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Antimicrobial evaluation of cinnamic and benzoic haloamides

Ricardo Carneiro Montes[1], Thiago Sampaio de Freitas[2], Maria do Socorro Costa[2], Fernanda de Sousa Oliveira[2], Fábia Ferreira Campina[2], Alana Rodrigues Ferreira[1], Sayonara de Oliveira Ferreira[1], Henrique Douglas Melo[2], Celidarque da Silva Dias[1] and Damião Pergentino de Sousa[1,*]

[1]Laboratory of Pharmaceutical Chemistry, Department of Pharmaceutical Sciences, Federal University of Paraíba, Cidade Universitária, João Pessoa, Paraíba, 58051-900, Brazil
[2]Microbiology and Molecular Biology Laboratory, Department of Biological Sciences, Regional University of Cariri, Juazeiro do Norte, 63040-005, Ceará, Brazil

ABSTRACT

*This study aimed to prepare a collection of para-halogenated benzylamides derivatives of structurally related benzoic and cinnamic acids by coupling reactions with 4-halobenzylamines, using BOP as a coupling agent. All compounds obtained were submitted to antimicrobial tests, using the method of the minimum inhibitory concentration (MIC), with gentamicin, amikacin, norfloxacin, penicillin as controls in the antibacterial assays. The study led to 22 amides (**1**-**22**) with yields ranging from 29-89%. Those compounds were confirmed by infrared spectra, ^{1}H and ^{13}C NMR spectra and by high resolution mass spectroscopy. The antimicrobial evaluation showed that only compound **4** have antibacterial activity and no amide derivative of vanillic acid showed biological activity. We conclude that antimicrobial assays of 4-methoxycinnamic acid derivatives may reveal new antibacterial agents.*

Keywords: Antimicrobial activity, *Escherichia coli*, phenylpropanoid, BOP, antibacterial, 4-methoxycinnamic amide, 4-chlorobenzylamine.

INTRODUCTION

Infectious diseases caused by bacteria are still a prominent global health problem, particularly in developing countries with low-income. In addition, the resistance of bacteria to conventional antibiotics is cause for serious concern to public health worldwide. There is an effort therefore by researchers to discover new antibacterial substances as drug candidates [1]. Cinnamic and benzoic derivatives constitute an interesting class of organic compounds with diverse pharmacologies; they have generated major reviews in the literature [2]. More specifically, cinnamic amides are known to be powerful antimicrobial agents, inhibiting the growth of gram-positive bacteria such as *Bacillus subtilis* [3] and *Staphylococcus aureus* [4], as well as Gram negative bacteria such as *Vibrio parahaemolyticus* [5] and *Escherichia coli* [6, 7]. They also inhibit the growth of fungi such as *Phytophthora infestans* [8], *Aspergillus niger* and *Candida albicans* [4]. Cinnamic acid derivatives are structurally similar to derivatives of benzoic acid (protocatechuic, vanillic, salicylic, and *p*-hydroxybenzoic acids), and also possess antifungal and antibacterial properties [9, 10]. Studies show that cinnamic amides with halogenated aromatic substituents display augmented antimicrobial activity [11]. Such reports led us to prepare a collection of structurally related cinnamic and benzoic amides through coupling reactions using benzotriazol-1-yloxy-tris(dimethylamino) phosphonium hexafluorophosphate (BOP) [12, 13] as coupling agent; subjecting the amides to antibacterial tests.

EXPERIMENTAL SECTION

Chemistry

Purification of the compounds was performed by column chromatography on silica gel 60, ART 7734 MERCK using solvent gradient Hex: EtOAc confirmed by analytical thin layer chromatography on silica gel 60 F_{254}, revealing ultraviolet light at two wavelengths (254 and 366 nm) using a Mineralight apparatus or H_2SO_2 in 5% ethanol. Infrared spectra (IR) were recorded in an FTIR spectrometer IR Prestige-21-Shimadzu model using KBr pellets. ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra were obtained in Varian MERCURY machines (200 and 50 MHz for ^{1}H and ^{13}C, respectively). Deuterated solvents were used ($CDCl_3$, MeOD or DMSO-d_6). Tetramethylsilane (TMS) was used for the internal standard. Chemical shifts (d) were measured in parts per million (ppm) and coupling constants (*J*) in Hz. Measurements of atomic mass for the compounds was carried out using a high-resolution mass spectra (HRMS) Ultraflex II TOF / TOF mass spectrometer equipped with a high performance solid state laser (λ = 355 nm), and reflector. The system was operated by the FlexControl 2.4 software package (Bruker Daltonik GmbH, Bremen, Germany).

*General procedures for the preparation of N-(4-halobenzyl)benzylamides (**1**)*

In a 100 ml flask equipped with magnetic stirring, the organic acid (1.35 mmol, 200 mg) was dissolved in 2.7 ml of dimethylformamide (DMF) and in 0.14 ml (1.35 mmol) of triethylamine. The solution was cooled in an ice bath (0 °C). Then, 1.35 mmol of 4-chlorobenzylamine was added. After this, 1.35 mmol solution of BOP in 10 ml of CH_2Cl_2 was added to the flask. The reaction was stirred at 0 ^{o}C for 30 min, and then for an additional period, at room temperature for 2-6 hours. After the reaction, the CH_2Cl_2 was removed under reduced pressure and the solution was poured into a separating funnel containing 10 ml water and 10 ml of ethyl acetate EtOAc. The product was extracted with three 10 ml portions of EtOAc (3 x 10 ml). The organic phase was washed sequentially with 10 ml HCl at 1 N, water, 10 ml of $NaHCO_3$ at 1 M, and 10 ml of water; dried with Na_2SO_4, filtered and concentrated in a rotary evaporator. The amide was purified by chromatography on a silica gel column using as the mobile phase an EtOAc:Hexane mixture gradient increasing in polarity [10].

*N-(4-chlorobenzyl) cinnamamide (**1**)*

Crystalline solid; 75% yield (274 mg), mp: 152-156 ^{o}C, IR υ_{max} (KBr, cm^{-1}): 3253 (N-H), 3080 (CH sp^2), 1654 (C=O), 1616 and 1489 (aromatic C=C), 1040 (stretching C-Cl), ^{1}H NMR (DMSO-d_6, 200 MHz): 7.71(*d*, *J*=16Hz, 1H, H-7); 7.53 (*m*, 2H, H-2, H-6); 7.39 (*m*, 3H, H-3,4,5); 7.31 (*m*, 4H, H-2', H-3', H-5', H-6'); 6.45(*d*, *J*=16Hz, 1H, H-8); 6.03 (*bs*, 1H, O=C-N**H**); 4.57 (*d*, *J*= 4.0Hz, H-7'). ^{13}C NMR (DMSO-d_6, 50 MHz) 166.0 (C=O); 141.9 (C-7); 136.9 (C-1'); 134.8 (C-1); 133.5 (C-4'); 130.0 (C-2', C-6'); 129.4 (C-3', C-5'); 129.0 (C-3, C-5, C-6); 128.0 (C-2, C-4); 120.2 (C-8); 43.3 (C-7') [14].

*(E)-N-(4-chlorobenzyl)-3-(3,4-dihydroxyphenyl) acrylamide (**2**)*

The product was prepared according to procedure 1. Dark amorphous solid; yield: 70% (228 mg), mp: 103-105 ^{o}C, IR υ_{max} (KBr, cm^{-1}): 3460 and 3406 (OH) 3230 (NH), 3018 (CH sp^2), 1651 (C=O) 1602 and 1490 (aromatic C=C), 1089 (C-Cl stretch). ^{1}H-NMR (CD_3OD, 200 MHz): 7.55 (*d*, *J*= 15.7 Hz, 1H; H-7), 7.24 (*m*, 4H; H-2', H-3', H-5', H-6')7.13 (*d*, *J*= 1.7 Hz, 1H; H-2); 7.02 (*dd*, *J*= 8.2, 1.8 Hz, 1H; H-5), 6.88 (*d*, *J*= 8.1 Hz, 1H; H-6), 6.52 (*d*, *J*= 15.7 Hz, 1H, H-8), 4.55 (*bs*, 2H; H-7'). ^{13}C NMR (CD_3OD, 50 MHz): 169.2 (C=O); 148.8 (C-4); 146.7 (C-3); 142.8 (C-7); 138.9 (C-1'); 131,4 (C-4'); 130.1 (C-2', C-6'); 129.6 (C-3'; C-5'); 128.2 (C-1); 122.2 (C-6); 118.0 (C-8); 115.0 (C-2); 116.4 (C-5); 43.5 (C-7') [15, 16]. HRMS (MALDI) calculated for $C_{16}H_{14}ClNNaO_3$ $[M + Na]^+$: 326.0560; found 326.0561.

*(E)-N-(4-chlorobenzyl)-3-(4-hydroxy-3-methoxyphenyl) (**3**)*

The product was prepared according to procedure 1. White amorphous solid; yield: 81% (273 mg), mp 129-131^{o}C, IR υ_{max} (KBr, cm^{-1}): 3414 (OH) 3275 (NH), 3010 (CH sp2), 1645 (C=O), 1612 and 1460 (aromatic C=C), 1039 (C-Cl stretch). ^{1}H-NMR (DMSO-d_6, 200 MHz): 7.59(*d*, *J*=16Hz, 1H; H-7) 7.28 (*dd*, *J*=2.0 Hz e 6.0Hz, 2H; H-2', H-6'); 7.22 (*dd*, *J*=2,0 Hz, 6,0Hz, 2H, H-3', H-5') 7.13 (*s*, 1H; H-2); 6.95 (*d*, *J*= 8,1 Hz;1H; H-5); 6.87 (*d*, *J*= 8,1 Hz; 1H; H-6); 6.27(*d*, *J*=16Hz, 1H; H-8); 4.36 (*d*, *J*= 6,0 Hz, 2H, H-7'); 3.79 (*s*, 3H, OC**H**$_3$). ^{13}C NMR (DMSO-d_6, 200 MHz): 165.5 (C=O), 148.4 (C-3), 147.8 (C-4), 139.6 (C-7), 138.7 (C-1'), 131.3 (C-4'), 129.2 (C-2', C-6'), 128.3 (C-3', C-5'), 126.3 (C-1), 121.7 (C-6), 118.6 (C-8), 115.7 (C-5), 110.8 (C-2), 55.6 (O**C**H$_3$), 41.6 (C-7') [17]. HRMS (MALDI) calculated for $C_{17}H_{16}ClNO_3$ $[M + H]^+$: 318.0887; found 318.0870.

*(E)-N-(4-chlorobenzyl)-3-(4-methoxyphenyl) acrylamide (**4**)*

The product was prepared according to procedure 1. Crystalline solid; yield: 91% (311 mg), mp 148-150 °C, IR υ_{max} (KBr, cm^{-1}): 3282 (N-H), 3041 (C-H sp2), 1647 (C=O), 1602 and 1462 (aromatic C=C), 1029 (stretching C-Cl). ^{1}H NMR (DMSO-d_6, 200 MHz): 8.59 (*t*, *J*= 6.0 Hz, 1H, O=C-N**H**), 7.50 (*t*, *J*= 7.3 Hz, 2H; H-2; H-6), 7.44 – 7.18 (*m*, 5H; H-7, H-2' H-3', H-5', H-6'), 6.97 (*d*, *J*= 8.7 Hz, 2H, H-3, H-5), 6.54 (*d*, *J*= 15.7 Hz, 1H; H-8), 4.38 (*d*, *J*= 6.0

Hz, 2H; H-7'), 3.77 (*s*, 3H, OC**H**$_3$). ^{13}C NMR (DMSO-d$_6$, 50 MHz): 165.5(C=O); 160.4 (C-4); 139.0 (C-7); 138.7 (C-1'); 131.4 (C-4'); 129.3 (C-2; C-6, C-2'; C-6'); 128.3(C-3', C-5'); 127.4 (C-1); 119.4 (C-8); 114.5 (C-3, C-5); 55.3 (O**C**H$_3$); 41.7 (C-7') [18]. HRMS (MALDI) calculated for $C_{16}H_{14}ClNO_2$ [M + H] $^+$: 324.0767; found 324.0771.

*(E)-N-(4-chlorobenzyl)-3- (2-hydroxyphenyl) acrylamide (**5**)*
The product was prepared according to procedure 1. Yellow amorphous solid; yield: 79% (277 mg), mp: 165-171 ^{o}C. υ_{max} IR (KBr, cm^{-1}): 3369 (OH), 3072 (C-H sp^2), 1649 (C=O), 1589 and 1458 (aromatic C=C), 1093 (C-Cl stretch). ^{1}H NMR (200 MHz, DMSO-d$_6$) 10.00 (*bs*, 1H, OH), 8.64 (*t, J*=5.83 Hz, 1H; O=C-N**H**), 7.78 (*d, J*=15.92 Hz; 1H; H-7), 7.49-7.15 (*m*, 6H; H-6, H-2', H-3', H-5', H-6'), 6.91-6.76 (*m*, 2H; H-4, H-5), 6.73 (*d, J*=15,92 Hz, 1H; H-8), 4.47 (*d, J*=5.86 Hz, 2H; H-7'). ^{13}C NMR (DMSO-d$_6$, 50 MHz) 165.8 (C=O); 156.4 (C-2); 138.8 (C-1'); 135.2 (C-4); 135.0; 130.6 (C-4'); 129.3(C-2', C-6'); 128.4 (C-6,); 128.3 (C-3', C-5'); 121.6 (C-1); 121.3 (C-5); 119.4 (C-8); 116.2; 41.7 (C-7') [19, 20]. HRMS (MALDI) calculated for $C_{16}H_{14}ClNO_2$ [M + H]$^+$: 288.0781; found 288.0785.

*(E)-N-(4-chlorobenzyl)-3-(3-hydroxyphenyl) acrylamide (**6**)*
The product was prepared according to procedure 1. Yellow crystalline solid; yield: 76% (268 mg), mp: 140-143 ^{o}C υ_{max} IR (KBr, cm^{-1}): 3460 (OH), 3075 (CH sp^2), 1649 (C=O), 1591 and 1448 (aromatic C=C), 1089 (C-Cl stretch). 1H-NMR (DMSO-d$_6$, 200 MHz): 9.61 (*s*, 1H, OH), 8.67 (*t, J*= 5.9 Hz, 1H, O=C-N**H**), 7.47 – 7.14 (*m*, 6H; H-5, H-7, H-2', H-3', H-5' e H-6'), 7.03 – 6.92 (*m*, 2H; H-4, H-6), 6.84 – 6.70 (*m*, 1H; H-2), 6.60 (*d, J*= 15.8 Hz, 1H; H-8), 4.38 (*d, J*= 5.9 Hz, 2H; H-7'). ^{13}C NMR (DMSO-d$_6$, 50 MHz): 165.1 (C=O); 157.7 (C-3); 139.4 (C-7); 138.5 (C-1'); 136.1 (C-1);131.4 (C-4'); 130.0 (C-5); 129.3 (C-2', C-6'); 128.3 (C-3', C-5'); 121.7 (C-8); 118.8 (C-6); 116.8 (C-2); 113.8 (C-4); 41.7 (C-7') [20]. HRMS (MALDI) calculated for $C_{16}H_{14}ClNNaO_2$ [M + Na]$^+$: 310.0611; found 310.0619.

*(E)-N-(4-chlorobenzyl)-3-(4-hydroxyphenyl) acrylamide (**7**)*
The product was prepared according to procedure 1. Crystalline solid; yield: 63% (221 mg), mp 157-160 °C. IV υ_{max} (KBr, cm-1): 3479 and 3369 (OH), 3072 (C-H sp^2), 1647 (C=O), 1589 and 1458 (aromatic C=C), 1093 (stretching C-Cl). ^{1}H NMR (200 MHz, DMSO-d$_6$): 9.87 (*bs*, 1H, OH); 8.53 (*t, J*=5.87 Hz, 1H, O=C-N**H**); 7.38 (*m*, 5H, H-2, H-6, H-7, H-2', H-6'); 7.28 (*m*, 3H; H-2; H-3'e H-5'); 6.77 (*d, J*=8.5 Hz, 2H; H-3 e H-5); 6.44 (*d, J*=15.73 Hz, 1H; H-8); 4.36 (*d, J* = 6.0 Hz, 2H; H-7'). ^{13}C NMR (DMSO-d$_6$, 50 MHz) 165.6 (C=O); 159.0 (C-4); 139.4 (C-7); 138.8 (C-1'); 131.4 (C-4'); 129.4 (C-2, C-6); 129.3 (C-2', C-6'); 128.3 (C-3', C-5'); 125.9 (C-1); 118.3 (C-8); 115.8 (C-3, C-5); 41.6 (C-7') [21]. HRMS (MALDI) calculated for $C_{16}H_{14}ClNNaO_2$ [M + Na]$^+$: 310.0611; found 310.0596.

*(E)-N- (4-chlorobenzyl)-3-(4-chlorophenyl) acrylamide (**8**)*
The product was prepared according to procedure 1. Crystalline solid; yield: 71% (240 mg), mp 157-161 °C. IV υ_{max} (KBr, cm^{-1}): 3414 (N-H), 3041 (C-H sp^2), 1651 (C=O), 1614 and 1487 (aromatic C=C), 1089 (stretching C-Cl). ^{1}H-NMR (DMSO-d$_6$, 200 MHz): 8.70 (*t, J*= 6.0 Hz, 1H, O=C-N**H**), 7.64 – 7.46 (*m*, 4H, H-2', H-3', H-5', H-6'), 7.45 – 7.25 (*m*, 5H; H-2, H-6, H-3, H-5, H-7), 6.69 (*d, J*= 15.8 Hz, 1H; H-8), 4.39 (*d, J*= 6.0 Hz, 2H). ^{13}C NMR (50 MHz, DMSO-d$_6$) 164.9 (C=O); 138.5 (C-1'); 137.9 (C-7); 134.0 (C-4); 133.8 (C-1); 131.5 (C-4'); 129.3 (C-2, C-6, C-2' e C-6'); 129.0 (C-3, C-5); 128.3 (C-3', C-5');122.7 (C-8); 41.7 (C-7') [22]. HRMS (MALDI) calculated for $C_{16}H_{13}Cl_2NNaO$ [M + Na]$^+$: 328.0272; found 328.0273.

*(E)-N-(4-chlorobenzyl)-3- (4-hydroxy-3,5-dimethoxyphenyl)-acrylamide (**9**)*
The product was prepared according to procedure 1. Yellow amorphous solid; yield: 60% (193 mg), mp: 182-185 ^{o}C, IR υ_{max} (KBr, cm^{-1}): 3414 (OH) and 3358 or NH), 3000 (CH sp^2), 1658 (C=O), 1624 and 1458 (aromatic C=C), 1091 (C-Cl stretch). ^{1}H-NMR (DMSO-d$_6$, 200MHz) 8.52 (*t, J*= 6.0 Hz; 1H, O=C-N**H**), 7.28-7.48 (*m*, 5H; H-7, H-2', H-3', H-5', H-6'), 6.86 (*s*, 2H; H-2, H-6), 6.55 (*d, J*= 15.8, 1H; H-8), 4.37(*d, J*=6 Hz, 2H; H-7') 3.79 (*s*, 6H; OC**H**$_3$). ^{13}C NMR (DMSO-d$_6$, 50 MHz) 165.6 (C=O); 148.1 (C-3, C-5); 140.0 (C-7) 138.7 (C-4); 137.4 (C-1'); 131.4; C-4'); 129.2 (C-2', C-6'); 128.4 (C-3', C-5'); 125.3 (C-1); 119.1 (C-8); 105.3 (C-2, C-6); 56.0 (O**C**H$_3$); 41.7 (C-7') [23]. HRMS (MALDI) calculated for $C_{18}H_{18}ClNNaO_4$ [M + Na]$^+$: 370.0822; found 370.0813.

*(E)-N-(4-chlorobenzyl)-3-(2-nitrophenyl) (**10**)*
The product was prepared according to procedure 1. White crystalline solid; yield: 79% (260 mg), mp: 164-167 ^{o}C, IR υ_{max} (KBr, cm-1): 3290 (NH), 3030 (CH sp^2), 1651 (C=O), 1624 and 1458 (aromatic C=C), 1525 and 1342 (C=O), 1091 (C-Cl stretch). ^{1}H NMR (DMSO-d$_6$, 200 MHz): 8.82 (*t, J*= 4.7 Hz, 1H, O=C-N**H**), 8.05 (*d, J*= 8.0 Hz, 1H; H-3), 7.78-7.75 (*m*, 2H; H-6, H-7), 7.72 – 7.57 (*m*, 2H; H-4, H-5), 7,40 (*m*, 4H, H-2', H-3', H-5' e H-6'), 6.67 (*d, J*= 5.6 Hz, 1H; H-8), 4.39 (*d, J*= 5.9 Hz, 1H; H-7'). ^{13}C NMR (DMSO-d$_6$,50 MHz): 164.3 (C=O); 148.4 (C-2); 138.3 (C-1'); 134.3 (C-7); 133.9 (C-5); 131.6 (C-4'); 130.4 (C-4); 130.0 (C-1); 129.4 (C-2', C-6'); 128.8 (C-6); 128.4 (C-3', C-5'); 126.6 (C-3); 124.7 (C-8); 41.8 (C-7') [24]. HRMS (MALDI) calculated for $C_{16}H_{13}ClN_2O_3$ [M + H] +: 317.0683; found 317.0683.

*(E)-N-(4-chlorobenzyl)-3- (3,4,5-trimethoxyphenyl) acrylamide (**11**)*
The product was prepared according to procedure 1. Crystalline solid; yield: 86% (260 mg), mp 146-150 °C, IR υ_{max} (KBr, cm-1): 3290 (N-H), 3070 (C-H sp^2), 1651 (C=O), 1614 and 1415 (aromatic C=C), 1029 (stretching C-Cl). ^{1}H NMR (DMSO-d_6, 200 MHz): 8.60 (*t, J*= 5.9 Hz, 1H; O=C-N**H**), 7,37 (*m*, 5H; H-7, H-2', H-3', H-5', H-6'), 6,90 (*s*, 2H; H-2, H-6), 6.64 (*d, J*= 15.7 Hz, 1H; H-8), 4.38 (*d, J*= 5.9 Hz, 1H; H-7'), 3.80 (*s*, 6H; *m*- OC**H$_3$**). 3.68 (*s*, 3H; *p*-OC**H$_3$**). ^{13}C NMR (DMSO-d_6, 50 MHz) 165.2 (C=O); 153.1 (C-3, C-5); 139.4 (C-7); 138.7 (C-4); 138.6 (C-1'); 131.4 (C-4'); 130.5 (C-1); 129.2 (C-2', C-6'); 128.4 (C-3', C-5'); 121.3 (C-8); 105.1 (C-2, C-6); 60.2 (C-4- O**C**H$_3$); 55.9 (C-3,5- O**C**H$_3$); 41.7 (C-7') [25]. HRMS (MALDI) calculated for $C_{19}H_{20}ClNO_4$ ([M + H] +: 384.0979, found 384.0913.

*N-(4-chlorobenzyl) benzamide or 4-benzoyl chlorobenzylamide (**12**)*
The product was prepared according to procedure 1. Crystalline solid; yield: 65% (260 mg), mp 136-139 °C, IR υ_{max} (KBr, cm^{-1}): 3300 (N-H), 3082 (C-H sp^2), 1637 (C=O), 1618 and 1490 (aromatic C=C), 1091 (stretching C-Cl). ^{1}H NMR (DMSO-d_6, 200 MHz): 9.10 (*t, J*= 5.9 Hz, 1H, O=C-N**H**), 7.89 (*dd, J*= 8.0 e 1.6 Hz, 2H; H-2, H-6), 7.64 – 7.09 (*m*, 7H; H-3, H-4, H-5, H-2', H-3', H-5', H-6'), 4.46 (*d, J*= 6.0 Hz, 2H; H-7'). ^{13}C NMR (DMSO-d_6, 50 MHz): 166.4 (C=O); 138.8 (C-1'); 134.2 (C-1); 131.4 (C-4); 131.3 (C-2', C-6'); 129.1 (C-3', C-5'); 128.3 (C-3 C-5); 127.3 (C-2, C-6); 42.1 (C-7') [26].

*N-(4-chlorobenzyl)-[1,1'-biphenyl]-4-carboxamide (**13**)*
The product was prepared according to procedure 1. White amorphous solid; yield: 56% (198 mg), mp 222-228 °C, IR υ_{max} (KBr, cm^{-1}): 3271 (N-H), 3078 (C-H sp^2), 1633 (C=O), 1606 and 1487 (aromatic C=C), 1089 (stretching C-Cl). ^{1}H NMR (DMSO-d_6, 200 MHz): 9.15 (*t, J*= 6.1 Hz, 1H, O=C-N**H**), 7.99 (*d, J*= 8.3 Hz, 2H; H-2, H-6), 7.84 – 7.65 (*m*, 4H; H-3, H-5, H-2'', H-6''), 7.56 – 7.29 (*m*, 7H; H-2', H-3', H-5', H-6', H-3'' H-4'', H-5''), 4.48 (*d, J*= 5.9 Hz, 2H). ^{13}C NMR (DMSO-d_6, 50 MHz) 166.1 (C=O); 143.0 (C-4); 139.2 (C-1''); 138.8 (C-1'); 133.0 (C-1); 131.4 (C-4'); 129.2 0 (C-2', C-6'); 128.4 (C-2, C-6); 128.1 (C-9, C-11); 127.0 (C-3', C-5'); 126.7 (C-10) (C-3, C-5, C-8, C-12); 42.1 (C-7') [27]. HRMS (MALDI) calculated for $C_{20}H_{16}ClNO_3$ $[M + H]^+$: 322.0988; found 322.0969.

*N-(4-Chlorobenzyl)-3,4,5-trihydroxybenzamide (**14**)*
The product was prepared according to procedure 1. Yellow amorphous solid; yield: 21% (73 mg), mp 96-100 °C, IR υ_{max} (KBr, cm^{-1}): 3400 (OH), 3400 (NH), 3000 (CH sp^2), 1614 (C=O), 1589 and 1494 (aromatic C=C), 1043 (stretching C-Cl). ^{1}H NMR (MeOD, 200 MHz): 8.12 (*s*, 1H; O=C-N**H**), 7.53 – 7.15 (*m*, 4H, H-2', H-3', H-5', H-6), 6.85 (*s*, 2H; H-2, H-6), 4.58 (*s*, 2H, H-7'), 4.46 (*s*, 2H; *m*-OH), 4.35 (*s*, 1H, *p*-OH). ^{13}C NMR (MeOD, 50 MHz): 170.5 (C=O), 146.7 (C-3, C-5), 139.4 (C-4), 136.1 (C-1), 134.2 (C-4), 130.5 (C-5', C-6'), 130.1 (C-3', C-5'), 125.9 (C-1), 107.8 (C-2, C-6), 43.6 (C-7') [28]. HRMS (MALDI) calculated for $C_{16}H_{12}ClNNaO_3$ $[M + Na]^+$: 316.0353; found 316.0373.

*N-(4-chlorobenzyl)-4-hydroxy-3-methoxybenzamide (**15**)*
The product was prepared according to procedure 1. White amorphous solid; yield: 44% (151 mg), mp: 75-77 °C, IR υ_{max} (KBr, cm-1): 3319 (O-H) 3251 (N-H), 3000 (C-H sp^2), 1639 (C=O), 1589 and 1487 (aromatic C=C), 1091 (stretching C-Cl). ^{1}H NMR (DMSO-d_6, 200 MHz): 8.12 (*d, J*= 9.2 Hz, 1H; O=C-N**H**), 7.56 – 7.22 (*m*, 7H; H-2, H-4, H-6, H-3', H-5', H-2', H-6'), 4.52 (*s*, 2H), 3.88 (*s*, 1H; OC**H$_3$**). ^{13}C NMR (DMSO-d_6, 50 MHz): 169.8 (C=O); 151.3 (C-3); 148.8 (C-4); 139.3 (C-1'); 133.8 (C-4'); 130.1 (C-2', C-6'); 129.5 (C-5', C-3'); 126.5 (C-1); 122.1 (C-5); 115.8 (C-6); 111.9 (C-2); 56.4 (3-OMe); 43.8 (C-7') [29]. HRMS (MALDI) calculated for $C_{16}H_{16}ClNO_4$ [M + Na]$^+$: 316.0530; found 316.0543.

*N-(4-chlorobenzyl)-4-hydroxy-3,5-dimethoxybenzamide (**16**)*
The product was prepared according to procedure 1. White amorphous solid; yield: 50% (164 mg), mp 110-115 °C, IR υ_{max} (KBr, cm^{-1}): 3493 (OH), 3277 (NH), 3084 (CH sp^2), 1666 (C=O), 1597 and 1492 (aromatic C=C), 1016 (stretching C-Cl). ^{1}H NMR (MeOD, 200 MHz): 8.15 (*s*, 1H, O=C-N**H**), 7.43 (*s*, 1H, OH), 7.35 – 7.27 (*m*, 4H; H-2', H-3', H-5', H-6'), 7.25 – 7.15 (*m*, 2H; H-2, H-6), 4.54 (*s*, 2H, H-7'), 3.88 (*s*, 6H; OC**H$_3$**). ^{13}C NMR (MeOD, 50 MHz): 149.0 (C-3, C-5), 169.8 (C=O), 139.3 (C-4), 133.8 (C-1'), 130.1 (C-2', C-6'), 129.5 (C-3', C-5'), 125.3 (C-4'), 106.4 (C-1), 106.0 (C-2, C-6), 43.9 (C-7'), 56.8 (O**C**H$_3$) [30]. HRMS (MALDI) calculated for $C_{16}H_{16}ClNNaO_4$ [M + Na]$^+$: 346.0636; found 346.0666.

*N-(4-chlorobenzyl)-4-hydroxybenzamide (**17**)*
The product was prepared according to procedure 1. White amorphous solid; yield: 73% (246 mg), mp 189-192 °C, IR υ_{max} (KBr, cm^{-1}): 3450 (OH), 3122 (NH), 3055 (CH sp^2), 1631 (C=O), 1593 and 1440 (aromatic C=C), 1085 (stretching C-Cl). ^{1}H NMR (DMSO-d_6, 200 MHz): 8.83 (*t, J*= 5.9 Hz, 1H; O=C-N**H**), 7.75 (*d, J*= 8.6 Hz, 2H, H-2, H-6), 7.59 – 7.43 (*m*, 4H, H-2'; H-3', H-5', H-6'), 6.80 (*d, J*= 8.8 Hz, 2H, H-3, H-5), 4.42 (*d, J*= 6.0 Hz, 2H; H-7'). ^{13}C NMR (DMSO-d_6, 50 MHz): 166.0 (C=O); 160.3 (C-4); 139.2 (C-1'); 133.4 (C-4'); 130.9 (C-2, C-6); 129.3 (C-

2', C-6'); 128.3 (C-3', C-5'); 124.9 (C-1); 114.9 (C-3, C-5); 41.7 (C-7') [31]. HRMS (MALDI) calculated for $C_{14}H_{12}ClNNaO_2$ $[M + Na]^+$: 286.0425; found 286.0432.

*3, 5-di-tert-butyl-N- (4-chlorobenzyl)-4-hydroxybenzamide (**18**)*
The product was prepared according to procedure 1. White amorphous solid; yield: 54% (203 mg), mp 184-186 °C, IR υ_{max} (KBr, cm^{-1}): 3450 (OH) and 3236 (NH), 3066 (CH sp^2), 1680 (C=O), 1544 and 1431 (aromatic C=C), 1012 (stretching C-Cl). ^{1}H NMR (DMSO-d_6, 200 MHz): 8.89 (*t, J*= 6.0 Hz, 1H; O=C-N**H**), 6.09 – 5.94 (*m*, 1H), 7.70 – 7.54 (*m*, 2H, H-2, H-6), 7.54-7.20 (*m*, 4H, H-2', H-3', H-5', H-6'), 4.42 (*d, J*= 5.9 Hz, 3H; H-7'), 1,39 (*s*, 18H; C(C**H$_3$**)$_3$). ^{13}C NMR (DMSO-d_6, 50 MHz): 167.0 (C=O); 156.9 (C-4); 140.0 (C-1'); 138.3 (C-3, C-5); 131.2 (C-4'); 129.2 (C-2', C-6'); 128.3 (C-5', C-3'); 125.3 (C-1); 124.2 (C-2, C-6); 42.0 (C-7'); 34.7 (3,5-(**C**$(CH_3)_3$; 30.3 (3,5-(C(**C**H_3)$_3$) [32] HRMS (MALDI) calculated for $C_{22}H_{28}ClNO_3$ $[M + H]^+$: 374.1877; found 374.1877.

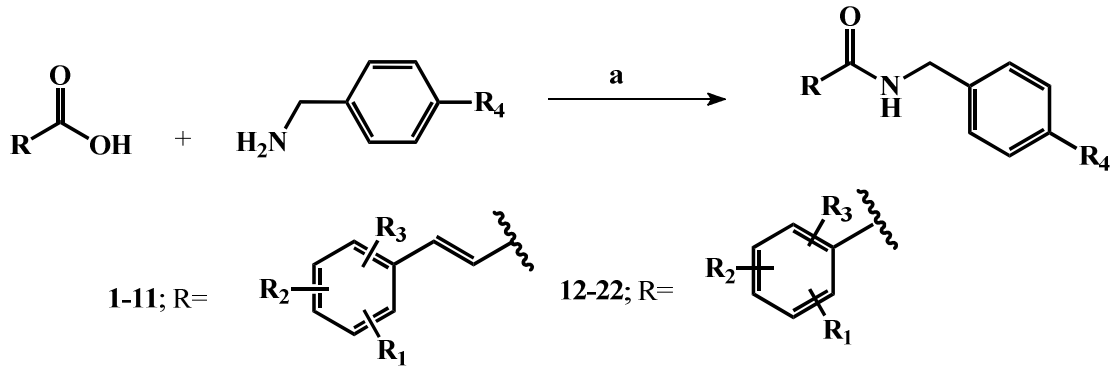
*N-(4-chlorobenzil) -2-hydroxy-5-methoxybenzamide (**19**)*
The product was prepared according to procedure 1. Crystalline solid; yield: 60% (180 mg), mp 137-140 °C, IR υ_{max} (KBr, cm^{-1}): 3360 (O-H and N-H), 3076 (C-H sp^2), 1651 (C=O), 1598 and 1435 (aromatic C=C), 1045 (stretching C-Cl). ^{1}H NMR (200 MHz, DMSO-d_6): 9.35 (*t, J*= 5.8 Hz, 1H, O=C-N**H**), 7.48 – 7.26 (*m*, 5H, H-6, H-2', H-3', H-5', H-6'), 7.04 (*dd, J*= 9.0, 3.0 Hz, 1H; H-3), 6.85 (*d, J*= 9.0 Hz, 1H; H-4), 4.49 (*d, J*= 5.9 Hz, 2H, H-7'), 3.72 (*s*, 3H; OC**H$_3$**). ^{13}C NMR (DMSO-d_6, 50 MHz): 168.7 (C=O); 154.0 (C-5); 151.7 (C-2); 115.2 (C-1); 138.2 (C-1'); 131.6 (C-4'); 129.3 (C-2', C-6'); 128.5 (C-5', C-3'); 121.2 (C-3); 118.4 (C-4); 111.3 (C-6); 55.8 (O**C**H_3); 41.9 (C-7') [33]. HRMS (MALDI) calculated for $C_{15}H_{14}ClNO_3$ $[M + H]^+$: 327.0512; found 327.0516.

N-(4-chlorobenzyl)-3-methyl-4-nitrobenzamide (**20**)
The product was prepared according to procedure 1. Crystalline solid; yield: 41% (143 mg), mp 148-151 °C, IR υ_{max} (KBr, cm^{-1}): 3278 (NH), 3080 (CH sp^2), 1637 (C=O), 1587 and 1423 (aromatic C=C), 1521 and 1355 (NO_{2arom}), 1089 (stretch C-Cl). ^{1}H NMR (DMSO-d_6, 200 MHz): 9.31 (*t, J*= 5.8 Hz, 1H, O=C-N**H**), 8.06 (*dd, J*= 8.4, 1H; H-5), 7.96 (*s*, 1H, H-2), 7.89 (*dd, J*= 8.4, 1.7 Hz, 1H; H-6), 7.42-7,31 (*m*, 4H, H-2', H-3', H-5', H-6'), 4,47 (*dd, J*= 5.9, 1.7 Hz, 2H, H-7'), 2.54 (*m*, 3H; C**H$_3$**). ^{13}C NMR (DMSO-d_6, 50 MHz): 164.8 (C=O); 150.5 (C-4); 138.3 (C-1); 138.1 (C-1'); 132.9 (C-4'); 131.8 (C-2', C-6'); 131.5 (C-3); 129.3 (C-3', C-5'); 128.4 (C-2); 126.2 (C-5); 124.6 (C-6); 42.3 (C-7'); 19.5 (**C**H_3) [34]. HRMS (MALDI) calculated for $C_{15}H_{13}ClN_2NaO_3$ $[M + Na]^+$: 327.0512; found 327.0516.

*N-(4-fluorobenzyl)-4-hydroxy-3-methoxibenzamide (**21**)*
The product was prepared according to procedure 1; 4-fluorobenzylamine used as the reagent. White amorphous solid; yield: 21% (90 mg), mp 161-165 °C, IR υ_{max} (KBr, cm^{-1}): 3304 (O-H), 3078 (N-H), 1631 (C=O), 1593 and 1423 (aromatic C=C), 1116 (C-F stretch). ^{1}H NMR ($CDCl_3$, 200 MHz): 9.60 (*s*, 1H; OH), 8.83 (*t, J*= 5.8 Hz, 1H; NH), 7.52 – 7.28 (*m*, 4H; H-2, H-6, H-2', H-6'), 7.22 –7.05 (*m*, 2H; H-3', H-5'), 6.81 (*d, J*= 8.2 Hz, 1H; H-5), 4.43 (*d, J*= 5.8 Hz, 2H; H-7'), 3.80 (*s*, 3H; OMe). ^{13}C NMR ($CDCl_3$, 50 MHz): 166.0 (C=O), 161.1 (*d, J*= 242.0 Hz; C-4'), 149.6 (C-4), 147.2 (C-3), 136.2 (C-1'), 129.2 (C-2', C-6'), 125.3 (C-1), 120.9 (C-6), 114.9 (C-3', C-5'), 115.2 (C-5), 111.3 (C-2), 55.7 (OMe), 42.0 (C-7') [29]. HRMS (MALDI) calculated for $C_{15}H_{14}FNNaO_3$ $[M + Na]^+$: 298.0855; found 298.0882.

*N- (4-bromobenzyl) -4-hydroxy-3-methoxybenzamide (**22**)*
The product was prepared according to procedure 1, with 4-bromobenzylamine used as reagent. Red amorphous solid; yield: 63% (252 mg), mp 119-122 °C. IV υ_{max} (KBr, cm^{-1}): 3304 (OH), 3078 (NH), 3003 (CH sp^2), 1631 (C=O), 1593 and 1423 (aromatic C=C), 1072 (stretching C-Br) ^{1}H NMR (DMSO-d_6, 200 MHz): 7.43 (*d, J*= 8.4 Hz, 3H; H-2, H-3', H-5'), 7.29 – 7.12 (*m*, 3H; H-6, H-2', H-6), 6.88 (*d, J*= 8.2 Hz, 1H; H-5), 6.65 (*t, J*= 5.2 Hz, 1H; NH), 6.21 (*s*, 1H; OH), 4.54 (*d, J*= 5.8 Hz, 2H, H-7'), 3.88 (*s*, 3H; OMe). ^{13}C NMR (DMSO-d_6, 50 MHz): 167.1 (C=O), 148.9 (C-4), 146.7 (C-3), 137.4 (C-1'), 131.7 (C-3', C-5'), 129.4 (C-2', C-6'), 126.1 (C-1), 119.8 (C-6, C-4'), 113.9 (C-5), 110.4 (C-2), 56.0 (OMe) ,43.4 (C-7'). [29]. HRMS (MALDI) calculated for $C_{15}H_{14}BrNO_3$ $[M]^+$: 335.0157; found 335.0156.

Procedure 1



Scheme 1: Preparation of amides. Reagents and conditions: (a) BOP, Et_3N, DMF, 30 min at 0 °C, follow 2-6 hours at room temperature. The ring substituents R_1, R_2, R_3 = H, Cl, OH, CH_3, OCH_3, NO_2, tert-Bu or C_6H_5 e R_4 = F, Cl, or Br

Study of antibacterial activity

Bacterial material and sample preparation

The microorganisms used in the tests were obtained from the Microbiology and Molecular Biology Laboratory (LMBM), of the Regional University of Cariri (URCA). Standard strains of bacteria were used; *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. For substance preparations, 10 mg (10,000 ug) of each substance were weighed to be tested, each one was placed into individual Eppendorf tubes, and diluted in 1 ml of dimethylsulfoxide (DMSO), composing solution concentrations equal to 10000 µg/mL. The final solution concentrations were 512 µg/mL for each of the substances. These final solutions were used for MIC testing. Due to photosensitive, the addition of DMSO was performed inside the laminar flow hood with appropriate lighting. To prepare the inoculum, bacterial cultures were seeded in Petri plates containing Heart Infusion Agar (HIA, Difco Laboratories Ltd.), and placed in an oven at 37° C, one day prior to growth, for 24 hours. Eppendorf tubes were prepared in triplicate for each bacterium and for each substance (2 bacteria x triplicate x 22 substances = 132 Eppendorf tubes), each containing 900 µL brain heart infusion (BHI 10% + 100 mL of inoculum, Difco Laboratories Ltd.), corresponding to 10% of the total solution.

The antibiotics amikacin, gentamicin, norfloxacin and penicillin were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), at concentrations of 512 µg/ml and diluted in sterile water were used as controls. Sodium resazurin (Sigma-Aldrich Co.) was used as an indicator of bacterial growth.

Determination of the minimum inhibitory concentration

Broth microdilution procedure was adopted [35]. Inoculates were prepared in Eppendorf tubes, each containing 1 mL of solution with 900 µL 10% BHI, and 100 µL of the bacterial suspension containing 10^6 CFU according to the McFarland scale. The plates were filled in alphabetical order, resulting in 8 wells per microdilution column. Adding 100 mL of the above solution to each well, serial microdilution in a 100 µL solution of each substance was performed, to be tested, a column in concentrations ranging 256-2 µg/mL. The microdilutions were performed in triplicate. The plates were brought to the incubator for 24 hours at 37° C. The determination of bacterial MIC was made using an addition of 20 µL of resazurin to each well with visual observation after 1 hour.

The MIC was determined by observing visible turbidity in each well and color change caused by resazurin, noting the lowest product concentration capable of inhibiting bacterial growth [35]. Assays were performed in triplicate and the results expressed as the geometric mean. Statistical analysis was performed using two-way ANOVA followed by the Bonferroni post-test with GraphPad Prism 5.0 software

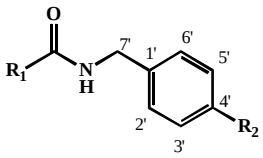
RESULTS AND DISCUSSION

Chemistry

Amides **1-22** (**scheme 1**) were prepared from an acid-base reaction, in which BOP generates a triazole intermediate from *in situ* carboxylic acid in order to replace the carboxyl oxygen of the amine nitrogen by forming a stable amide [13]. Compounds **1-22** were identified based on IR, ^1H and ^{13}C NMR and HRMS spectra. The spectroscopic techniques were sufficient to confirm the formation of amide, because of its very characteristic signal [14]. NH protons from amide group were assigned as a triplet at the 8.50-8.70 ppm region of the 6.0-5.7 Hz engaging with the methylene hydrogens (NH- **CH_2**) which formed a doublet assigned to 4:59 to 4:36 in the ppm range. For compounds

1-11 we observed 7.0-8.0 ppm (J = 15.7 Hz, 1H) in the region characteristic of olefinic proton H-7 and H-8 signals forming *trans* conformation structures.

Table 1: Data of cinnamic and benzoic amides 1-22



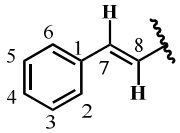
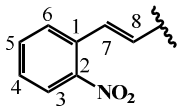
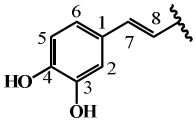
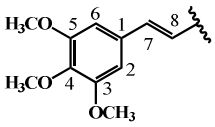
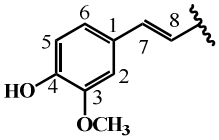
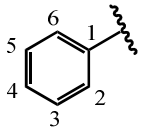
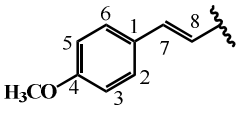
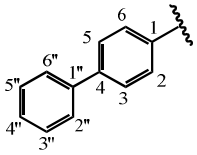
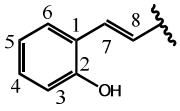
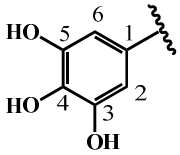
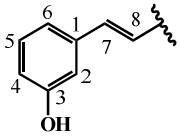
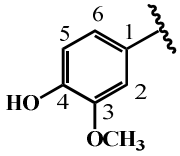
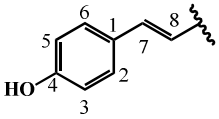
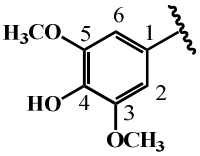
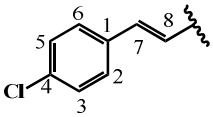
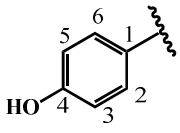
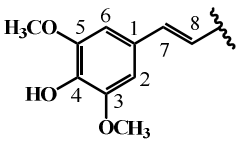
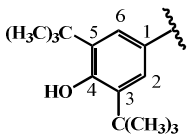
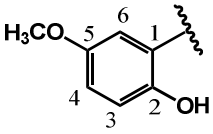
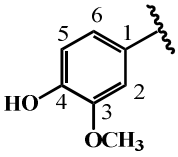
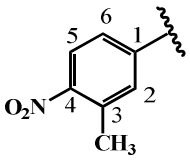
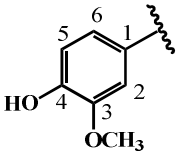
Compound	R_1	R_2	time (h)	Yield (%)	Compound	R_1	R_2	time (h)	Yield (%)
1		Cl	3	75	**10**		Cl	2	79
2		Cl	7	70	**11**		Cl	3	86
3		Cl	4	81	**12**		Cl	3	65
4		Cl	2	91	**13**		Cl	6	56
5		Cl	5	79	**14**		Cl	3	21
6		Cl	4	76	**15**		Cl	6	44
7		Cl	3	63	**16**		Cl	3	50
8		Cl	2	71	**17**		Cl	3	73
9		Cl	6	60	**18**		Cl	3	54

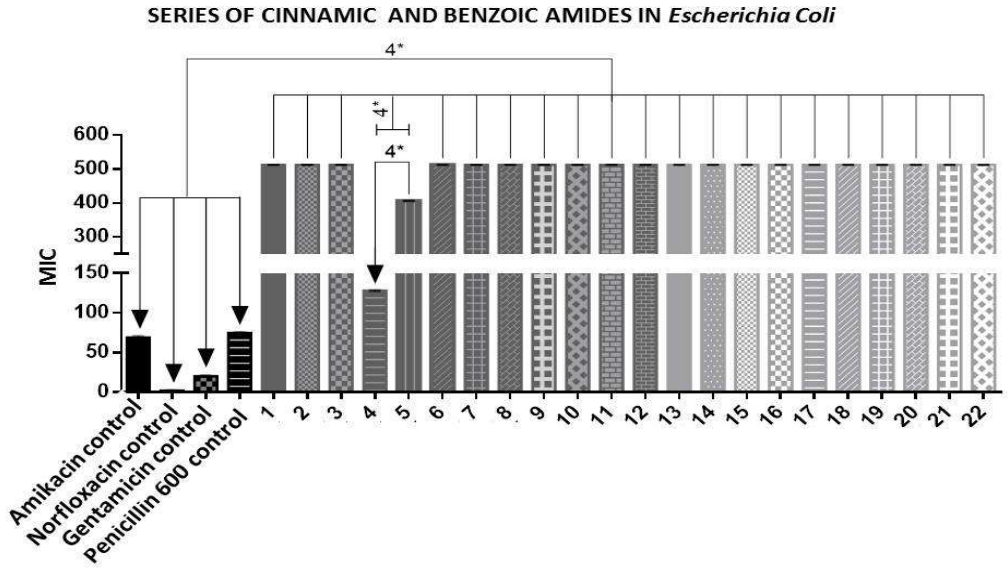
Table 1: Continuation

Compound	R_1	R_2	time (h)	Yield (%)	Compound	R_1	R_2	time (h)	Yield (%)
19		Cl	6	60	**21**		F	2	27
20		Cl	4	41	**22**		Br	2	63

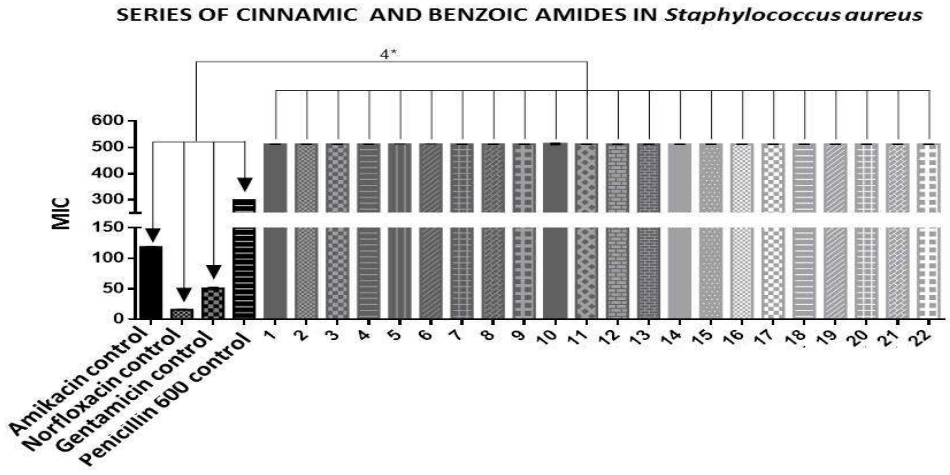
Antibacterial activity

The bioassay results for the antibacterial activity of compounds **1-22** are shown in **graphics 1** and **2** respectively. Antibacterial assays were performed using broth microdilution [36] procedures and standard strains of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. The controls used were gentamycin, amikacin, norfloxacin, and benzathine penicillin at 512 µg/mL. The amide 4-(4-methoxy-cinnamoyl-4 chlorobenzinamide) gave moderate to good activity, the other compounds (**1-3** and **5-22**) did not show inhibition against the bacterial strains used. The literature reports antibacterial activity of *ortho-*, and *meta* and *para-trans*-methoxycinnamic acids against bacterial strains, including strains of *E. coli*, [37-38], but this is the first study reporting on a methoxycinnamic amide with antibacterial activity.

Compound **4** was compared with amides **3** and **9** in which there is a hydroxyl group with a methoxyl on the aromatic ring. It was observed that the simple presence of a hydroxyl group or a methoxyl groups position on the aromatic ring results in amides with no antibacterial activity. Interestingly, amide **11**, a tri-methoxylated derivative, was also inactive against the bacteria tested. This result suggests that the presence of a single methoxyl in position 4 of the ring is fundamental to the amide's bioactivity. Methoxylated amides **15**, and **16** were not bioactive, confirming the chemical requirement for bioactivity. The literature reports that certain cinnamic derivatives with a methoxyl in position 4 have antibacterial activity. For example, ehretiolide, a cinnamic ester isolated from the roots of *Ehretia longiflora* showed inhibitory activity against *M. tuberculosis* growth H37Rv (MIC = 41 uM) [39]. 4-Methoxycinnamaldehyde was bioactive against *Escherichia coli* MTCC 43 (8.3 mM) and *Staphylococcus aureus* MTCC 121 (8.3 mM) [40]. These data demonstrate that it is possible to develop methoxylated cinnamic derivatives (at position 4) with potential applications as new antibacterial agents.



Graphic 01. Result of antibacterial microdilution test of amides 1- 22, using strains of *Escherichia coli*



Graphic 02. Result of antibacterial microdilution test of amides 1-22, using strains of *Staphylococcus aureus*

CONCLUSION

In short, a series of cinnamic and benzoic amides with aromatic *para*-halogenated moieties were prepared and evaluated against bacteria. One of the compounds demonstrated good activity against a gram-negative bacterium *E. coli*. This study reveals for the first time the antibacterial activity of a cinnamic amide with a 4-methoxycinnamic ring. These results suggest the need for further chemical and biological studies with 4-methoxycinnamic acid derivatives which may someday be used as ingredients in the development of future therapeutic approaches to the growing problem of microbial pathogens.

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