 369.1168.

(3) 4-Chlorobenzyl Caffeate (**11**). Yellow amorphous solid, 26.43% yield; IR υmax (KBr, cm^{-1}): 3338, 3053, 2964, 1677, 1600 and 1435, 1258 and 1175, 1011; ^{1}H-NMR (DMSO-d_6, 200 MHz): δH 5.18 (2H; s), 6.33 (1H; d; J = 16.0 Hz), 6.76 (1H; d; J = 8.0 Hz), 7.02 (1H; dd; J = 8.0 Hz, 2.0 Hz), 7.07 (1H; d; J = 2.0 Hz), 7.44 (4H; brs), 7.53 (1H; d; J = 16.0 Hz); ^{13}C-NMR (DMSO-d_6, 50 MHz): δC 64.5, 113.6, 114.9, 115.8, 121.6, 125.5, 128.5, 129.9, 132.6, 135.5, 145.6, 145.8, 148.5, and 166.3 [51]; HRMS (MALDI) calculated for $C_{16}H_{13}ClO_4$ $[M]^+$: 304.0502, found 304.3855.

(4) (Di-(4-chlorobenzyl)) Caffeate (**13**). White amorphous solid, 11.82% yield; IR υmax (KBr, cm^{-1}): 3332, 3059, 2976, 1695, 1606 and 1442, 1277 and 1169, 1011; ^{1}H-NMR (DMSO-d_6, 200 MHz): δH 5.16 (2H; s), 5.19 (2H; s), 6.41 (1H; d; J = 16.0 Hz), 7.00 (1H; d; J = 8.4 Hz); 7.10 (1H; d; J = 2.0 Hz); 7.14 (1H; s), 7.49–7.43 (8H; m), 7.56 (1H; d; J = 16.0 Hz); ^{13}C-NMR (DMSO-d_6, 50 MHz): δC 64.6, 68.9, 113.7, 114.8, 115.0, 121.2, 127.2, 128.4, 128.5, 129.5, 129.9, 132.4, 132.7, 135.4, 136.0, 145.2, 147.0, 148.8, and 166.3 [51, 52]; HRMS (MALDI) calculated for $C_{16}H_{13}ClO_4$ $[M+H]^+$: 429.0660, found 429.0667.

(5) (Di-(4-methoxybenzyl)) Caffeate (**14**). Orange amorphous solid, 36.98% yield; IR υmax (KBr, cm^{-1}): 3344, 3059, 2831, 1702, 1620 and 1435, 1188 and 1112; ^{1}H-NMR (DMSO-d_6, 200 MHz): δH 3.74 (3H; s), 3.74 (3H; s), 5.07 (2H; s); 5.11 (2H; s), 6.36 (1H; d; J = 15.8 Hz), 6.94–6.86 (5H; m), 7.02 (1H; dd, J = 8.0 Hz, 2.0 Hz), 7.10 (1H; s), 7.22 (2H; d; J = 8.6 Hz), 7.35 (2H; d; J = 8.6 Hz), 7.51 (1H; d; J = 16.0 Hz); ^{13}C-NMR (DMSO-d_6, 50 MHz): δC 55.1, 55.2, 65.4, 69.6, 113.6, 113.8, 113.9, 114.6, 115.1, 121.3, 127.1, 128.4, 128.9, 129.8, 130.3, 144.9, 147.2, 149.2, 159.1, 159.3, and 166.5 [52, 53]; HRMS (MALDI) calculated for $C_{25}H_{24}O_6$ $[M]^+$: 420.1573, found 420.0234.

(6) 4-Methylbenzyl Caffeate (**16**). Yellow amorphous solid, 50.95% yield; IR υmax (KBr, cm^{-1}): 3332, 3027, 2976, 1677, 1600 and 1435, 1271 and 1163; ^{1}H-NMR (DMSO-d_6, 200 MHz): δH 2.29 (3H; s), 5.13 (2H; s), 6.30 (1H; d; J = 16.0 Hz), 6.75 (1H; d; J = 8.0 Hz), 7.00 (1H; dd; J = 8.2 Hz, 2.0 Hz), 7.06 (1H; d; J = 2.0 Hz), 7.18 (2H; d; J = 8.0 Hz), 7.29 (2H; d; J = 8.0 Hz), 7.50 (1H; d; J = 16.0 Hz); ^{13}C-NMR (DMSO-d_6, 50 MHz): δC 21.9, 65.3, 113.8, 114.9, 115.8, 121.6, 125.5, 128.3, 129.1, 133.5, 137.4, 145.5, 145.6, 148.6, and 166.5 [50]; HRMS (MALDI) calculated for $C_{17}H_{16}O_4$ $[M]^+$: 284.1049, found 284.4357 (Scheme 1).

4. Antibacterial Assay

The microbiological testing used single strains of *Staphylococcus aureus* (ATCC-25619) and *Escherichia coli* (ATCC-2536). The strains belonged to the Bacterioteca of the Laboratory of Toxicological Tests, Institute of Research in Drugs and Medications, Paraíba Federal University. The strains were maintained in an appropriate culture medium, BHI (Difco Laboratories/France/USA), and stored at 4°C and 35°C. The microorganism suspension was prepared according to McFarland tube 0.5, corresponding to approximately 10^8 CFU·ml^{-1} [54, 55].

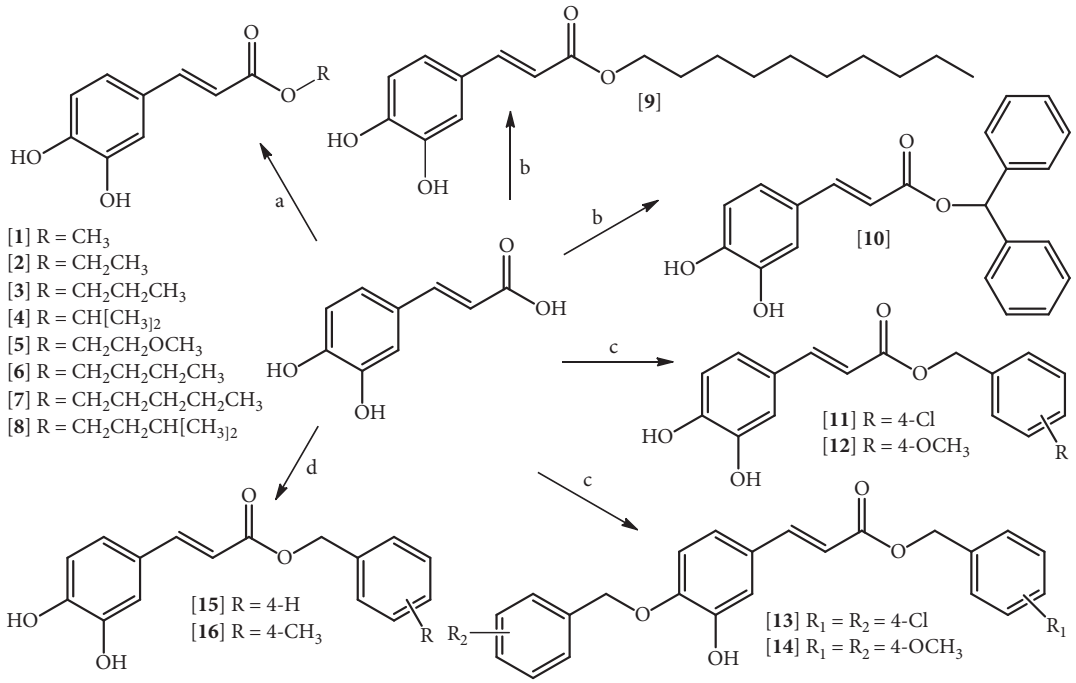
Culture Medium. The antifungal activity assays were performed in brain heart infusion (BHI) broth liquid medium (Difco Laboratories/France/USA), which was prepared and used according to the manufacturer's instructions.

Determination of the Minimum Inhibitory Concentration (MIC). The MIC values were determined by the microdilution method [54] using 96-well "U"-shaped microtiter plates in duplicate. In each plate well, 100 μl of twice concentrated BHI liquid medium was added, except the first line which received 160 μl. Then, 40 μl of product solution (also doubly concentrated) was placed in the first row of the plate wells. By serial dilution (ratio of two), concentrations from 200–12.5 μg/ml were obtained, such that in the first line of the plate was the highest concentration and in the latter lower concentration. Finally, another 100 μl of the BHI medium was added to all wells, and 10 μl of inoculum was added to the wells in each plate column that referred specifically to a strain. The same was also done in the culture medium with the bacterial drug chloramphenicol (100 mg/ml). The plates were incubated at 37°C for 24 h for further reading; this was performed with the addition of 10 μl of a 0.01% (w/v) solution of resazurin (Sigma), a colorimetric metabolic activity indicator. For each strain, the MIC was defined as the lowest concentration capable of inhibiting bacterial growth in the wells, as visually observed compared with the control. All of the tests were performed in duplicate, and the results were expressed as a geometric mean of the MIC values obtained from both tests [56].

5. 3D-QSAR Modeling

Otherwise noted, default parameters were employed for all software. One initial 3D conformer per compound was generated with OpenEye Omega [57]. Each compound's initial conformation was subjected to 200 cycles (100 ps each) of quenched molecular dynamics simulations with Open3DAlign [58] using an implicit solvent model. Molecules alignment was also performed with Open3DAlign by means of its mixed algorithm considering multiple conformers for the template and candidate compounds.

Bioactivities were converted to pIC_{50} values ($pIC_{50} = -\log_{10} IC_{50}$), and QSAR models were separately trained for *Escherichia coli* and *Staphylococcus aureus* using Open3DQSAR [59]. The box step for the grid was set to 0.5 Å, and van der Waals (VDW) and electrostatic molecular interaction fields (MIFs) were computed. The dielectric constant was set to 4. Grid points providing VDW values larger than 10^4 kcal/mol were removed from both MIFs. Values of the grid exceeding 30 kcal/mol or below −30 kcal/mol were set to 30 kcal/mol and −30 kcal/mol, respectively, for both



Scheme 1: Synthesis of the caffeic acid derivatives: (a) ROH, H_2SO_4, reflux; (b) Et_3N, RX, acetone, reflux; (c) DMF, K_2CO_3, RX, room temperature; (d) ROH, THF, TPP, DIAD, 0°C to room temperature.

MIFs. In addition, all grid points with an absolute value lower than 0.05 were set to zero, and variables with standard deviation lower than 0.1 were excluded from the calculations. Variables spanning up to four levels were also excluded. After these variables filtering steps, those remaining in the dataset were scaled according to the BUW algorithm as implemented in Open3DQSAR.

We considered up to 10 principal components (PCs) for models training. The optimal number of PCs considered in the final model was selected according to the r^2 value of the leave-one-out (LOO) cross validation of the model (q^2_{LOO}). With this optimal number of PCs, variables were further grouped using the Smart Region Definition procedure implemented in Open3DQSAR. The grouped variables were subjected to a selection procedure according to the fractional factorial design using leave-many-out (LMO) cross validation with 20 runs and 5 groups. Only selected variables in the previous step remained on the dataset. Finally, given the limited number of available samples, the PLS model was recomputed and its quality was evaluated in 100 cycles of LMO cross validation.

6. Results and Discussion

6.1. Antibacterial Activity and Structure-Activity Relationship (SAR). In the current work, a set of sixteen caffeic acid ester derivatives were evaluated for their in vitro antibacterial activity against strains of *Staphylococcus aureus* and *Escherichia coli*. Methyl caffeate (**1**), ethyl caffeate (**2**), propyl caffeate (**3**), isopropyl caffeate (**4**), methoxy-ethyl caffeate (**5**), butyl caffeate (**6**), pentyl caffeate (**7**), isopentyl caffeate (**8**), decyl caffeate (**9**), diphenylmethyl caffeate (**10**), 4-chlorobenzyl caffeate (**11**), 4-methoxybenzyl caffeate (**12**), (di-(4-chlorobenzyl)) caffeate (**13**), (di-(4-methoxybenzyl)) caffeate (**14**), benzyl caffeate (**15**), and 4-methylbenzyl caffeate (**16**) each with differing substitutions on the ester side chain (R_1) and the hydroxyl of the *para* position of the caffeic ring (R_2) were tested. The results were expressed as minimal inhibitory concentration (MIC) in μM. The structure-activity relationship (SAR) study verified which chemical structures influenced the antibacterial activity. In addition, a quantitative structure-activity relationship (QSAR) was performed to verify contributions of steric and electrostatic factors towards the *Escherichia coli* and *Staphylococcus aureus* activity.

The results obtained in this study revealed that five compounds presented strong to optimum antibacterial activity; the compounds were found to be more active against the *Escherichia coli* strain. Of the sixteen ester derivatives evaluated, propyl caffeate (**3**), butyl caffeate (**6**), and pentyl caffeate (**7**) were found to exhibit the best antibacterial activity of the products with alkyl side chains, with an MIC value of 0.2 μM against the *Escherichia coli* strain and only butyl caffeate (**6**) showed the same MIC for the *Staphylococcus aureus* strain, whereas of the products with aryl side chains, the best MIC results against the *Escherichia coli* strain were 0.23 μM for (di-(4-chlorobenzyl)) caffeate (**13**) and 0.29 μM for diphenylmethyl caffeate (**10**), both being less active against *Staphylococcus aureus*. Table 1 summarizes the in vitro susceptibilities of the two *Escherichia coli* and *Staphylococcus aureus* strains against all of the test compounds.

Of the screened ester derivatives, certain structural features were observed that may increase antibacterial activity, giving information on functional groups that might be important to antibacterial effect, such as alkyl ester side chain, especially a median linear carbon chain, benzyl radicals with two aromatic rings without substituents, and

Table 1: Minimal inhibitory concentration (MIC) values (expressed in μM) of the compounds **1**–**16**.

Compounds	MIC (μM)	
	Staphylococcus aureus (ATCC-25619)	*Escherichia coli* (ATCC-2536)
1	0.52	0.52
2	0.48	0.48
3	0.45	0.22
4	0.45	0.45
5	0.84	0.84
6	0.21	0.21
7	0.40	0.20
8	0.80	0.40
9	0.62	0.62
10	0.58	0.29
11	1.31	1.31
12	0.67	0.33
13	0.93	0.23
14	0.95	0.48
15	0.74	0.37
16	1.41	0.70
Chloramphenicol	0.31	0.31

electron-withdrawal substituents in the *para* position such as chloro.

Data analyses revealed that introduction of alkyl substituents as short chains and the presence of a heteroatom and long (10 carbon atoms) or branching chains resulted in decreased antibacterial effect. For example, butyl caffeate (**6**: butyl group) was found to be the most bioactive compound for both strains (MIC = 0.20 μM), whereas methyl caffeate (**1**; methyl group), ethyl caffeate (**2**; ethyl group), isopropyl caffeate (**4**; isopropyl group), methoxyethyl caffeate (**5**; methoxyethyl group), isopentyl caffeate (**8**; isopentyl group), and decyl caffeate (**9**; decyl group) presented increased MICs (decreased activities). This reduction in antibacterial activity probably occurs due to steric hindrance caused by the presence of bulky groups and also poorly lipophilic short-chain groups. Activity achieved a maximum for the esters with a median linear carbon chain conferring lipophilicity to the molecules.

The compounds presenting aryl radicals resulted in decreased antibacterial effect, such as aromatic ring substituents with electron donor groups in the *para* position such as methyl and methoxy or with electron withdrawal groups such as chloro (except compound (**13**) which was active against *E. coli*). Those without substituents also resulted in decreased antibacterial effect, except compound (**10**) which was active against *E. coli*. The presence of an electron-withdrawing group such as di-(4-chlorobenzyl) caffeate (**13**; di-(4-chlorobenzyl) group) provided more activity against the *Escherichia coli* strain, which probably occurred due to the *para* chloro substituent in the caffeic ring, which was the only difference compared to 4-chlorobenzyl caffeate (**11**; chlorobenzyl group), and presented similarity to the reference drug having two chloro substituents. Compound (**10**) presented good results against *Escherichia coli* due to the presence of bulky groups without substituents with two aromatic rings, such as with diphenylmethyl caffeate (**10**; diphenylmethyl group); this was compared to benzyl caffeate (**15**; benzyl group), which presented a better bond to its target.

The antimicrobial activity of caffeic acid derivatives has been reported in other studies [43, 60–65]. For example, Merkl et al. [66] investigated the antibacterial activity of a series of caffeic acid esters (methyl, ethyl, propyl, and butyl caffeate) against *E. coli* DMF 7503. All of the esters screened showed potent antibacterial activity against the test strain, principally propyl and butyl caffeate, with MIC values of 5.00 mM. The MIC values of the other caffeic acid esters tested were roughly 10.00 mM [67]. In the study of Meyuhas et al. [42], the phenethyl caffeate exhibited antibacterial activity against *S. aureus* (48 mM) and *E. coli* (254 mM). This compound has structural similarity to ester **15**, which showed strong antibacterial action (Table 1). The ester derivatives of caffeic acid were prepared using short linear carbon chain radicals, such as methyl and ethyl caffeate, and from them, compounds synthesized with (4-chloro-benzyl) radicals on the two hydroxyls of the *meta* and *para* positions of the caffeic ring. The antibacterial activities of the compounds were evaluated against *E. coli* and *S. aureus*; the esters, methyl and ethyl caffeates, showed activity for both strains, while their by-products were only active for the *E. coli* strain. Derivatives thus prepared are promising candidates for treating bacterial pathologies, and their test results corroborate those already published in the literature.

6.2. Quantitative Structure-Activity Relationship (QSAR) Studies. The statistical parameters of the 3D-QSAR models obtained for the *E. coli* and *S. aureus* inhibitory activities are summarized in Table 2. As can be seen, both models have good statistical parameters. Furthermore, for both *E. coli* and *S. aureus*, bioactivity is mainly sterically driven. Also, given the lower number of PCs included in the *S. aureus* model and its statistical parameters, it can be considered statistically more robust.

6.2.1. Scaffold. Bioactivity is mostly steric-guided. Models show 77% and 73% contributions of the steric factor to the *Escherichia coli* and *Staphylococcus aureus* activities, respectively (Figure 1).

6.2.2. Electrostatic Molecular Interaction Field. The electrostatic contribution to bioactivity shows that certain substitutions at R_2 can improve bioactivity. In general, the electronegative molecular interaction field next to the O-R_2 bond indicates a positive contribution to bioactivity of substitutions at this position. This positive contribution cannot be exploited when R_2 = H. This later observation holds for both *Escherichia coli* and *Staphylococcus aureus*. Figures 2 and 3 summarize the electrostatic contribution to bioactivity in both strains.

The comparisons of compounds **11** and **13** and of compounds **12** and **14** reveal some interesting details. In the case of *Escherichia coli*, there is an electronegative molecular interaction field close to the 4-chlorobenzyl substitution at

Table 2: Statistical parameters of the 3D-QSAR models for the antibacterial activity against *E. coli* and *S. aureus*.

	E. coli	*S. aureus*
No. PCs[a]	4	2
R^{2b}	0.95	0.86
q^{2}_{LOO}[c]	0.70	0.76
q^{2}_{LMO}[d]	0.68	0.74
VDW contrib.[e]	77%	73%
Ele. contrib.[f]	23%	27%

[a]Number of PCs in the final model. [b]Coefficient of determination. [c]LOO cross-validation performance. [d]LMO cross-validation performance. [e, f]Contribution of VDW and electrostatic factors to bioactivity, respectively.

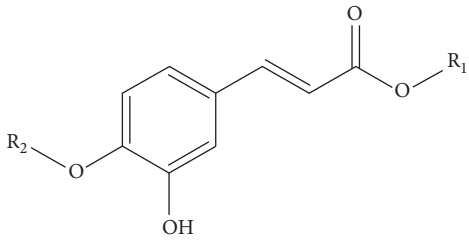


Figure 1

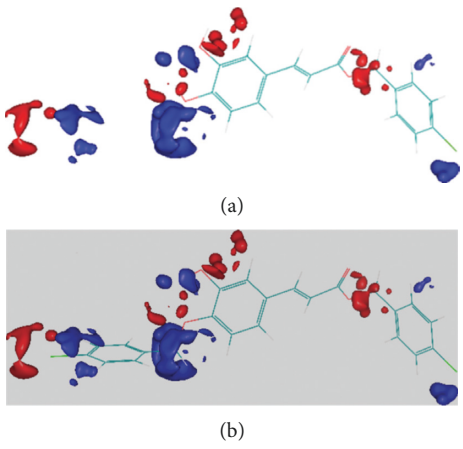


Figure 2: Electrostatic molecular interaction field for compounds **11** and **13** in *Escherichia coli*.

R_2, indicating that benzyl groups with electronegative *para*-substitutions can have an additional positive contribution to bioactivity against this strain. In contrast, substitutions at R_2 such as 4-methoxybenzyl present in compound **14** are unable to increase the bioactivity of R_2-substituted compounds. This can be clearly exemplified for compounds **12** and **14**.

The lack of any additional electrostatic molecular interaction field surrounding R_2 in the *Staphylococcus aureus* model indicates that no increase in the anti-*S. aureus* bioactivity could be obtained with specific substitutions at R_2.

6.2.3. Steric Molecular Interaction Field. The analysis of the steric molecular interaction field shows that there is a steric

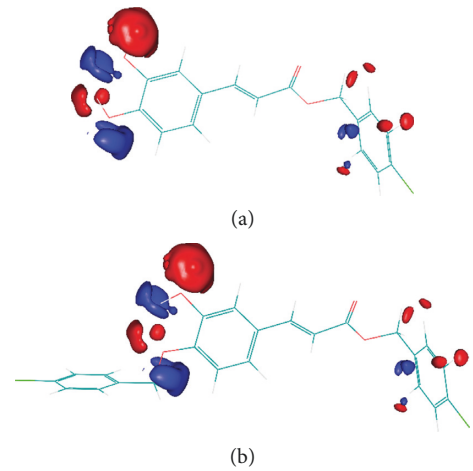


Figure 3: Electrostatic molecular interaction field for compounds **11** and **13** in *Staphylococcus aureus*.

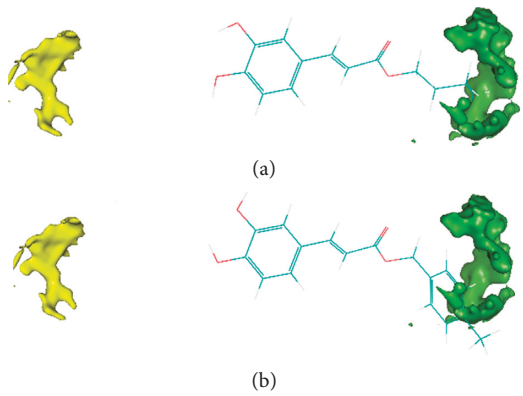
favorable region around R_1 in both *E. coli* and *S. aureus* models. Compounds with small aliphatic substituents at R_1 are favored, while too bulky or too long substituents such as long aliphatic chains and aromatic substitutions are disfavored at this position. This effect is more pronounced in the *Staphylococcus aureus* model where there is a large unfavorable steric region close to R_1. For example, the R_1 substituent of compound **6** completely falls into the steric favorable region defined by both strains models. On the other hand, the 4-methylbenzyl substituent of compound **16** falls beyond the steric favorable region in *Escherichia coli* and in the steric unfavorable region defined by the *Sthaphylococcus aureus* model. In addition, bulky substitutions at R_2 (such as those present in compound **14**) decrease anti-*E. coli* bioactivity. The influence of the steric factor in bioactivity is summarized in Figures 4 and 5.

Considering all these observations, for dual *Escherichia coli* and *Staphylococcus aureus* bioactivity, small aliphatic substitutions are essential at R_1. Furthermore, basing on the available data, substitutions at R_2 could be beneficial for dual bioactivity. This last hypothesis should be confirmed by testing more compounds substituted at R_2 with small groups.

Our QSAR studies confirmed the biological activity of the tested compounds, connecting with chemical structures for (di-(4-chlorobenzyl)) caffeate (**13**), through the electronegative electrostatic molecular interaction field at R_2 as against *Escherichia coli* and for butyl caffeate (**6**), due to the small aliphatic radical completely falling into the sterically favorable region as defined by both strain models. In contrast to these, 4-methylbenzyl caffeate (**16**) exceeded the sterically favorable regions of these models and presented the worst bacterial activity against the tested strains.

7. Conclusion

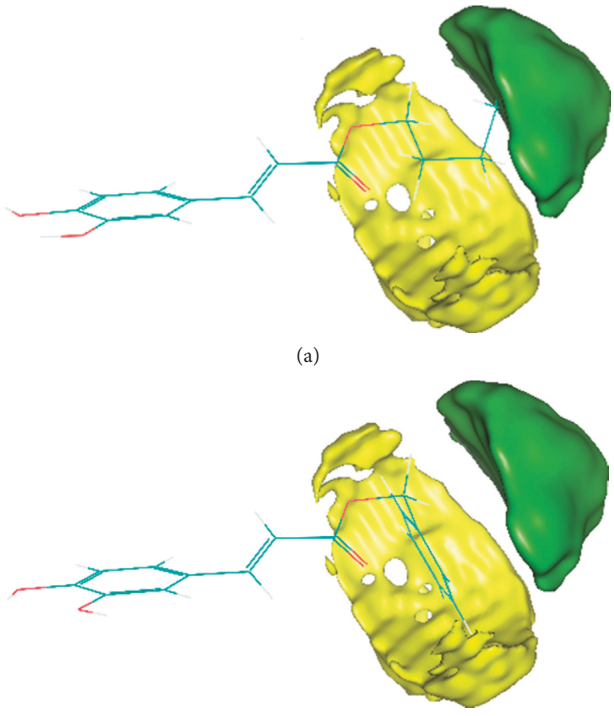
The present study investigated the antibacterial activity of sixteen caffeic acid ester derivatives against a Gram-positive *Staphylococcus aureus* strain and a Gram-negative



(a)

(b)

Figure 4: Steric contribution to *Escherichia coli* bioactivity for compounds **6** and **16**.



(a)

(b)

Figure 5: Steric contribution to *Staphylococcus aureus* bioactivity for compounds **6** and **16**.

Escherichia coli strain. Based on the findings of the present investigation, we conclude that the propyl, butyl, pentyl (di-(4-chlorobenzyl)), and diphenylmethyl caffeate derivatives present the highest antibacterial activity against the *Escherichia coli* strain; only butyl caffeate was active against the *Staphylococcus aureus* strain. Further, the QSAR study demonstrated that small aliphatic substituents in R_1 (butyl caffeate) and substituents in the aromatic ring, with electron withdrawal groups in the *para* position in R_2 ((di-(4-chlorobenzyl)) caffeate), result in bioactive compounds. This preliminary study revealed that certain structural features are important for antibacterial action. Additional studies should be performed to optimize antimicrobial activity in this class of compounds for eventual synthesis of new antibacterial agents.

Data Availability

The data used to support the findings of this study have been deposited in the Federal University of Paraíba repository at https://repositorio.ufpb.br/jspui/handle/tede/9074.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Spectroscopy data of known compounds. ^{13}C-NMR and ^{1}H-NMR spectra of the unpublished compounds: methoxyethyl caffeate, diphenylmethyl caffeate, 4-chlorobenzyl caffeate, (di-(4-chlorobenzyl)) caffeate, (di-(4-methoxybenzyl)) caffeate, and 4-methylbenzyl caffeate. (*Supplementary Materials*)

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