Synthesis and evaluation of arylamidine derivatives for new antimicrobial and cytotoxic activities

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ABSTRACT
A series of arylamidines 3a-j was designed, synthesized and investigated for antimicrobial activity. Structures of the compounds were confirmed by IR, 1H-NMR and 13C-NMR and a 2D spectroscopic study was performed. A preliminary screening of the antimicrobial tests clearly showed that three out of ten arylamidines, viz, 3f, 3g and 3i, were effective against all the gram-negative bacteria: Klebsiella pneumoniae, Pseudomonas aeruginosa and Salmonella enterica; and against the yeast, candida albicans. Further, the Minimum Inhibitory Concentrations (MIC) against the bacteria and yeast were determined. All compounds 3a-d, 3f, 3g, 3i and 3j were also investigated for their low cytotoxic effects on tested cell lines. Compounds 3d and 3f were the most effective derivatives against HL-60 and HEp-2 cells, respectively, with IC50 value (2µg/mL), and low normal cells toxicity.

Key words: arylamidines, 2D spectroscopy, gram-negative bacteria, yeast, cytotoxicity.

INTRODUCTION
Amidine is an integral part of the chemical structure of the pyrimidine and pyrimidinone class of compounds, having a range of pharmacological activities: anti-inflammatory (Falcão et al. 2006, Natarajan et al. 2014) anticonvulsant (Samuel et al. 2016), antitumor (Marzano et al. 2010), antimicrobial (Pizzuti 2005) and anticarcinogenic (Makarov et al. 2005), antiviral (Andrews and Mansur 2014), antibacterial (Arafa et al. 2005), and antiprotozoal (Adiyala et al. 2014). A chemical interest in amidine derivatives is also reported in the literature. The synthesis of a series of 31 α-amino amidines derived from the reaction of aromatic aldehydes, aromatic amines and isonitrile and nitriles carried out using molecular iodine as
the catalyst (Tahghighi et al. 2011). Tahghighi et al. (2011) conducted a synthesis of 10 amidine derivatives and determined their antileishmanial activity against the promastigote form of L. major, of which three products showed promising results (Asano et al. 2004). Driven by the chemical similarity of amidines with the 4-anilinoquinazolines that inhibit the epidermal growth factor receptor (EGFR) tyrosine kinase (Melo et al. 2002), we synthesized two benzamidines and found that they strongly inhibit tyrosine EGFR kinase. Our research group has been synthesizing amidine derivatives for the past 30 years. Numerous pyrimidine derivatives (Falcão et al. 2006, Francisco 2007, Do Monte et al. 2016) containing amidines as part of their structures, have shown us promising results with their anti-inflammatory, anticonvulsant, anti-tumor and antimicrobial activity. The literature also has reports on the pharmacological potential of amidines. So, considering that their antimicrobial properties have not been reported in the literature, we evaluated the antimicrobial activities of 10 arylamidine, 08 of which had been obtained by our research group previously (Falcão et al. 2006, Francisco 2007, Do Monte et al. 2016). We also conducted a one- and two-dimensional nuclear magnetic resonance study for the compound 4-nitrobenzimidamide.

MATERIALS AND METHODS

MATERIALS AND INSTRUMENTS

All reagents were obtained from commercial (Sigma-Aldrich) sources and used without further purification. All melting points were recorded on a BUCHI B-540 apparatus and were not corrected. The IR spectra were recorded on a Perkin Elmer Spectrum 400, using the KBr wafer technique. The spectra of compound 3a-j were acquired using a VARIAN Unity Plus 300 spectrometer operating at 300 MHz and 75 MHz for $^1$H and $^{13}$C nuclei, respectively. Reactions were monitoring by thin layer chromatography (TLC) with precoated silica gel on aluminum sheets (60 mesh containing fluorescent indicator F$_{254}$ Merck). Visualization of the spots was carried out under ultraviolet light (UV) at 365 and 254 nm.

SYNTHESIS

Preparation of derivative arylamidine, 3a-j: An appropriate amount of bisnitrile (42.86 mmol) 1a-g was dissolved in ethanol grade for HPLC (10 mL) and refluxed for 4 hours at room temperature in the presence of Hydrochloric acid gas. Gas ammonia was added to the then formed intermediate imidate, then refluxed, for 2 hours while the solution was maintained in an ice bath. After the reaction was completed, the solvent evaporated to give a solid mass, which was crystallized and recrystallized with solvent n-pentane to generate the corresponding arylamidine. The data for 3b-j are described below:

4-methoxybenzamidine (3b)

This compound was obtained as colorless crystals from n-pentane in 89.90% yield, m.p. 205-207, $R_f$ = 0 (n-hexane-ethylacetate 8:2); IR, KBr, $\gamma_{\text{max}}$ cm$^{-1}$: 3107 (NH$_{2\text{asym}}$), 3019 (NH$_{2\text{sym}}$), 1602 (C = N); $^1$H-NMR (DMSO-d$_6$, 300 MHz), $\delta$: 7.80 (d, 2H, $J$ 9.0 Hz, H3 and H5); 7.13 (d, 2H, $J$ 9.0 Hz, H2 and H6); 5.85 (b, 2H, C7-NH$_2$) and 3.89 (s, -OMe). $^{13}$C-NMR (DMSO-d$_6$, 300 MHz), $\delta$: 166.0 (1C, -OMe). 4-methylbenzamidine (3c)

This compound was obtained as colorless crystals from n-pentane in 90% yield, m.p. 199-201, $R_f$ = 0 (n-hexane-ethylacetate 7:3); IR, KBr, $\gamma_{\text{max}}$ cm$^{-1}$: 3123 (NH$_{2\text{asym}}$), 2990 (NH$_{2\text{sym}}$), 1676 (C = N); $^1$H-NMR (DMSO-d$_6$, 300 MHz), $\delta$: 7.73 (d, 2H, J 9.0 Hz, H3 and H5); 7.43 (d, 2H, J 9.0 Hz, H2 and H6); 4.90 (b, 2H, C7-NH$_2$) and 2.45 (s, -Me). $^{13}$C-NMR (DMSO-d$_6$, 300 MHz), $\delta$: 166.0 (1C, -C7); 120.8 (1C, C1); 160.0 (1C, C4); 131.0 (2C, C2 and C6); 115.8 (2C, C3 and C5) and 56.2 (1C, -OMe).
SYNTHESIS AND EVALUATION: AMIDINE DERIVATIVES

4-diethylaminobenzamidine (3d)

This compound was obtained as colorless crystals from n-pentane in 89% yield, m.p. 201-203, R_f = 0 (n-hexane-ethylacetate 8:2); IR, KBr, γ_max cm⁻¹: 3369 (NH₂asymm.), 3019 (NH₂symm.), 1654 (C = N); 1H-NMR (DMSO-d₆, 300 MHz), δ: 8.44 (d, 2H, J 9.0 Hz, H3 and H5); 8.06 (d, 2H, J 9.0 Hz, H2 and H6); 5.12 (b, 2H, C7-NH₂) and 3.30 (s, -NC₂H₆). 13C-NMR (DMSO-d₆, 300 MHz), δ: 166.0 (1C, C7); 147.8 (1C, C1); 135.0 (2C, C2 and C6); 131.8 (1C, C4); 125.3 (2C, C3 and C5) and 49.0 (1C, -NC₂H₆).

4-aminobenzamidine (3e)

This compound was obtained as colorless crystals from n-pentane in 92.90% yield, m.p. 136-138, R_f = 0 (n-hexane-ethylacetate 8:2); IR, KBr, γ_max cm⁻¹: 3080 (NH₂asymm.), 3004 (NH₂symm.), 1659 (C = N); 1H-NMR (DMSO-d₆, 300 MHz), δ: 7.80 (d, 2H, J 9.0 Hz, H3 and H5); 7.13 (d, 2H, J 9.0 Hz, H2 and H6); 4.85 (b, 2H, C7-NH₂) and 3.89 (s, -OMe). 13C-NMR (DMSO-d₆, 300 MHz), δ: 166.0 (1C, C7); 147.8 (1C, C1); 135.0 (2C, C2 and C6); 131.8 (1C, C4); 125.3 (2C, C3 and C5) and 49.0 (1C, -NC₂H₆).

4-trifluoromethylbenzamidine (3f)

This compound was obtained as colorless crystals from n-pentane in 82.90% yield, m.p. 215-217, R_f = 0 (n-hexane-ethylacetate 7:3); IR, KBr, γ_max cm⁻¹: 3289 (NH₂asymm.), 3198 (NH₂symm.), 1612 (C = N); 1H-NMR (DMSO-d₆, 300 MHz), δ: 8.00 (d, 2H, J 9.0 Hz, H3 and H5); 7.09 (d, 2H, J 9.0 Hz, H2 and H6) and 4.85 (b, 2H, C7-NH₂); 13C-NMR (DMSO-d₆, 300 MHz), δ: 159.6 (1C, C7); 134.5 (1C, C1); 134.0 (1C, C4); 132.0 (2C, C2 and C6); 131.3 (2C, C3 and C5) and 128.0 (1C, C8).

4-bromomethylbenzamidine (3g)

This compound was obtained as colorless crystals from n-pentane in 76% yield, m.p. 203-205, R_f = 0 (n-hexane-ethylacetate 8:2); IR, KBr, γ_max cm⁻¹: 3213 (NH₂asymm.), 3104 (NH₂symm.), 1615 (C = N); 1H-NMR (DMSO-d₆, 300 MHz), δ: 7.97 (d, 2H, J 9.0 Hz, H3 and H5); 7.09 (d, 2H, J 9.0 Hz, H2 and H6) and 4.83 (b, 2H, C7-NH₂); 4.70 (s, 1H, H7); 1H-NMR (DMSO-d₆, 300 MHz), δ: 166.0 (1C, C7) 140.6 (1C, C1); 134.2 (2C, C3 and C5); 130.1 (2C, C2 and C6) and 119.0 (1C, C4).

4-chlorobenzamidine (3h)

This compound was obtained as colorless crystals from n-pentane in 90.75% yield, m.p. 219-221, R_f = 0 (n-hexane-ethylacetate 8:2); IR, KBr, γ_max cm⁻¹: 3092 (NH₂asymm.), 3001 (NH₂symm.), 1593 (C = N); 1H-NMR (DMSO-d₆, 300 MHz), δ: 7.72 (d, 2H, J 9.0 Hz, H3 and H5); 7.58 (d, 2H, J 9.0 Hz, H2 and H6) and 4.84 (b, 2H, C7-NH₂); 13C-NMR (DMSO-d₆, 300 MHz), δ: 166.0 (1C, C7) 140.6 (1C, C4); 134.2 (2C, C3 and C5); 130.1 (2C, C2 and C6) and 119.0 (1C, C1).

2,6-dichlorobenzamidine (3i)

This compound was obtained as colorless crystals from n-pentane in 81% yield, m.p. 225-227, R_f = 0 (n-hexane-ethylacetate 8:2); IR, KBr, γ_max cm⁻¹: 3319 (NH₂asymm.), 3114 (NH₂symm.), 1525 (C = N); 1H-NMR (DMSO-d₆, 300 MHz), δ: 7.82-7.72 (m, 2H, H3 and H5); 7.65-7.60 (t, J 7.5 Hz, 1H, H2) and 4.83 (b, 2H, C7-NH₂); 13C-NMR (DMSO-d₆, 300 MHz), δ: 168.6 (1C, C7); 135.2 (1C, C3); 130.5 (1C, C2); 129.6 (1C, C5) 129.0 (1C, C4) and 123.0 (1C, C1).

3-chlorobenzamidine (3j)

This compound was obtained as colorless crystals from n-pentane in 66% yield, m.p. 216-218, R_f = 0 (n-hexane-ethylacetate 8:2); IR, KBr, γ_max cm⁻¹: 3424 (NH₂asymm.), 3114 (NH₂symm.), 1698 (C = N); 1H-NMR (DMSO-d₆, 300 MHz), δ: 7.82-7.72 (m, 2H,
H5 and H6); 7.65-7.60 (t, J 7.5 Hz, 1H, H2) and 4.83 (b, 2H, C7-NH). 13C-NMR (DMSO-d6 300 MHz), δ: 168.6 (1C, C7); 135.2 (1C, C3); 130.5 (1C, C2); 129.6 (1C, C5) 129.0 (1C, C4) and 123.0 (1C, C1).

TEST OF THE DRUGS (MINIMUM INHIBITORY CONCENTRATION - MIC)

To determine the MIC, the each compound was dissolved individually in a solution containing 20% dimethyl-sulfoxide (DMSO) and 80% Tween-80. The culture medium employed was Mueller Hinton Broth (Difco). The microorganisms used in the present test were: *Salmonella enteric* UFPEDA 414; *Klebsiella pneumoniae* UFPEDA 396, *Pseudomonas aeruginosas* UFPEDA 416 and *candida albicans* UFPEDA 1007 from the culture collection of the Department of Antibiotics, Federal University of Pernambuco, all having been maintained in Mueller Hinton agar. The MIC test was carried out using the micro dilution method in micro 96-well plates containing 100 mL Mueller Hinton Broth, according to the procedure recommended by the Standard Clinical Laboratory Institute, CLSI (2009). The bacterial suspensions were formed in sterilized distilled water and the turbidity adjusted to 0.5 on the McFarland scale (1.5x10^8 UFC/mL). The concentrations of all eleven compounds tested were from 1.0 to 0.00195 µg/mL, the antibiotic gentamicin at a concentration of 0.00195 µg/mL and the antifungal amphotericin B at a concentration of 0.001mg / mL. These were employed as standards, having the same concentrations as recommended by CLSI (2010). Overall, 12 wells (columns) were used: In the first column, the broth alone; in the second, solvent and the broth. From columns 3 through 11, in 1.0 mL of the solvent, the broth was employed as follows: 1.0mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, 0.0625 mg/mL, 0.03125 µg/mL, 0.015625 mg/mL, 0.0078125 mg/mL, 0.00390625 mg/mL and 0.001953125 mg/mL. In the 12th well, both broth and bacteria were added. In the second to eleventh wells, 20mL of bacteria were added. The plates were incubated at 35°C for 24 hours. The plates with bacteria were incubated at 35°C for 24 hours and plates with yeast were incubated at 30°C for 48 hours. When the plate cultivation period ended, a reading was taken with the naked eye and afterwards a sterilized aqueous solution of resazurine (0.1%) was added. After 4 hours of incubation, the reading was taken again. Resazurine facilitates the verification of microbial proliferation: a blue color indicates that there is no bacterial proliferation, while a red color indicates the presence of living cells in the process of proliferation. Thus, it was possible to determine the minimum concentration responsible for the inhibition of the microorganisms (Do Monte et al. 2016).

MTT ASSAY

The cytotoxicity of all compounds 3a-j was measured using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT). The cytotoxicity test was performed using the following cell lines: NCI-H292 (human lung muco epidermoid carcinoma cells), MCF-7 (human breast adenocarcinoma), Hep-2 (human larynx epidermoid carcinoma), HL-60 (promyelocytic leukemia cells) and HT-29 (human Colorectal Adenocarcinoma Cell Line). All cell lines were obtained from the Cell Bank of Rio de Janeiro (Rio de Janeiro, Brazil). The NCI-H292, MCF-7 and Hep-2 cells were grown in Dulbecco’s modified Eagle’s medium (Sigma-Aldrich, St Louis, MO), while the HL-60 cell were maintained in RPMI medium (Sigma-Aldrich, St Louis, MO). The media were supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich, St Louis, MO) and 1% penicillin/streptomycin (Thermo Fisher Scientific, Carlsbad, CA) at 37°C in a 5% CO₂ atmosphere. The cells were seeded in 96-well plates and incubated for 24 hours. Subsequently,
the ten compounds 3a-j were dissolved in DMSO (Sigma–Aldrich, St Louis, MO) (0.1%) and added to the wells to yield a final concentration of 500 µg/mL. The drug doxorubicin (5 mg/mL) was used as the standard. After 72 hours of incubation, 25 µL of MTT solution (5 mg/mL) was added to each well, and the wells were then incubated for 3 hours. The MTT + culture medium were aspirated and 100 µL of DMSO was then added. The absorbance was measured at 560 nm in a spectrophotometer. The experiments were performed in quadruplicate, and the percentages of inhibition were calculated with GraphPad Prism software 5.0 (DEMO). The following intensity scale was used to assess the potential of the cytotoxic samples: cytotoxic activity (95 to 100% inhibition), moderate cytotoxic activity (cell growth inhibition ranging from 70 to 90%), and no cytotoxicity (growth inhibition lower than 50%) (Freire et al. 2016, Rodrigues et al. 2014, Houghton et al. 2007, Alley et al. 1988).

RESULTS AND DISCUSSION

CHEMISTRY

All derivatives of arylamidines 3a-j (Figure 1) were crystalline and stable compounds with recrystallized melting points, prepared with satisfactory yield, and characterized based on their physical, analytical and spectral data. The compounds 3b, 3h and 3i had been previously synthesized by the working group (Do Monte et al. 2016, Francisco 2007, Falcão et al. 2006, Melo et al. 2002) and reported in the literature. The compounds 3a, 3c, 3d-g and 3j have also been reported in the literature (Do Monte 2016).

Syntheses of the compounds 3a-j are known in the literature. There is no study of 2D of assignments ¹H NMR and ¹³C of compounds NMR spectrum. Given this, we made assignments to compound 3a using experiments IR and 2D, ¹H-¹H COSY (Correlation Spectroscopy), and Heteronuclear Single-Quantum Coherence (HSQC). As all compounds have one aromatic and quaternary carbons, a similar strategy was used to attribute the signals of all compounds 3b-j, that were characterized by their spectroscopic data, such as, IR, ¹H-NMR, and ¹³C-NMR. We observed one COSY correlation: δ 8.37 with δ 8.17 ppm. Therefore, there is one AA’BB’ system, with coupling constant equal to 6.0 Hz. Also, one band centered at δ 5.06 ppm was observed and assigned to the hydrogen of the amino group of carbon C7. The ¹3C NMR spectrum presented 7 signals, to assign all the others, it was necessary obtain 2D spectra. In the HSQC spectrum, the following correlations to the AA’BB’ systems were observed: δ_H 8.37 ppm – δ_C 124.4 ppm; δ_H 8.17 ppm – δ_C 134.2 ppm. But a complete assignment was only possible using the HMBC experiment, with which the correlations were observed and the attributions made. So, δ_H 8.37 ppm (H2 and H6 nuclei) correlates δ_C 150.0 ppm (C1 nucleus), 133.3 ppm (C4 nucleus) and δ_C 124.4 ppm (C3 and C6 nuclei) and δ_H 8.17 ppm – δ_C 150.0 ppm (C1 nucleus), 133.3 ppm (C4 nucleus), 134.2 ppm (C2 and C6 nuclei) and 164.3 ppm (C7 nucleus).

A similar strategy was used to attribute the signals of the o,m, and p-arylamidines groups of all compounds 3b-j.

R: -4NO2Ph(3a); -4OMePh (3b); -4MePh (3c); -4NC2H5Ph(3d); 4NH2Ph(3e); -4CF3Ph(3f); -BrCH2Ph(3g); -ClPh(3h); -2,4ClPh(3i); 3ClPh(3j).

Figure 1 - Synthesis of compounds 3a-j from 1a-j and 2a-j.
ANTIMICROBIAL ACTIVITY

Compounds 3a-j were screened for antimicrobial activity. (Mueller Hinton Broth – Difco) from 0.00195 mg/mL to 1.0mg/mL (Table I) was used as the nutrient. We used the antibiotic gentamicin concentration of 0.004 mg/ml, and the antifungal Amphotericin B in a concentration of 1ug / ml as the controls. The compounds 3f, 3i and 3g were tested for their activities against Gram-negative bacteria (*Klebsiella pneumoniae, Salmonella enteric* and *Pseudomonas aeruginosa*) and against yeast (*Candida albicans*). The results revealed that most of the synthesized compounds showed inhibition against the tested microorganisms, as shown in Table I. The compounds 3f and 3g were moderately active with a large bacterial spectrum. Compound 3i was only moderately active against *pseudomonas aeruginosa*. None of the above compounds showed better results than the controls tested.

### TABLE I

<table>
<thead>
<tr>
<th>Compounds</th>
<th>R</th>
<th>Klebsiella pneumoniae</th>
<th>Salmonella enteric</th>
<th>Pseudomonas aeruginosa</th>
<th>C. Albicans</th>
</tr>
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<tbody>
<tr>
<td>3a</td>
<td>p-NO2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3b</td>
<td>p-CH3O</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3c</td>
<td>p-CH3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3d</td>
<td>p-(CH3)2N</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3e</td>
<td>p-NH2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3f</td>
<td>p- CF3</td>
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<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>3g</td>
<td>p-CH2Br</td>
<td>1.0</td>
<td>1.0</td>
<td>0.5</td>
<td>0.5</td>
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<td>3h</td>
<td>p-Cl</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3i</td>
<td>o,p-Cl</td>
<td>—</td>
<td>—</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>3j</td>
<td>m-Cl</td>
<td>—</td>
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### TABLE II

Cytotoxic Activity of Compounds 3a-j against Cell Lines.

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<tr>
<th>Compounds</th>
<th>IC50 (µg/mL)</th>
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<tr>
<td>3a</td>
<td>10.4 (8.7-11.9)</td>
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<tr>
<td>3b</td>
<td>11.9 (8.7-16.2)</td>
</tr>
<tr>
<td>3c</td>
<td>13.9 (12.4-15.7)</td>
</tr>
<tr>
<td>3d</td>
<td>&gt; 25 µg/mL</td>
</tr>
<tr>
<td>3e</td>
<td>&gt; 25 µg/mL</td>
</tr>
<tr>
<td>3f</td>
<td>1.3 (1.0-1.7)</td>
</tr>
<tr>
<td>3g</td>
<td>30.7 (20.4-46.1)</td>
</tr>
<tr>
<td>3h</td>
<td>26.3 (19.6-35.2)</td>
</tr>
<tr>
<td>3i</td>
<td>21.6 (20.4-22.9)</td>
</tr>
<tr>
<td>3j</td>
<td>&gt; 25 µg/mL</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>0.7 (0.3-1.4)</td>
</tr>
<tr>
<td>HEp-2</td>
<td>0.06 (0.05-0.08)</td>
</tr>
<tr>
<td>HL-60</td>
<td>0.4 (0.3-0.5)</td>
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<tr>
<td>HT-29</td>
<td>0.11 (0.08-0.15)</td>
</tr>
<tr>
<td>MCF-7</td>
<td>0.01 (0.04-0.3)</td>
</tr>
<tr>
<td>NCIH-292</td>
<td>—</td>
</tr>
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</table>

*An Acad Bras Cienc* (2017) 89 (2)
SYNTHESIS AND EVALUATION: AMIDINE DERIVATIVES

CYTOTOXICITY EVALUATION

The cellular cytotoxicity of the compounds 3a-j was verified by the MTT assay. In the MTT assay, the 3a-d, 3f, 3g, 3i and 3j samples were non-toxic at a concentration of IC$_{50}$ low of 25µg/mL (Table II), since the cell inhibition was below standard (Table III). Compounds 3d and 3f were the most effective derivatives against HL-60 and HEp-2 cells, respectively, with an IC$_{50}$ value low of 2µg/mL, making them good candidates for use in cancer treatment because of their low toxicity against cells.

CONCLUSIONS

We synthesized ten derivatives of the arylamines 3a-j in their crystalline state. All compounds were characterized by their infrared $^1$H-NMR and $^{13}$C-NMR spectra and a 2D spectroscopy study was performed. Bioassays of all compounds were carried out and we were able to determine the Minimum Inhibition Concentration (MIC) against Gram-negative bacteria and yeast. The compounds 3f and 3g were moderately active with a large bacterial spectrum; 3i was only moderately active against pseudomonas aeruginosa. All cells (ED$_{50}$ = 25 µg/mL) showed low toxicity towards compounds 3a-d, 3f, 3g, 3i and 3j. The best results were for compounds 3d and 3f. But, only compounds 3f, 3g and 3i are good candidates for microbial infections and cancer treatment because of their high toxicity.

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