

## Ultrasound-Promoted Synthesis, Structural Characterization and *in vitro* Antimicrobial Activity of New 5-Aryl-3-(2-hydroxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboximidamides

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This work aims the synthesis of amidino pyrazolines by the cyclocondensation reaction between 3-(hetero)aryl-1-(2-hydroxyphenyl)enones and aminoguanidine hydrochloride under sonochemical conditions. Thirteen new compounds were synthesized in 15-83% of yield after sonication for 30 min without purification by chromatography. Furthermore, antimicrobial activities of the synthesized compounds were investigated by *in vitro* minimum inhibitory concentration (MIC) in a panel of four bacterial strains and four *Candida* yeasts. As result, the 5-(4-bromophenyl)-3-(2-hydroxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboximidamide hydrochloride was identified as the most active against gram-positive *S. aureus* and *E. faecalis* with MIC of 7.8  $\mu\text{g mL}^{-1}$ , similar to the value showed by chloramphenicol, which was used as standard.

**Keywords:** amidino pyrazoline, antibacterial activity, antifungal activity, cyclocondensation reaction, ultrasonic irradiation

### Introduction

Pyrazoles and their non-aromatic analogous pyrazolines are important scaffolds for the development of new biologically active compounds.<sup>1</sup> Although pyrazole-containing natural products are rare, many synthetic molecules bearing this heterocycle are clinically approved pharmaceuticals such as celecoxib (anti-inflammatory activity),<sup>2</sup> sildenafil (treatment of erectile dysfunction),<sup>3</sup> and rimonabant (treatment of obesity),<sup>4</sup> as well as agrochemicals such as chlorantraniliprole (insecticide),<sup>5</sup>

fipronil (insecticide),<sup>6</sup> and pyraclostrobin (fungicide).<sup>7</sup> The related pyrazolines are motifs commonly found in compounds with potent bioactivities such as antibacterial,<sup>8</sup> antifungal,<sup>9</sup> antiviral,<sup>10</sup> anti-inflammatory,<sup>11</sup> antioxidant,<sup>12</sup> anticancer,<sup>13</sup> antidepressant and anti-anxiety.<sup>14</sup>

In the same context, amidine-containing compounds show recognized bioactivities. Pentamidine is included on the World Health Organization's List of Essential Medicines for the basic health system indicated for the treatment of first stage human African trypanosomiasis and as a second-line therapy for leishmaniasis.<sup>15</sup> In this concern, amidine-containing molecules have been pointed as prominent prototypes in the search for new antimicrobial agents.<sup>16</sup>

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In the last years, the ultrasonic irradiation as green energy source has been applied in the field of pharmaceutical and green chemistry. The beneficial effect of sonication on organic synthesis are attributed to cavitation which creates special local conditions in the reaction medium. As result, reactions have been completed in shorter reaction times and products have been obtained in higher yields than under conventional methods.<sup>17</sup> Recently, we demonstrated that ultrasonic irradiation was valuable in promoting the cyclocondensation reaction of chalcones and aminoguanidine hydrochloride for the synthesis of amidino pyrazolines.<sup>18</sup> This method prompted us to synthesize series of pyrazolines in short reaction times and good yields using ethanol as green solvent despite the low solubility of aminoguanidine in such medium.

In continuation to this program, in this work we sought to promote the cyclocondensation reaction of phenolic chalcones and aminoguanidine hydrochloride under ultrasonic irradiation, aiming the synthesis of water soluble phenolic amidino pyrazolines hydrochlorides. Furthermore, searching for antimicrobial compounds, all new products were tested against gram-positive and gram-negative bacteria and *Candida* type yeasts.

## Experimental

### General

All the chemicals were used without purification as purchased from commercial suppliers (Sigma-Aldrich, St. Louis, USA). The sonicated reactions were carried out with a microtip probe connected to a 500 W Sonics Vibracell ultrasonic processor operating at 20 kHz at 20% of the maximum power output. Melting point values were determined in open capillary on an Instrutherm DF-3600 II apparatus and are uncorrected. Infrared spectra (FTIR) were acquired on a JASCO-4100 spectrophotometer. <sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance (NMR) spectra were acquired on a Bruker Avance III HD instrument (300 MHz for <sup>1</sup>H and 75 MHz for <sup>13</sup>C) in 5 mm sample tubes at 298 K in dimethyl sulfoxide (DMSO-*d*<sub>6</sub>). A hybrid high-resolution and high accuracy microTof (Q-TOF) (Bruker® Scientific) was used for detection, with electrospray ionization (ESI) source (MicrOTOF-QII Bruker® Scientific) in positive mode. The compounds **3a-3m** were dissolved individually in a solution of 50% (v/v) chromatographic grade acetonitrile (Tedia, Fairfield, OH, USA), 50% (v/v) deionized water and 0.1% formic acid. The solutions were infused directly into the ESI source by means of a syringe pump (Harvard Apparatus) at a flow rate of 180 μL min<sup>-1</sup>. The drying temperature was 200 °C and

nitrogen was used for drying gas, in a 10 L min<sup>-1</sup> flow. The ESI(+)-MS and tandem ESI(+)-MS/MS were acquired under the following conditions: capillary and cone voltages were set to +3500 V and +40 V, respectively, with a desolvation temperature of 100 °C. For ESI(+)-MS/MS, the energy for the collision induced dissociations (CID) was optimized for each component. The software Compass DataAnalysis version 4.3 (Bruker® Scientific) was used for spectrum analysis. The diagnostic ions were identified by the comparison of their ESI(+)-MS/MS dissociation patterns with compounds with theoretical mass for the respective elementary formula. The data were collected in the *m/z* range of 70-2000 at the speed of two scans *per* second, providing the resolution of 50,000 (full width at half maximum, FWHM) at *m/z* 200. No important ions were observed below *m/z* 100 or above *m/z* 700, therefore ESI(+)-MS data is shown in the *m/z* 100-700 range. Diffraction data were collected on a Bruker D8 Venture Photon 100 diffractometer equipped with an Incoatec IμS high brilliance Mo-Kα X ray tube with two-dimensional Montel micro-focusing optics. The structure was solved by direct methods using SHELXS.<sup>19</sup> Subsequent Fourier-difference map analyses yielded the positions of the non-hydrogen atoms. Refinements were carried out with the SHELXL package.<sup>19</sup> All refinements were made by full-matrix least-squares on F<sup>2</sup> with anisotropic displacement parameters for all non-hydrogen atoms. Hydrogen atoms were included in the refinement in calculated positions but the atoms (of hydrogens) that are commenting performing special bond were located in the Fourier map. Drawings were done using ORTEP-31 for Windows<sup>20</sup> crystal data and more details of the data collection and refinements of the compound **3a** is presented in Table 1.

### Chemistry

#### Procedure for the synthesis of 4,5-dihydro-1*H*-pyrazole-1-carboximidamides **3a-m**

A mixture of 3-(hetero)aryl-1-(2-hydroxyphenyl) enone **1a-1m** (1.0 mmol), aminoguanidine hydrochloride **2** (0.22 g, 2.0 mmol) and potassium hydroxide (0.11 g, 2.0 mmol) in ethanol (15 mL) was sonicated for 30 min. After the excess of aminoguanidine hydrochloride had been filtered off, the resulting solution was quenched with 10% hydrogen chloride (15 mL). The mixture was extracted with chloroform (3 × 30 mL) and the combined organic layer was dried over MgSO<sub>4</sub>. The solvent was evaporated and the residue was purified by recrystallization from ethyl acetate-methanol (10:1) to afford the 4,5-dihydro-1*H*-pyrazole-1-carboximidamides **3a-3l** as crystalline solids. For the extraction of **3m**, after removal of excess

**Table 1.** Crystal data and structure refinement for **3a**

Chemical formula	C <sub>18</sub> H <sub>23</sub> ClN <sub>4</sub> O <sub>2</sub>
Molecular weight / (g mol <sup>-1</sup> )	362.85
Crystal system, space group	monoclinic, P2 <sub>1</sub> /c
Temperature / K	100(2)
<i>a</i> , <i>b</i> , <i>c</i> / Å	11.4290(5), 15.2989(9), 10.3794(5)
β / degree	102.713(2)
Volume / Å <sup>3</sup>	1770.36(16)
<i>Z</i> , D <sub>calc</sub> / (g cm <sup>-3</sup> )	4, 1.361
Radiation type	Mo Kα
μ / mm <sup>-1</sup>	0.236
Crystal size / mm	0.20 × 0.19 × 0.18
F (000)	768
θ range / degree	2.26-29.19
T <sub>min</sub> , T <sub>max</sub>	0.9688, 0.9444
Index ranges	-15 ≤ <i>h</i> ≤ 15, -18 ≤ <i>k</i> ≤ 20, -14 ≤ <i>l</i> ≤ 13
Reflections collected / unique	10281 / 4774 [R(int) = 0.0761]
Completeness to theta maximum / %	99.6
Refinement method	full-matrix least-squares on <i>F</i> <sup>2</sup>
Data / restraints / parameters	4774 / 0 / 226
Goodness-of-fit (GOF) on <i>F</i> <sup>2</sup>	1.082
Final <i>R</i> indices [I > 2σ( <i>I</i> )]	<i>R</i> <sub>1</sub> = 0.0602, <i>wR</i> <sub>2</sub> = 0.1455
<i>R</i> indices (all data)	<i>R</i> <sub>1</sub> = 0.1199, <i>wR</i> <sub>2</sub> = 0.2111
Δρ <sub>max</sub> , Δρ <sub>min</sub> / (e Å <sup>-3</sup> )	0.315 and -0.379

*a*-*c*: unit cell parameters; *Z*: formula unit *per* unit cell; D<sub>calc</sub>: calculated density; μ: absorption coefficient; F (000): structure factor in the zeroth-order case; R(int): internal *R*-value; *F*<sup>2</sup>: squared structure factor; *R*<sub>1</sub>: *R*-value; I > 2σ(*I*): final *R* indices based on the reflections observed; *wR*<sub>2</sub>: *R*-value for *F*<sup>2</sup>.

of aminoguanidine, the filtrate was treated with a saturated solution of NaCl and extracted with chloroform (3 × 30 mL). The combined organic layer was acidified with some drops of concentrated hydrogen chloride, dried over MgSO<sub>4</sub> and the solvent was evaporated.

### 3-(2-Hydroxyphenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazole-1-carboximidamide (**3a**)

Whitish solid; 77% yield; mp 294-296 °C; FTIR (attenuated total reflectance, ATR) *v* / cm<sup>-1</sup> 3282, 3167, 3105, 1646, 1615, 1596, 1585, 1493; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 10.00 (s, 1H), 8.00 (bs, 4H), 7.78-7.75 (m, 1H), 7.40-7.28 (m, 4H), 7.23-7.21 (m, 2H), 7.05-7.02 (m, 1H), 6.92-6.87 (m, 1H), 5.91-5.85 (m, 1H, H<sub>X</sub>), 4.12 (dd, 1H, *J* 18.4, 11.2 Hz, H<sub>M</sub>), 3.45-3.38 (m, 1H, H<sub>A</sub>); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ 158.1, 156.9, 153.0, 139.9, 132.7, 129.7, 129.1, 128.2, 125.3, 119.5, 117.1, 115.8, 59.4, 46.0; high resolution mass spectrometry (HRMS, ESI-TOF MS) *m/z*, calcd. for [C<sub>16</sub>H<sub>17</sub>N<sub>4</sub>O]<sup>+</sup>: 281.1397, found: 281.1393.

### 3-(2-Hydroxyphenyl)-5-(*o*-tolyl)-4,5-dihydro-1*H*-pyrazole-1-carboximidamide (**3b**)

Yellowish solid; 64% yield; mp 282-284 °C; FTIR (ATR) *v* / cm<sup>-1</sup> 3273, 3117, 3032, 2940, 1646, 1617, 1590, 1490; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 9.97 (s, 1H), 8.06 (bs, 4H), 7.77-7.73 (m, 1H), 7.35-7.13 (m, 4H), 7.04-7.01 (m, 1H), 6.91-6.86 (m, 1H), 6.77-6.74 (m, 1H), 5.91 (dd, 1H, *J* 11.2, 2.9 Hz, H<sub>X</sub>), 4.17 (dd, 1H, *J* 18.1, 11.1 Hz, H<sub>M</sub>), 3.27 (dd, 1H, *J* 18.1, 2.9 Hz, H<sub>A</sub>), 2.41 (s, 3H); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ 158.1, 156.8, 153.0, 137.4, 135.0, 132.7, 131.3, 129.8, 127.8, 126.2, 122.4, 119.5, 117.1, 115.7, 57.8, 44.9, 19.2; HRMS (ESI-TOF MS) *m/z*, calcd. for [C<sub>17</sub>H<sub>19</sub>N<sub>4</sub>O]<sup>+</sup>: 295.1553, found: 295.1546.

### 3-(2-Hydroxyphenyl)-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazole-1-carboximidamide (**3b**)

Yellowish solid; 83% yield; mp 273-274 °C; FTIR (ATR) *v* / cm<sup>-1</sup> 3272, 3166, 3065, 2938, 1645, 1616, 1588, 1493; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 9.96 (s, 1H), 7.96 (bs, 4H), 7.77-7.74 (m, 1H), 7.37-7.32 (m, 1H), 7.20-7.17 (m, 2H), 7.12-7.09 (m, 2H), 7.04-7.01 (m, 1H), 6.93-6.88 (m, 1H), 5.81 (dd, 1H, *J* 11.2, 3.0 Hz, H<sub>X</sub>), 4.09 (dd, 1H, *J* 18.3, 11.1 Hz, H<sub>M</sub>), 3.37-3.36 (m, 1H, H<sub>A</sub>), 2.26 (s, 3H); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ 158.0, 156.8, 152.9, 137.5, 136.9, 132.6, 129.7, 129.6, 125.2, 119.5, 117.1, 115.8, 59.3, 45.9, 20.7; HRMS (ESI-TOF MS) *m/z*, calcd. for [C<sub>17</sub>H<sub>19</sub>N<sub>4</sub>O]<sup>+</sup>: 295.1553, found: 295.1551.

### 3-(2-Hydroxyphenyl)-5-(2-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carboximidamide (**3d**)

Yellowish solid; 73% yield; mp 290-293 °C; FTIR (ATR) *v* / cm<sup>-1</sup> 3309, 3194, 3108, 2966, 2939, 1644, 1618, 1594, 1494; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 10.00 (s, 1H), 7.96 (bs, 4H), 7.76-7.73 (m, 1H), 7.35-7.29 (m, 2H), 7.10-7.02 (m, 2H), 6.96-6.86 (m, 3H), 5.84 (dd, 1H, *J* 11.1, 3.1 Hz, H<sub>X</sub>), 4.07 (dd, 1H, *J* 18.3, 11.2 Hz, H<sub>M</sub>), 3.43 (s, 3H), 3.31 (dd, 1H, *J* 18.3, 3.2 Hz, H<sub>A</sub>); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ 158.6, 156.8, 156.2, 153.0, 132.5, 129.6, 129.5, 126.6, 125.0, 120.3, 119.4, 117.0, 115.8, 111.8, 56.3, 55.7, 44.7; HRMS (ESI-TOF MS) *m/z*, calcd. for [C<sub>17</sub>H<sub>19</sub>N<sub>4</sub>O<sub>2</sub>]<sup>+</sup>: 311.1503, found: 311.1494.

### 3-(2-Hydroxyphenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carboximidamide (**3e**)

Yellowish solid; 80% yield; mp 274-276 °C; FTIR (ATR) *v* / cm<sup>-1</sup> 3266, 3178, 3066, 2953, 2835, 1645, 1615, 1587, 1510, 1492; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 9.98 (s, 1H), 7.96 (bs, 4H), 7.78-7.74 (m, 1H), 7.38-7.32 (m, 2H), 7.18-7.15 (m, 2H), 7.04-7.02 (m, 1H), 6.95-6.89 (m, 3H), 5.82-5.77 (m, 1H, H<sub>X</sub>), 4.08 (dd, 1H, *J* 18.3, 11.1 Hz, H<sub>M</sub>), 3.72 (s, 3H), 3.38-3.37 (m, 1H, H<sub>A</sub>); <sup>13</sup>C NMR

(75 MHz, DMSO- $d_6$ )  $\delta$  159.1, 158.1, 156.8, 152.9, 132.6, 131.8, 129.7, 126.7, 119.5, 117.1, 115.9, 114.4, 59.0, 55.2, 45.9; HRMS (ESI-TOF MS)  $m/z$ , calcd. for  $[C_{17}H_{19}N_4O_2]^+$ : 311.1503, found: 311.1485.

5-(4-Fluorophenyl)-3-(2-hydroxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboximidamide (**3f**)

Whitish solid; 74% yield; mp 272-275 °C; FTIR (ATR)  $\nu / \text{cm}^{-1}$  3363, 3275, 3080, 2949, 2829, 1647, 1615, 1594, 1509, 1492;  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  9.98 (s, 1H), 8.00 (bs, 4H), 7.78-7.75 (m, 1H), 7.38-7.19 (m, 5H), 7.04-7.01 (m, 1H), 6.93-6.88 (m, 1H), 5.87 (dd, 1H,  $J$  11.3, 3.0 Hz,  $H_X$ ), 4.12 (dd, 1H,  $J$  18.5, 11.2 Hz,  $H_M$ ), 3.39-3.38 (m, 1H,  $H_A$ );  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ )  $\delta$  161.8 (d,  $J$  244.4 Hz), 158.0, 156.8, 152.9, 136.1 (d,  $J$  3.0 Hz), 132.7, 129.7, 127.6 (d,  $J$  8.4 Hz), 119.5, 117.1, 115.9 (d,  $J$  21.7 Hz), 115.8, 58.8, 44.9; HRMS (ESI-TOF MS)  $m/z$ , calcd. for  $[C_{16}H_{16}FN_4O]^+$ : 299.1303, found: 299.1301.

5-(2-Bromophenyl)-3-(2-hydroxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboximidamide (**3g**)

Whitish solid; 60% yield; mp 279-282 °C; FTIR (ATR)  $\nu / \text{cm}^{-1}$  3352, 3274, 3196, 3141, 3064, 2957, 2838, 1656, 1613, 1592, 1494;  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  10.10 (s, 1H), 8.07 (bs, 4H), 7.83-7.72 (m, 2H), 7.44-7.39 (m, 1H), 7.35-7.26 (m, 2H), 7.03-7.00 (m, 1H), 6.93-6.85 (m, 2H), 5.84 (dd, 1H,  $J$  11.2, 3.0 Hz,  $H_X$ ), 4.24 (dd, 1H,  $J$  18.4, 11.1 Hz,  $H_M$ ), 3.40-3.34 (m, 1H,  $H_A$ );  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ )  $\delta$  157.7, 156.9, 153.1, 137.9, 133.8, 132.7, 130.0, 129.6, 128.2, 125.1, 121.5, 119.4, 117.1, 115.7, 60.1, 45.5; HRMS (ESI-TOF MS)  $m/z$ , calcd. for  $[C_{16}H_{16}BrN_4O]^+$ : 359.0502, found: 359.0498.

5-(3-Bromophenyl)-3-(2-hydroxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboximidamide (**3h**)

Yellowish solid; 44% yield; mp 308-309 °C; FTIR (ATR)  $\nu / \text{cm}^{-1}$  3313, 3178, 3121, 2934, 2847, 1638, 1615, 1603, 1590, 1494;  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  10.00 (s, 1H), 8.03 (bs, 4H), 7.78-7.75 (m, 1H), 7.55-7.51 (m, 1H), 7.46-7.45 (m, 1H), 7.39-7.32 (m, 2H), 7.19-7.17 (m, 1H), 7.04-7.01 (m, 1H), 6.93-6.88 (m, 1H), 5.86 (dd, 1H,  $J$  11.0, 3.0 Hz,  $H_X$ ), 4.13 (dd, 1H,  $J$  18.5, 11.3 Hz,  $H_M$ ), 3.31 (m, 1H,  $H_A$ );  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ )  $\delta$  158.0, 156.8, 152.9, 142.4, 132.7, 131.4, 131.1, 129.7, 128.5, 124.1, 122.0, 119.5, 117.1, 115.7, 58.9, 45.9; HRMS (ESI-TOF MS)  $m/z$ , calcd. for  $[C_{16}H_{16}BrN_4O]^+$ : 359.0502, found: 359.0501.

5-(4-Bromophenyl)-3-(2-hydroxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboximidamide (**3i**)

Yellowish solid; 68% yield; mp 277-279 °C; FTIR

(ATR)  $\nu / \text{cm}^{-1}$  3394, 3192, 3101, 2956, 2836, 1656, 1617, 1590, 1490;  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  9.99 (s, 1H), 8.01 (bs, 4H), 7.78-7.75 (m, 1H), 7.61-7.58 (m, 2H), 7.37-7.32 (m, 1H), 7.19-7.15 (m, 2H), 7.03-7.01 (m, 1H), 6.93-6.88 (m, 1H), 5.85 (dd, 1H,  $J$  11.2, 3.0 Hz,  $H_X$ ), 4.13 (dd, 1H,  $J$  18.4, 11.3 Hz,  $H_M$ ), 3.46-3.38 (m, 1H,  $H_A$ );  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ )  $\delta$  158.0, 156.9, 152.9, 139.2, 132.7, 132.0, 129.7, 121.3, 119.5, 117.1, 115.8, 58.9, 45.8; HRMS (ESI-TOF MS)  $m/z$ , calcd. for  $[C_{16}H_{16}BrN_4O]^+$ : 359.0502, found: 359.0492.

3-(2-Hydroxyphenyl)-5-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazole-1-carboximidamide (**3j**)

Whitish solid; 63% yield; mp 294-296 °C; FTIR (ATR)  $\nu / \text{cm}^{-1}$  3393, 3289, 3222, 3153, 3079, 2943, 2843, 1657, 1616, 1592, 1495;  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  9.93 (bs, 1H), 8.04 (bs, 4H), 7.79-7.77 (m, 3H), 7.45-7.43 (m, 2H), 7.38-7.32 (m, 1H), 7.03-7.01 (m, 1H), 6.93-6.88 (m, 1H), 5.98 (dd, 1H,  $J$  11.4, 3.0 Hz,  $H_X$ ), 4.18 (dd, 1H,  $J$  18.5, 11.4 Hz,  $H_M$ ), 3.49-3.40 (m, 1H,  $H_A$ );  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ )  $\delta$  157.9, 156.9, 153.0, 144.3, 132.7, 129.7, 128.7 (q,  $J$  31.7 Hz), 126.3, 126.0 (q,  $J$  4.0 Hz), 124.1 (q,  $J$  272.4 Hz), 119.4, 117.1, 115.7, 59.1, 45.9; HRMS (ESI-TOF MS)  $m/z$ , calcd. for  $[C_{17}H_{16}F_3N_4O]^+$ : 349.1271, found: 349.1252.

3-(2-Hydroxyphenyl)-5-(3-nitrophenyl)-4,5-dihydro-1H-pyrazole-1-carboximidamide (**3k**)

Brownish solid; 15% yield; mp 311-312 °C; FTIR (ATR)  $\nu / \text{cm}^{-1}$  3296, 3208, 3103, 2934, 2864, 1645, 1616, 1603, 1529, 1494;  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  10.02 (s, 1H), 8.21-8.18 (m, 1H), 8.11-8.10 (m, 1H), 8.06 (bs, 4H), 7.80-7.64 (m, 3H), 7.38-7.32 (m, 1H), 7.03-7.00 (m, 1H), 6.94-6.88 (m, 1H), 6.02 (dd, 1H,  $J$  11.4, 3.2 Hz,  $H_X$ ), 4.20 (dd, 1H,  $J$  18.6, 11.4 Hz,  $H_M$ ), 3.53-3.46 (m, 1H,  $H_A$ );  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ )  $\delta$  158.0, 156.9, 152.9, 148.0, 141.8, 132.8, 132.0, 130.9, 129.7, 123.2, 120.7, 119.5, 117.1, 115.7, 58.9, 46.0; HRMS (ESI-TOF MS)  $m/z$ , calcd. for  $[C_{16}H_{16}N_5O_3]^+$ : 326.1248, found: 326.1245.

3-(2-Hydroxyphenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carboximidamide (**3l**)

Yellowish solid; 78% yield; mp 264-267 °C; FTIR (ATR)  $\nu / \text{cm}^{-1}$  3321, 3200, 3107, 2940, 2869, 1615, 1589, 1493;  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  10.00 (s, 1H), 8.04 (bs, 4H), 7.81-7.78 (m, 1H), 7.49-7.47 (m, 1H), 7.39-7.34 (m, 1H), 7.22-7.21 (m, 1H), 7.04-6.90 (m, 3H), 6.22 (dd, 1H,  $J$  10.8, 2.5 Hz,  $H_X$ ), 4.08 (dd, 1H,  $J$  18.4, 10.8 Hz,  $H_M$ ), 3.60 (dd, 1H,  $J$  18.3, 2.5 Hz,  $H_A$ );  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ )  $\delta$  158.2, 156.9, 152.9, 141.9, 132.8, 129.7, 126.9, 126.5, 126.2, 119.5, 117.1, 115.8, 55.9, 45.8; HRMS

(ESI-TOF MS)  $m/z$ , calcd. for  $[C_{14}H_{15}N_4OS]^+$ : 287.0961, found: 287.0954.

### 3-(2-Hydroxyphenyl)-5-(pyridin-2-yl)-4,5-dihydro-1H-pyrazole-1-carboximidamide (**3m**)

Whitish solid; 24% yield; mp 276-278 °C; FTIR (ATR)  $\nu / \text{cm}^{-1}$  3332, 3262, 3208, 3097, 3051, 2813, 2720, 1670, 1618, 1589, 1495;  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  9.97 (s, 1H), 8.05 (bs, 4H), 8.03-8.00 (m, 2H), 7.77-7.74 (m, 1H), 7.57-7.50 (m, 2H), 7.37-7.31 (m, 1H), 7.04-7.01 (m, 1H), 6.92-6.87 (m, 1H), 6.02 (dd, 1H,  $J$  11.5, 3.3 Hz,  $H_X$ ), 4.15 (dd, 1H,  $J$  18.4, 11.5 Hz,  $H_M$ ), 3.61 (dd, 1H,  $J$  18.4, 3.3 Hz,  $H_A$ );  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ )  $\delta$  157.7, 156.8, 156.3, 153.3, 148.2, 139.6, 132.6, 129.7, 124.2, 121.9, 119.5, 117.1, 115.7, 59.5, 44.5; HRMS (ESI-TOF MS)  $m/z$ , calcd. for  $[C_{15}H_{16}N_5O]^+$ : 282.1349, found: 282.1330.

### Biology

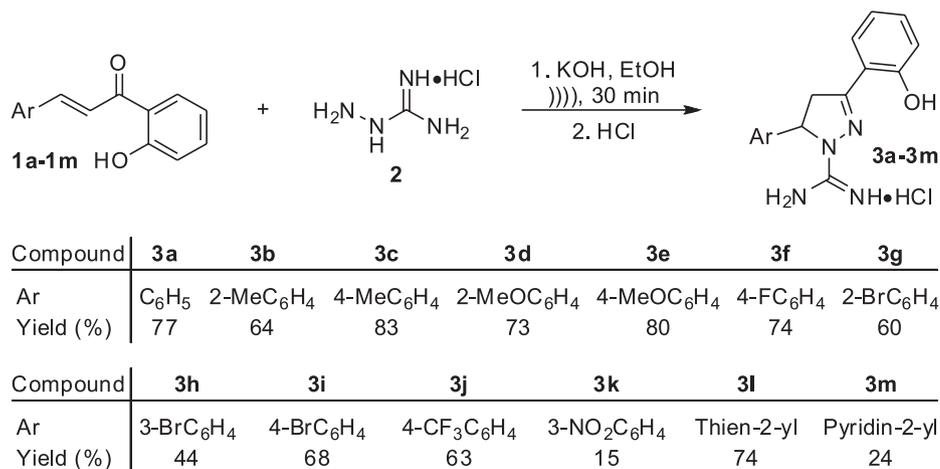
The minimum inhibitory concentrations (MIC) of the 4,5-dihydro-1H-pyrazole-1-carboximidamides **3a-3m** were determined by the broth microdilution test, following the guidelines of the Clinical and Laboratory Standards Institute (2012) and document M27-A3 with some adaptations.<sup>21</sup> The microorganisms are strains from American Type Culture Collection. Two gram-negative bacteria: *Salmonella enteritidis* (ATCC 13076) and *Escherichia coli* (ATCC 25922); and two gram-positive: *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 51299), in addition to four yeasts: *Candida krusei* (ATCC 6558), *Candida tropicalis* (ATCC 750), *Candida albicans* (ATCC 90028) and *Candida glabrata* (ATCC 2001) were used. Dimethyl sulfoxide was used as solvent for the antimicrobial tests.

## Results and Discussion

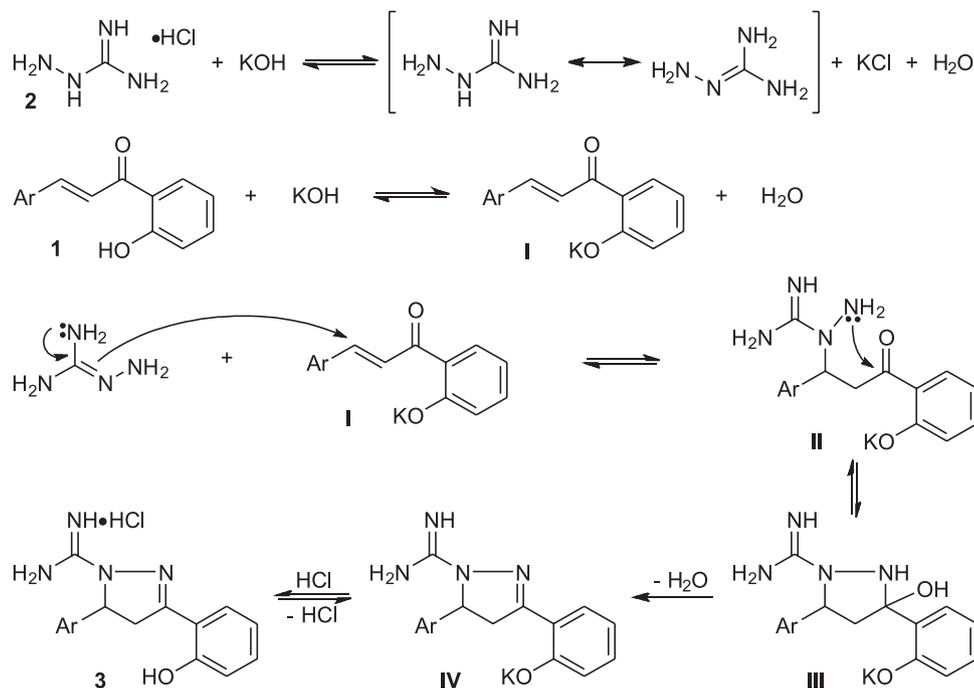
### Synthesis and structural characterization

3-(Hetero)aryl-1-(2-hydroxyphenyl)enones **1a-1m** were prepared according to previously reported procedures.<sup>22</sup> Sonication of **1a-1m** and aminoguanidine hydrochloride **2** for 30 min in the presence of potassium hydroxide using ethanol as solvent, and further treatment with 10% hydrogen chloride before extraction, afforded the novel 4,5-dihydro-1H-pyrazole-1-carboximidamides hydrochlorides **3a-3m** in acceptable to good yields (15-83%) (Scheme 1). The method determined here showed some advantages including the short reaction time, non-chromatographic purification and hydroxyl protection/deprotection steps not required. Unfortunately, all the new compounds were water-insoluble. Consequently, dimethyl sulfoxide was used as the solvent for the bactericidal and fungicidal tests.

The mechanism of the cyclocondensation reaction between aminoguanidine and  $\alpha,\beta$ -unsaturated ketones for synthesis of amidino pyrazolines remains experimentally unestablished. However, we propose the mechanism showed in Scheme 2 based on a previously reported mechanism<sup>23</sup> that considered the hard and soft acids and bases (HSAB) concept and the structure of aminoguanidine obtained by quantum chemical studies.<sup>24</sup> In the first step, the KOH neutralizes the aminoguanidine salt **2** to give aminoguanidine free base (tautomer forms). In the same line, the phenol is deprotonated by the KOH leading to the formation of the phenoxide intermediate **I**. In the next step, the Aza-Michael-type addition with the internal imine nitrogen gives the intermediate **II**. Subsequently, **III** is formed after an intramolecular nucleophilic attack by nitrogen, which is dehydrated to give the product **IV**. The product **3** is formed after the reaction media is acidified.



**Scheme 1.** Ultrasound-promoted synthesis of 4,5-dihydro-1H-pyrazole-1-carboximidamides **3a-3m**.



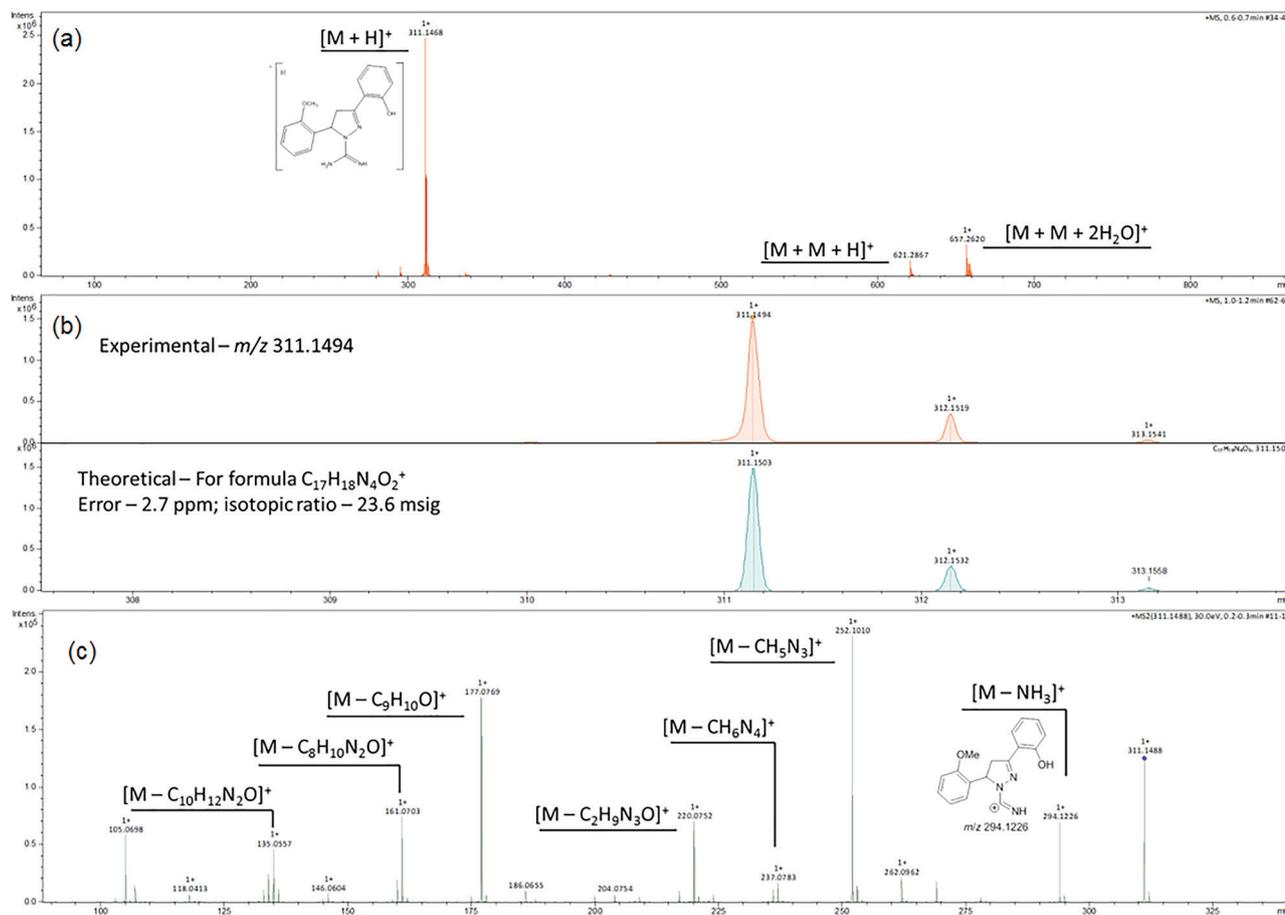
**Scheme 2.** Proposed mechanism for the cyclocondensation reaction between 3-(hetero)aryl-1-(2-hydroxyphenyl)enones and aminoguanidine hydrochloride in the presence of KOH.

The structure elucidation of compounds **3a-3m** was performed from analysis of their spectroscopic and spectrometric data (FTIR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and HRMS). The IR spectrum of pyrazole **3d** taken as representative example of the series showed a pair of absorption bands at 3309 and 3194  $\text{cm}^{-1}$  related to the N–H of protonated amidine group. No prominent band was seen in the region where stretching vibration of the OH oscillator should appear. The  $^1\text{H}$  NMR spectrum of **3d** showed a singlet at 10.00 ppm assigned to the OH proton and a broad singlet at 7.96 ppm associated to the protons of the protonated amidine group. The signals attributed to the eight aromatic protons appear in the region 7.76–6.86 ppm. The signals assigned to the protons of the AMX system appear as three double-doublets at 3.31 ppm with coupling constant values  $^2J = 18.3$  Hz and  $^3J = 3.2$  Hz for proton  $\text{H}_A$ ; 4.07 ppm, with coupling constant values  $^2J = 18.3$  Hz and  $^3J = 11.2$  Hz, for proton  $\text{H}_M$ ; and 5.84 ppm, with coupling constant values  $^3J = 11.1$  Hz and  $^3J = 3.1$  Hz, for proton  $\text{H}_X$ . The methoxy group generated a singlet at 3.43 ppm. Furthermore, the  $^{13}\text{C}$  NMR spectrum showed all the expected signals.

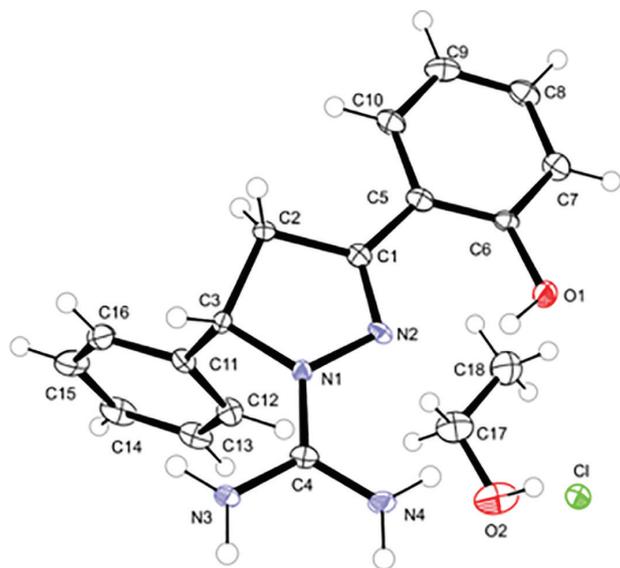
The thirteen products **3a-3m** were analyzed by HRMS with electrospray ionization (ESI). Spectra were acquired in positive mode ESI(+)-MS and in ESI(+)-MS/MS for evaluation of fragmentation pathway. The exact mass/charge ( $m/z$ ) of the hydrogen adduct  $[\text{M} + \text{H}]^+$  of all compounds are present with errors less than 10 ppm relative to that theoretically expected for elemental formula, as

for instance for the **3d** with  $m/z$  311.1494 for the formula  $[\text{C}_{17}\text{H}_{18}\text{N}_4\text{O}_2 + \text{H}]^+$  (theoretical  $m/z$  311.1503). Furthermore, for some compounds it is possible to identify the dimers formation, as for instance, with the  $m/z$  621.2867 and 657.2620, which are the  $[\text{M} + \text{M} + \text{H}]^+$  and  $[\text{M} + \text{M} + 2\text{H}_2\text{O}]^+$ , respectively, for the compound **3d**. In the same line, the fit of isotopic ratio (theoretical *versus* experimental) assist in the confirmation of elementary composition of synthesized compounds, being in the **3d** example equal to 23.6 msig. Errors of exact  $m/z$  below 10 ppm, as well as differences of isotopic ratios less than 25 msig, for HRMS with ionization by ESI and mass separation by quadrupole-time of flight (Q-TOF), confirm the elemental composition.<sup>25</sup> Moreover, the fragmentation pathway has important information about the molecular structure. For the title compounds, it is similar to that proposed by Kéki *et al.*,<sup>26</sup> for 1,3,5-trisubstituted pyrazolines, as well as dos Santos *et al.*<sup>27</sup> for dihydropyrimidines. The Figure 1 demonstrates this HRMS tools used to characterize the synthesized amidino pyrazolines, using as example the compound **3d**. For the other compounds, see the Supplementary Information (SI) section.

Finally, the molecular structure of compound **3a** was confirmed by single X-ray diffraction analysis (Figure 2). It shows a protonated amidino-nitrogen atom resulting in a delocalized positive charge in the N3–C4–N4 system, according to the symmetric bond length between N3–C4 = 1.325(4) Å and C4–N4 = 1.323(4) Å. These values



**Figure 1.** The HRMS analysis of compound **3d**. In (a) the full spectra; in (b) the expansion between 308 and 314 u.a.m. highlighting the exact mass and isotopic ratio; and in (c) the analysis in MS-MS mode (fragmentation pathway).



**Figure 2.** Molecular structure of **3a**. Thermal ellipsoids drawn at the 50% probability level.

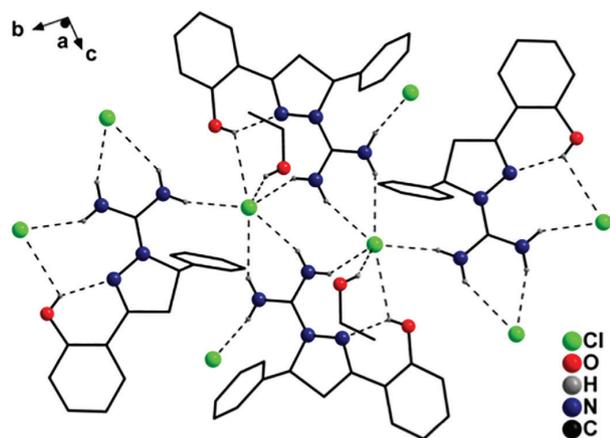
are intermediate between single and double C–N bond lengths and are very close to those reported for the protonated amidino group in the aminoguanidine hydrochloride

structure.<sup>28</sup> The electroneutrality is achieved by a chloride anion. Additionally, an ethanol molecule is observed as a solvate. Crystal data, data collection and structure refinement details are summarized in Table 1. Other crystallographic data for **3a** are shown in the SI section.

The one-dimensional (1-D) growth of **3a** in the *bc* plane is represented in Figure 3. The intermolecular H-bonds of type N–H...Cl<sup>−</sup> involving the chloride ion and the hydrogen of the protonated amidino group measure between 2.3801(7) and 2.7397(7) Å. For the interactions of type O–H...Cl<sup>−</sup>, the distances are 2.8394(7) Å for the one involving the phenolic hydrogen and 2.5001(8) Å for the ethanolic one. The intramolecular H-bonds of type O–H...N between pyrazoline nitrogen and phenolic hydrogen measure 2.0531(24) Å. These lengths are shorter than the sum of the Cl/H, O/H and N/H van der Waals radii (3.0, 2.67 and 2.75 Å, respectively).<sup>29</sup>

#### Antibacterial activity

All the novel compounds were screened for their *in vitro* antibacterial activity against gram-positive



**Figure 3.** Supramolecular assembly of **3a** in the *bc* plane and the intermolecular Cl...H and N...H interactions (dotted lines). For clarity, some of the hydrogen atoms are not shown.

and gram-negative bacteria. The minimum inhibitory concentrations (MIC) were determined and reported in Table 2. Gram-negative bacteria *E. coli* and *S. Enteritidis* can be found in the gastrointestinal tract of humans and other animals, causing diarrhea and other systemic diseases.<sup>30</sup> Gram-positive bacteria constitute important clinical risk and are responsible for high mortality rates associated with bloodstream infections. In addition, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterococcus* spp. resistant to vancomycin (VRE) show the importance of the development of new antibiotics.<sup>31</sup> In this concern, compounds **3a-3f,3k** showed selectivity for gram-positive bacteria *Staphylococcus aureus* and *Enterococcus faecalis* with MIC values in the range 31.2-62.5  $\mu\text{g mL}^{-1}$ . On the other hand, compound **3j** was

active against the four tested strains (MIC 31.2  $\mu\text{g mL}^{-1}$ ). Compound **3h** also showed discrete inhibition against both gram-positive and gram-negative bacteria. Compound **3i** was the most active, showing inhibitory activity against *Staphylococcus aureus* (MIC 7.8  $\mu\text{g mL}^{-1}$ ) and *Enterococcus faecalis* (MIC 7.8  $\mu\text{g mL}^{-1}$ ). These MIC values are lower than those observed for the licensed antibiotic chloramphenicol against *S. aureus* and *E. faecalis*. This compound also showed moderate inhibition against *Salmonella enteritidis* (MIC 62.5  $\mu\text{g mL}^{-1}$ ) and *Escherichia coli* (MIC 62.5  $\mu\text{g mL}^{-1}$ ). These data show that bromine at 4-position of the benzene ring is essential for molecule activity. When this atom is shifted to 3-position there is an 8-fold decrease in compound activity against *S. aureus* and *E. faecalis* strains. Additionally, the change of bromine by a fluorine atom attached at 4-position produced a molecule 16-fold less active against *S. aureus* and *E. faecalis*. Electron donating groups at 4-position, like methyl and methoxyl, cause at least a 4-fold decrease in compound activity against *S. aureus* and *E. faecalis*. These results indicate that compounds **3h-3j** may be used as prototype for new broad-spectrum antibiotics.

#### Antifungal activity

The MIC against four *Candida* type yeasts were determined and reported in Table 3. These *Candida* species were chosen because they are important opportunistic pathogens besides provide infections due to their virulence factors and resistance to antifungal agents.<sup>32</sup> Most of amidino pyrazolines did not show high antifungal activity.

**Table 2.** *In vitro* antibacterial activity (MIC values)

Compound	Ar	MIC / ( $\mu\text{g mL}^{-1}$ )			
		<i>S. enteritidis</i> ATCC 13076	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 25923	<i>E. faecalis</i> ATCC 51299
<b>3a</b>	C <sub>6</sub> H <sub>5</sub>	250	250	62.5	62.5
<b>3b</b>	2-MeC <sub>6</sub> H <sub>4</sub>	250	250	31.2	62.5
<b>3c</b>	4-MeC <sub>6</sub> H <sub>4</sub>	125	125	31.2	62.5
<b>3d</b>	2-MeOC <sub>6</sub> H <sub>4</sub>	250	250	31.2	62.5
<b>3e</b>	4-MeOC <sub>6</sub> H <sub>4</sub>	250	250	62.5	62.5
<b>3f</b>	4-FC <sub>6</sub> H <sub>4</sub>	125	125	125	125
<b>3g</b>	2-BrC <sub>6</sub> H <sub>4</sub>	125	250	250	125
<b>3h</b>	3-BrC <sub>6</sub> H <sub>4</sub>	62.5	62.5	62.5	62.5
<b>3i</b>	4-BrC <sub>6</sub> H <sub>4</sub>	62.5	62.5	7.8	7.8
<b>3j</b>	4-CF <sub>3</sub> C <sub>6</sub> H <sub>4</sub>	31.2	31.2	31.2	31.2
<b>3k</b>	3-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	62.5	125	31.2	31.2
<b>3l</b>	thiophen-2-yl	250	250	125	250
<b>3m</b>	pyridin-2-yl	1000	1000	500	1000
Chloramphenicol	–	4	4	32	16
Ampicillin	–	1	4	4	0.5

MIC: minimum inhibitory concentration.

**Table 3.** *In vitro* antifungal activity (MIC values)

Compound	MIC / ( $\mu\text{g mL}^{-1}$ )				
	Ar	<i>C. krusei</i> ATCC 6558	<i>C. tropicalis</i> ATCC 750	<i>C. albicans</i> ATCC 90028	<i>C. glabrata</i> ATCC 2001
<b>3a</b>	C <sub>6</sub> H <sub>5</sub>	250	250	250	1000
<b>3b</b>	2-MeC <sub>6</sub> H <sub>4</sub>	125	125	500	500
<b>3c</b>	4-MeC <sub>6</sub> H <sub>4</sub>	31.2	62.5	125	250
<b>3d</b>	2-MeOC <sub>6</sub> H <sub>4</sub>	125	125	250	250
<b>3e</b>	4-MeOC <sub>6</sub> H <sub>4</sub>	250	500	500	1000
<b>3f</b>	4-FC <sub>6</sub> H <sub>4</sub>	250	250	500	500
<b>3g</b>	2-BrC <sub>6</sub> H <sub>4</sub>	250	250	250	250
<b>3h</b>	3-BrC <sub>6</sub> H <sub>4</sub>	125	125	250	250
<b>3i</b>	4-BrC <sub>6</sub> H <sub>4</sub>	62.5	62.5	125	125
<b>3j</b>	4-CF <sub>3</sub> C <sub>6</sub> H <sub>4</sub>	125	125	125	250
<b>3k</b>	3-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	125	62.5	125	125
<b>3l</b>	thiophen-2-yl	500	500	500	500
<b>3m</b>	pyridin-2-yl	–	–	–	–
Fluconazole	–	32	2	0.5	2
Amphotericin B	–	0.25	0.06	0.06	0.06

MIC: minimum inhibitory concentration.

Only **3c** and **3i** moderately inhibited the growth of *C. krusei* and *C. tropicalis*. Compound **3k** also showed moderate activity against *C. tropicalis*.

## Conclusions

In summary, a concise and greener method for the synthesis of amidino pyrazolines under sonochemical conditions was achieved. The 13 new compounds were obtained in moderate to good yields without additional purification. The structural characterization was unequivocal using FTIR, <sup>1</sup>H and <sup>13</sup>C NMR, HRMS and X-ray diffraction. Concerning the antimicrobial tests, some pyrazolines exhibited promising *in vitro* antibacterial activity. In this sense, the compound **3i** (Ar = 4-BrC<sub>6</sub>H<sub>4</sub>) presented the most promising values of MIC, which was comparable with the standard antibiotic chloramphenicol against *S. aureus* and *E. faecalis*. In this line, future studies can highlight these amidino pyrazoline as drugs prototypes.

## Supplementary Information

Crystallographic data (excluding structure factors) for the structure in this work were deposited in the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 1880378. Copies of the data can be obtained, free of charge, via [www.ccdc.cam.ac.uk/conts/retrieving.html](http://www.ccdc.cam.ac.uk/conts/retrieving.html) or from the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033. E-mail: [deposit@ccdc.cam.ac.uk](mailto:deposit@ccdc.cam.ac.uk).

Supplementary data are available free of charge at <http://jbcs.sbq.org.br> as PDF file.

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## Author Contributions

Danilo Y. de Albuquerque was responsible for the investigation, methodology and writing original draft; Andressa C. Damim for the investigation and methodology; Eliandro Faoro for the formal analysis, software, validation, visualization, writing-review and editing; Gleison A. Casagrande for the conceptualization, writing-review and editing; Davi F. Back for the investigation, data curation and formal analysis; Sidnei Moura for the investigation, data curation, formal analysis, writing-review and editing; Claudio M. P. de Pereira for the conceptualization and resources; Kelly M. P. de Oliveira for the conceptualization, data curation, methodology, writing-review and editing; Lucas Pizzuti for the conceptualization, data curation, funding acquisition, project administration, writing original draft, writing-review and editing.

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