

Synthesis and Antimicrobial Activity of Glycosylated 2-Aryl-5-amidinobenzimidazoles

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A series of new glycosylated 2-aryl-5-amidinobenzimidazoles derived from four different carbohydrates (*D*-glucose, *D*-galactose, *N*-acetyl-*D*-glucosamine and lactose) were synthesized by the condensation of the appropriate 4-formyl-3-methoxyphenyl glycoside with 4-amidino- or 4-*N*-isopropylamidino-*ortho*-phenylenediamine hydrochloride. All the compounds were properly characterized by high resolution mass spectrometry, uni- and bidimensional ¹H and ¹³C nuclear magnetic resonance and then were evaluated for their antibacterial and antifungal potential. Considering the antifungal potential of them, two derivatives were active against *Candida parapsilosis* at 96.4 μmol L⁻¹ and another was active against this same strain at 83.5 μmol L⁻¹. In addition, one benzamidine showed activity against *Candida glabrata* at 97 μmol L⁻¹. Considering the antibacterial potential of these compounds, six of them showed better activity against three different stains: three of them with IC₅₀ of 96.4, 97 and 83.5 μmol L⁻¹ against Gram-positive *Micrococcus luteus*, the other two with IC₅₀ 96.5 and 96.4 μmol L⁻¹ against Gram-positive *Enterococcus faecalis* and one against Gram-negative *Escherichia coli* at 90.5 μmol L⁻¹. These findings suggest this structural pattern can be employed for design of more potent agents for discovery of new antimicrobial drug candidates.

Keywords: 2-aryl-5-amidinobenzimidazoles, glycosides, antibacterial, antifungal

Introduction

Microbial infections represent a serious public health problem and are associated with a large number of deaths worldwide. The microbial resistance has increased these numbers and made the available antimicrobial therapy poorly effective.¹ Although a small number of *Candida* species are pathogenic to humans, candidiasis is considered the main cause of hospital infections, mainly due to use of immunosuppressive drugs or modern medical procedures that leave the patient more susceptible to contamination by these microorganisms.^{2,3} In addition, few antibacterial drugs have been discovered in the last 50 years, making infections caused by Gram-positive or Gram-negative bacteria also a serious health threat.⁴

Among the various known potentially antimicrobial heterocyclic compounds, benzimidazoles occupy a prominent position and there are several reports of synthesis of benzimidazoles with antibacterial⁵ and antifungal⁶ potential. The benzimidazole nucleus is considered an important pharmacophore since it can act as a bioisostere group of different molecular constituents of the microorganisms.⁷

On the other hand, amidine derivatives have also occupied an important position among the biologically active compounds, since this strongly basic group is easily protonated in physiological conditions, allowing important interactions with several targets of different microorganisms.⁸ Göker *et al.*⁹ reported the synthesis and antimicrobial activity of a series of benzamidines derived from flavones and benzopyranones. Some benzamidines were active against methicillin-resistant

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Staphylococcus aureus and *Staphylococcus epidermidis*, *Escherichia coli* and *Enterococcus faecalis* in the range of 1.56-50 $\mu\text{g mL}^{-1}$. Other benzamidines synthesized in this study showed antifungal potential against *Candida albicans* and *Candida krusei* in the range of 3.12-50 $\mu\text{g mL}^{-1}$.

In this context, we report here the synthesis and antimicrobial potential of new glycosylated 2-aryl-5-amidinobenzimidazole derivatives. Four different carbohydrates (*D*-glucose, *D*-galactose, *N*-acetyl-*D*-glucosamine and lactose) were used, aimed to investigate the influence of the carbohydrate moiety on the biological activity of the compounds. There are several reports of biologically active glycosylated compounds, especially against microorganisms, and the presence of the saccharide unit has proved essential for their activity.^{4,10} We envisaged the synthesis of the target molecules according to the retrosynthetic shown in Scheme 1. The aryl glycosides and the substituted *ortho*-phenylenediamines need for the synthesis can be easily obtained by procedures described in the literature.⁹

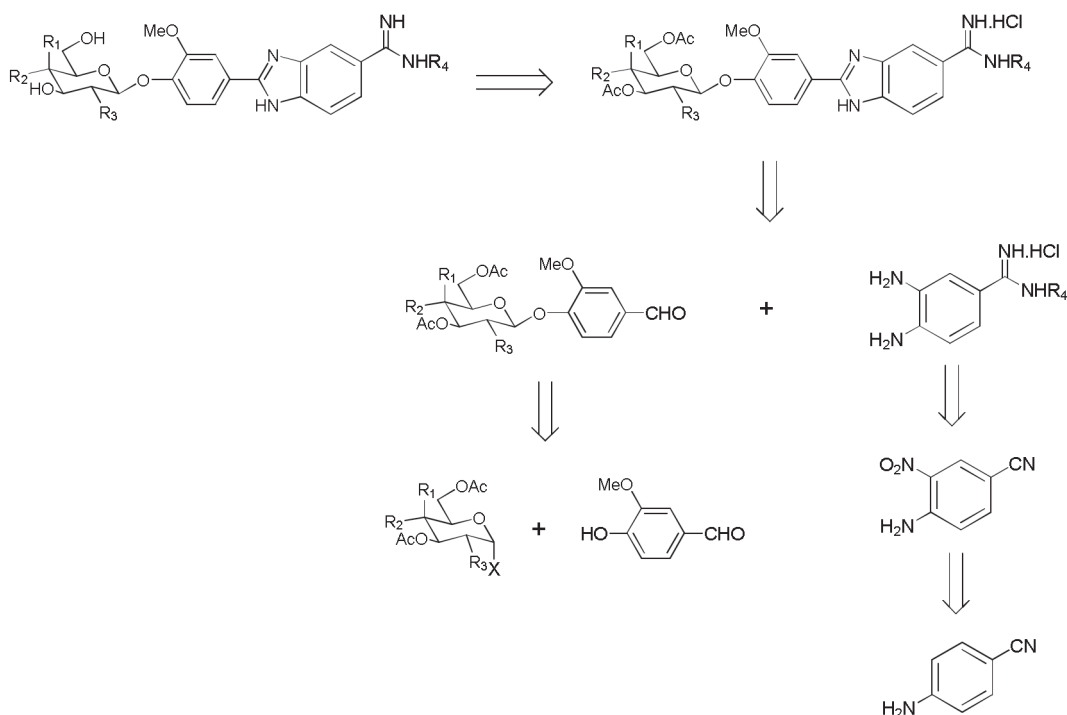
Experimental

Chemistry

The proposed 2-aryl-5-amidinobenzimidazoles were synthesized from the reaction of two 3,4-diamino-

benzamidines in combination with four glycosylated aldehydes, followed by deacetylation of peracetylated derivatives initially obtained.

Melting points of synthesized compounds were determined on Microquímica MOAs 301 apparatus and are uncorrected. Infrared spectroscopy was performed on Spectrum One, PerkinElmer spectrophotometer. ¹H and ¹³C nuclear magnetic resonance (NMR) spectra were obtained on Bruker Avance DRX-200 (200 MHz FT NMR) and DRX-400 (400 MHz FT NMR) spectrometers in deuterated chloroform or dimethyl sulfoxide (DMSO). Chemical shifts (δ) were reported in parts *per million* (ppm) with reference to tetramethylsilane (TMS) as internal standard and coupling constants (*J*) were reported in hertz (Hz). The following abbreviations were used for the ¹H multiplicities: singlet (s), doublet (d), triplet (t), quartet (qr), quintet (q), multiplet (m) and broad signal (br s). The specific optical rotations $[\alpha]_D$ were measured on PerkinElmer 341 polarimeter, at 20 °C. High resolution mass spectra were acquired using a liquid chromatography mass spectrometry-ion trap-time of flight (LCMS-IT-TOF) mass spectrometer and the samples were solubilized in MeOH + 0.1% formic acid, following manual injection. Reaction courses and product mixtures were monitored by thin-layer chromatography (TLC) on silica gel-G TLC plates (Merck) and column grade silica gel (0.063-0.200 mm mesh size) was employed for chromatography.



Scheme 1. Retrosynthetic analysis for the target compounds.

Synthesis of acetamidobenzonitrile (**1**)

4-Aminobenzonitrile (8.46 mmol) was added, slowly, to 8 mL of acetic anhydride and the temperature was maintained between 35 and 40 °C. After the complete addition, the suspension was poured into a bath of ice/water and the yellow solid obtained was collected by filtration and washed with water, affording the desired product.

Synthesis of 4-amino-3-nitrobenzonitrile (**2**)

Potassium nitrate (15.6 mmol) was dissolved in 8 mL of concentrated H₂SO₄ and the mixture was cooled to below 0 °C. To this solution, it was added, slowly, 7.8 mmol of **1** and the temperature was maintained at 0 °C for 3.5 h. The mixture was poured into a bath of ice/water and the yellow solid obtained was collected by filtration and washed with a small amount of water. The obtained product was suspended in 30 mL of 2 mol L⁻¹ H₂SO₄ and heated under reflux for 3 h. The suspension was cooled to room temperature and the yellow solid was collected by filtration and washed with small amount of cold water, to afford the desired product in 74% yield.

Synthesis of 3-nitrobenzamidine (**3**)

A sample of **2** (6.13 mmol) was suspended in 55 mL of dry methanol and the suspension was saturated with HCl_(g) for 30 min. After 4 days stirring at room temperature, the suspension was filtrated and the solid was washed with 120 mL of ethyl ether. The yellow solid obtained was dissolved in dry methanol and saturated with NH₃ during 6 h. The solution was concentrated to half and to the resulting solution was added 60 mL of ethyl ether. The resulting precipitated was collected by filtration and washed with 50 mL of ethyl acetate, affording the interest product.

4-Amino-3-nitrobenzamidine hydrochloride (**3**)

This product was obtained as a yellow solid (60% yield); mp > 300 °C; IR (ATR) ν / cm⁻¹ 3453, 3420 (NH₂), 3134, 3052 (NH.HCl amidine), 1685 (C=N), 1628 (NH₂); ¹H NMR (200 MHz, DMSO-*d*₆) δ 9.38 (br s, 2H, H amidine), 9.12 (br s, 2H, H amidine), 8.62 (s, 1H, H arom.), 8.17 (s, 2H, NH₂), 7.87 (d, *J* 9.0 Hz, 1H, H arom.), 7.19 (d, *J* 9.0 Hz, 1H, H arom.); ¹³C NMR (50 MHz, DMSO-*d*₆) δ 163.5, 149.1, 133.6, 129.6, 127.6, 119.4, 113.2.

Synthesis of 3-nitrobenzamidine (**5**)

A sample of **2** (6.13 mmol) was suspended in 55 mL of dry methanol and the suspension was saturated with HCl_(g)

for 30 min. After 4 days stirring at room temperature, the suspension was filtrated and the solid was washed with 120 mL of ethyl ether. The yellow solid obtained was dissolved in dry methanol and to this solution was added 1 mL of isopropylamine; the mixture was heated under reflux for 3 h. The solvent was removed and the solid resulting was washed with 150 mL of ethyl ether and 120 mL of ethyl acetate.

4-Amino-3-nitro-*N*-isopropylbenzamidine hydrochloride (**5**)

This product was obtained as a yellow solid (63% yield); mp 259.5-261.6 °C; IR (ATR) ν / cm⁻¹ 3456, 3161 (NH₂), 3047, 2942 (NH.HCl amidine), 1685 (C=N); ¹H NMR (200 MHz, DMSO-*d*₆) δ 8.44 (br s, 1H, H arom.), 8.08 (br s, 2H, NH₂), 7.76 (br s, 1H, H arom.), 7.20 (br s, 1H, H arom.), 4.07 (m, 1H, CH), 1.23 (d, *J* 5.6 Hz, 6H, CH₃); ¹³C NMR (50 MHz, DMSO-*d*₆) δ 159.5, 148.6, 134.2, 129.3, 127.2, 119.2, 115.5, 44.9, 21.5.

General procedure for the synthesis of diaminobenzamidines (**4** and **6**)

A solution of **3** (4.6 mmol) or **5** (3.81 mmol) in 60 mL of ethanol and 10% Pd-C was hydrogenated until the required quantity of H₂ was taken up. The Pd-C was removed by filtration and the ethanol was concentrated affording the desired products, which were used without previous purification.

3,4-Diaminobenzamidine hydrochloride (**4**)

This product was obtained in 93% yield as a yellow solid; mp 230-235 °C; IR (ATR) ν / cm⁻¹ 3466, 3420 (NH₂), 3200, 3115, 3031 (NH.HCl amidine), 1641 (C=N); ¹H NMR (200 MHz, DMSO-*d*₆) δ 8.80 (s, 2H, H amidine), 8.69 (s, 2H, H amidine), 7.00 (d, *J* 8.4 Hz, 1H, H arom.), 6.95 (s, 1H, H arom.), 6.59 (d, *J* 8.2 Hz, 1H, H arom.), 5.44 (br s, 2H, NH₂); 3.43 (sl, 2H, NH₂); ¹³C NMR (50 MHz, DMSO-*d*₆) δ 165.5, 141.7, 134.0, 118.9, 114.0, 112.7.

3,4-Diamino-*N*-isopropylbenzamidine hydrochloride (**6**)

This product was obtained in 98% yield as a yellow solid; mp 227-229 °C; IR (ATR) ν / cm⁻¹ 3379, 3207 (NH₂), 3082, 2972, 2941 (NH.HCl amidine), 1689 (C=N); ¹H NMR (200 MHz, DMSO-*d*₆) δ 8.71 (s, 2H, H amidine), 6.85 (br s, 2H, H arom.), 6.56 (d, *J* 8.2 Hz, 1H, H arom.), 5.53 (s, 2H, NH₂), 4.92 (s, 2H, NH₂), 4.04 (m, 1H, CH), 1.20 (d, *J* 5.6 Hz, 6H, CH₃); ¹³C NMR (50 MHz, DMSO-*d*₆) δ 162.0, 140.7, 134.3, 118.3, 116.0, 112.8, 112.6, 44.4, 21.6.

General procedure for the synthesis of peracetylated glycosides (**11-13**)

A solution of the corresponding glycosyl bromide (1 equiv.) in acetone (20 mL) was added to a solution of 4-hydroxy-3-methoxybenzaldehyde (vanillin) (3 equiv.) in 1.0 mol L⁻¹ lithium hydroxide (10 mL) and the solution was stirred for 2 h at room temperature. The completion of reaction was monitored by TLC, when acetone was removed, and the resulting suspension was extracted with dichloromethane (3 × 50 mL). The crude product was washed with 10% sodium hydroxide (3 × 30 mL), water and dried over anhydrous sodium sulfate. After filtration and removal of the solvent under reduced pressure, the crude product was recrystallized from isopropyl alcohol, affording the title compounds.

4-Formyl-2-methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranoside (**11**)

This product was obtained in 57% yield as a white solid; mp 136.1-137.3 °C; [α]_D -39.2° (*c* 0.51 CH₂Cl₂); IR (ATR) ν / cm⁻¹ 1753, 1737 (C=O ester), 1694 (C=O aldehyde); ¹H NMR (400 MHz, CDCl₃) δ 9.89 (s, 1H, CHO), 7.43-7.40 (m, 2H, H-9 and H-11), 7.21 (d, *J* 8.0 Hz, 1H, H-8), 5.34-5.28 (m, 2H, H-2 and H-3), 5.13 (t, *J* 6.8 Hz, 1H, H-4), 5.09 (d, *J* 6.4 Hz, 1H, H-1), 4.27 (dd, 1H, H-6, ²*J* 12.4 Hz, ³*J* 5.2 Hz), 4.18 (dd, ²*J* 12.4 Hz, ³*J* 2.4 Hz, 1H, H-6'), 3.86 (s, 3H, OCH₃), 3.85-3.70 (m, 1H, H-5), 2.07-2.04 (4s, 12H, COCH₃); ¹³C NMR (100 MHz, CDCl₃) δ 190.8, 170.5-169.2, 151.1, 151.0, 132.8, 125.3, 118.2, 110.8, 99.7, 72.4, 72.2, 71.0, 68.2, 61.9, 56.1, 20.6-20.5.

4-Formyl-2-methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranoside (**12**)

This product was obtained in 60% yield as a white solid; mp 123.1-123.8 °C; [α]_D -8.1° (*c* 0.49 CH₂Cl₂); IR (ATR) ν / cm⁻¹ 1752, 1740 (C=O ester), 1693 (C=O aldehyde); ¹H NMR (400 MHz, CDCl₃) δ 9.89 (s, 1H, CHO), 7.43-7.40 (m, 2H, H-11 and H-9), 7.25 (d, *J* 8.4 Hz, 1H, H-8), 5.55 (t, *J* 9.2 Hz, 1H, H-2), 5.46 (d, *J* 2.8 Hz, 1H, H-4), 5.12 (dd, *J*_(ax-ax) 10.4 Hz, *J*_(ax-eq) 3.6 Hz, 1H, H-3), 5.05 (d, *J* 8.0 Hz, 1H, H-1), 4.23 (dd, ²*J* 11.8 Hz, ³*J* 6.8 Hz, 1H, H-6), 4.16 (dd, ²*J* 11.2 Hz, ³*J* 6.4 Hz, 1H, H-6'), 4.07-4.03 (m, 1H, H-5), 3.90 (s, 3H, OCH₃), 2.17-2.02 (4s, 12H, COCH₃); ¹³C NMR (100 MHz, CDCl₃) δ 190.8, 170.3-169.3, 151.2, 150.9, 132.7, 125.3, 117.9, 110.7, 100.35, 71.2, 70.6, 68.4, 66.8, 61.3, 56.1, 20.6-20.5.

4-Formyl-2-methoxyphenyl 2,3,6,2',3',4',6'-hepta-*O*-acetyl-β-*D*-lactoside (**13**)

This product was obtained in 52% yield as a white solid;

mp 88.6-90.1 °C; [α]_D -12.5° (*c* 0.48 CH₂Cl₂); IR (ATR) ν / cm⁻¹ 1741 (C=O ester), 1687 (C=O aldehyde); ¹H NMR (400 MHz, CDCl₃) δ 9.88 (s, 1H, CHO), 7.42-7.39 (m, 2H, H-11 and H-9), 7.17 (d, *J* 8.0 Hz, 1H, H-8), 5.35 (d, *J* 2.8 Hz, 1H, H-4'), 5.32 (t, *J* 8.8 Hz, 1H, H-3), 5.22 (t, *J* 8.8 Hz, 1H, H-2), 5.14-5.08 (m, 2H, H-1 and H-2'), 4.97 (dd, ²*J* 12.0 Hz, ³*J* 3.2 Hz, 1H, H-3'), 4.53-4.51 (m, 2H, H-1' and H-6), 4.17-4.06 (m, 3H, H-6', H-6'' and H-6'''), 3.93-3.89 (m, 3H, H-4 and H-5'), 3.88 (s, 1H, OCH₃), 3.79-3.75 (m, 1H, H-5), 2.15-1.97 (s, 21H, COCH₃); ¹³C NMR (100 MHz, CDCl₃) δ 190.8, 170.3-169.1, 151.1, 150.9, 132.7, 125.3, 117.8, 110.7, 101.13, 99.3, 76.0, 73.0, 72.4, 71.3, 70.9, 70.7, 69.1, 66.6, 61.8, 60.8, 56.1, 20.7-20.5.

Synthesis of peracetylated glycoside (**14**)

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl chloride (5.44 mmol) was solubilized in acetonitrile (50 mL). To this solution were added 4-hydroxy-3-methoxybenzaldehyde (10.8 mmol), K₂CO₃ (23.7 mmol), polyethyleneglycol 4000 (0.27 mmol) and the mixture was stirred for 3 h at room temperature, when the completion of reaction was observed by TLC. The suspension obtained was filtered and the filtrate was concentrated to residue. The crude product was solubilized in chloroform (60 mL) and the organic layer was washed with 10% NaOH and water and dried over anhydrous sodium sulfate. After filtration and removal of the solvent under reduced pressure, the crude product was recrystallized from isopropyl alcohol.

4-Formyl-2-methoxyphenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside (**14**)

This product was obtained in 54% yield as a white solid; mp 197.1-198.2 °C; [α]_D -15.0° (*c* 0.53 CH₂Cl₂); IR (ATR) ν / cm⁻¹ 1736 (C=O ester), 1690 (C=O aldehyde), 1671 (C=O amide); ¹H NMR (400 MHz, CDCl₃) δ 9.88 (s, 1H, CHO), 7.42 (s, 1H, H-11), 7.41 (dd, ³*J* 10.4 Hz, ⁴*J* 1.6 Hz, 1H, H-9), 7.22 (d, *J* 8.0 Hz, 1H, H-8), 5.86 (d, *J* 8.0 Hz, 1H, NH), 5.50 (t, *J* 9.6 Hz, 1H, H-3), 5.40 (d, *J* 8.0 Hz, 1H, H-1), 5.13 (t, *J* 9.6 Hz, 1H, H-4), 4.27 (dd, ²*J* 12.0 Hz, ³*J* 5.4 Hz, 1H, H-6), 4.16 (dd, ²*J* 12.0 Hz, ³*J* 2.4 Hz, 1H, H-6'), 4.06 (qr, *J* 8.4 Hz, 1H, H-2), 3.90 (s, 3H, OCH₃), 3.88-3.84 (m, 3H, H-5), 2.06-2.04 (s, 9H, OCOCH₃), 1.96 (s, 3H, NHCOCH₃); ¹³C NMR (100 MHz, CDCl₃) δ 190.8, 170.6-170.5, 169.4, 151.0, 150.8, 132.6, 125.5, 118.3, 110.6, 98.9, 72.2, 71.6, 68.5, 62.0, 56.1, 55.0, 23.3, 20.6-20.6.

General procedure for the synthesis of peracetylated benzamidines (**15-22**)

To a solution of the corresponding glycoside (**11-14**) (1 equiv.) in ethanol (35 mL), it was added the 3,4-diaminobenzamidine **4** or **6** (1 equiv.) and *p*-benzoquinone (1 equiv.). The mixture was heated at 70 °C for 4 h, when the completion of reaction was observed by TLC. The solution was concentrated to residue and the pure product was obtained by column chromatography on silica gel.

2-[4-(2,3,4,6-Tetra-*O*-acetyl- β -*D*-glucopyranosyloxy)-3-methoxyphenyl]benzimidazole-5-carboxamidine hydrochloride (**15**)

This product was obtained in 64% yield as a brown solid after purified by chromatography (dichloromethane/methyl alcohol 87:13); mp 196.1-199.3 °C; $[\alpha]_D -50.0^\circ$ (*c* 0.40 MeOH); IR (ATR) ν / cm^{-1} 3340, 3119 (NH.HCl amidine), 1740 (C=O ester), 1675 (C=N); $^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ 13.75 (br s, 1H, NH benzimidazole), 9.31 (br s, 2H, H amidine), 9.04 (br s, 2H, H amidine), 8.20-7.68 (m, 5H, H-9, H-11, H-15, H-16 and H-18), 7.29 (d, *J* 8.8 Hz, 1H, H-8), 5.53 (d, *J* 8 Hz, 1H, H-1), 5.41 (t, *J* 9.6 Hz, 1H, H-3), 5.11 (t, *J* 8.8 Hz, 1H, H-2), 5.03 (t, *J* 9.6 Hz, 1H, H-4), 4.27-4.21 (m, 2H, H-6 and H-5), 4.11 (d, *J* 10.4 Hz, 1H, H-6'), 3.90 (s, 3H, OCH₃), 2.05-1.98 (4s, 12H, COCH₃); $^{13}\text{C NMR}$ (100 MHz, DMSO- d_6) δ 169.9-168.9, 166.0, 149.8, 147.5, 124.8, 119.6, 117.6, 111.3, 98.1, 71.8, 70.9, 70.6, 68.0, 61.6, 56.1, 20.4-20.2; HRMS (ESI) *m/z*, calcd. for C₂₉H₃₃O₁₁N₄ [M + H]⁺: 613.2140, found: 613.2174.

2-[4-(2,3,4,6-Tetra-*O*-acetyl- β -*D*-galactopyranosyloxy)-3-methoxyphenyl]benzimidazole-5-carboxamidine hydrochloride (**16**)

This product was obtained in 61% yield as a brown solid after purified by chromatography (dichloromethane/methyl alcohol 87:13); mp 191.5-194.1 °C; $[\alpha]_D -13.6^\circ$ (*c* 0.44 MeOH); IR (ATR) ν / cm^{-1} 3117 (NH.HCl amidine), 1741 (C=O ester), 1675 (C=N); $^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ 13.80-13.71 (br s, 1H, NH benzimidazole), 9.32 (br s, 2H, H amidine), 9.09 (br s, 2H, H amidine), 8.22 (m, 5H, H-9, H-11, H-15, H-16 and H-18), 7.29 (d, *J* 8.4 Hz, 1H, H-8), 5.44 (d, *J* 6.4 Hz, 1H, H-1), 5.35 (s, 1H, H-4), 5.31-5.24 (m, 2H, H-2 and H-3), 4.45-4.42 (m, 1H, H-5), 4.18-4.09 (m, 2H, H-6 and H-6'), 3.91 (s, 3H, OCH₃), 2.17-1.96 (4s, 12H, COCH₃); $^{13}\text{C NMR}$ (100 MHz, DMSO- d_6) δ 169.3-169.0, 166.7, 149.3, 147.6, 119.1, 98.8, 70.4, 70.1, 68.3, 67.2, 62.7, 56.2, 20.5-20.3; HRMS (ESI) *m/z*, calcd. for C₂₉H₃₃O₁₁N₄ [M + H]⁺: 613.2140, found: 613.2162.

2-[4-(2,3,6,2',3',4',6'-Hepta-*O*-acetyl- β -*D*-lactosyloxy)-3-methoxyphenyl]benzimidazole-5-carboxamidine hydrochloride (**17**)

This product was obtained in 63% yield as a brown solid after purified by chromatography (dichloromethane/methyl alcohol 95:5 to 85:15); mp 197.2-199.5 °C; $[\alpha]_D -47.6^\circ$ (*c* 0.42 MeOH); IR (ATR) ν / cm^{-1} 3348, 3119 (NH.HCl amidine), 1740 (C=O ester), 1675 (C=N); $^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ 13.70 (br s, 1H, H benzimidazole), 9.17 (br s, 4H, H amidine), 8.15 (br s, 1H, H-18), 7.95 (s, 1H, H-11), 7.84 (d, *J* 7.0 Hz, 1H, H-9), 7.77 (br s, 1H, H-15), 7.67 (d, *J* 7.0 Hz, 1H, H-16), 7.26 (d, *J* 8.4 Hz, 1H, H-8), 5.48 (d, *J* 8.0 Hz, 1H, H-1), 5.30 (t, *J* 8.0 Hz, 1H, H-3), 5.25 (d, *J* 3.2 Hz, 1H, H-4'), 5.18 (dd, 2J 10.0 Hz, 3J 3.6 Hz, 1H, H-3'), 5.02 (t, *J* 9.0 Hz, 1H, H-2), 4.88 (t, *J* 9.2 Hz, 1H, H-2'), 4.80 (d, *J* 8.0 Hz, 1H, H-1'), 4.38 (d, 2J 10.4 Hz, 1H, H-6), 4.30-4.25 (m, 1H, H-5'), 4.23-4.03 (m, 4H, H-5, H-6', H-6'', H-6'''), 3.94-3.89 (m, 1H, H-4), 3.89 (s, 3H, OCH₃), 2.10-1.91 (s, 21H, COCH₃); $^{13}\text{C NMR}$ (100 MHz, DMSO- d_6) δ 170.13-169.04, 166.0, 149.7, 147.6, 124.6, 119.6, 117.2, 111.3, 99.8, 97.8, 76.1, 72.1, 71.9, 70.9, 70.2, 69.7, 68.8, 67.0, 62.0, 60.8, 56.1, 20.6-20.2; HRMS (ESI) *m/z*, calcd. for C₄₁H₄₉O₁₉N₄ [M + H]⁺: 901.2986, found: 901.3029.

2-[4-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- β -*D*-glucopyranosyloxy)-3-methoxyphenyl]benzimidazole-5-carboxamidine hydrochloride (**18**)

This product was obtained in 65% yield as a brown solid after purified by chromatography (dichloromethane/methyl alcohol 88:12); mp 218.4-220.2 °C; $[\alpha]_D -47.3^\circ$ (*c* 0.38 MeOH); IR (ATR) ν / cm^{-1} 3110 (NH.HCl amidine), 1740 (C=O ester), 1663 (C=O amide); $^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ 13.78 (br s, 1H, H benzimidazole), 9.20 (br s, 3H, H amidine), 8.14-7.66 (m, 6H, NH, H-9, H-11, H-15, H-16 and H-18), 7.32 (d, *J* 8.4 Hz, 1H, H-8), 5.47 (d, *J* 8.0 Hz, 1H, H-1), 5.26 (t, *J* 10.0 Hz, 1H, H-3), 4.94 (t, *J* 9.6 Hz, 1H, H-4), 4.22 (dd, 2J 11.6 Hz, 3J 4.4 Hz, 1H, H-6), 4.15-4.98 (m, 3H, H-2, H-5 and H-6'), 3.89 (s, 3H, OCH₃), 2.06-2.00 (3s, 9H, OCOCH₃), 1.86 (s, 3H, NHCOCH₃); $^{13}\text{C NMR}$ (100 MHz, DMSO- d_6) δ 169.9, 169.6-169.3, 166.1, 149.6, 147.9, 124.2, 119.1, 116.7, 111.5, 97.9, 72.3, 70.9, 68.3, 61.6, 56.2, 53.3, 22.6, 20.5-20.3; HRMS (ESI) *m/z*, calcd. for C₂₉H₃₄O₁₀N₅ [M + H]⁺: 612.2300, found: 612.2322.

2-[4-(2,3,4,6-Tetra-*O*-acetyl- β -*D*-glucopyranosyloxy)-3-methoxyphenyl]benzimidazole-5-[*N*-(isopropyl)carboxamidine] hydrochloride (**19**)

This product was obtained in 60% yield as a brown solid after purified by chromatography (dichloromethane/methyl alcohol 92:8); mp 187.5-190.0 °C; $[\alpha]_D -33.3^\circ$

(*c* 0.42 MeOH); IR (ATR) ν / cm^{-1} 3072 (NH.HCl amidine), 1743 (C=O ester), 1660 (C=N); ^1H NMR (400 MHz, DMSO- d_6) δ 13.88 (br s, 1H, NH benzimidazole), 9.50 (br s, 3H, H amidine), 7.97 (m, 2H, H-15 and H-18), 7.87 (d, *J* 8.4 Hz, 1H, H-16), 7.77 (br s, 1H, H-11), 7.55 (d, *J* 8.4 Hz, 1H, H-9), 7.29 (d, *J* 8.8 Hz, 1H, H-8), 5.53 (d, *J* 8 Hz, 1H, H-1), 5.42 (t, *J* 9.6 Hz, 1H, H-3), 5.11 (t, *J* 8.8 Hz, 1H, H-2), 5.03 (t, *J* 9.6 Hz, 1H, H-4), 4.26-4.23 (m, 2H, H-6 and H-5), 4.12-4.10 (m, 2H, H-6' and H-21), 3.91 (s, 3H, OCH₃), 2.05-1.98 (4s, 12H, COCH₃), 1.32 (d, *J* 6.4 Hz, 1H, H-22); ^{13}C NMR (100 MHz, DMSO- d_6) δ 169.9-168.9, 167.0, 149.8, 147.4, 124.9, 119.6, 117.7, 111.3, 98.2, 71.8, 70.9, 70.6, 68.0, 61.6, 56.1, 44.9, 21.3, 20.4-20.2; HRMS (ESI) *m/z*, calcd. for C₃₂H₃₉O₁₁N₄ [M + H]⁺: 655.2610, found: 655.2611.

2-[4-(2,3,4,6-Tetra-*O*-acetyl- β -*D*-galactopyranosyloxy)-3-methoxyphenyl]benzimidazole-5-[*N*-(isopropyl)carboxamidine] hydrochloride (**20**)

This product was obtained in 61% yield as a brown solid after purified by chromatography (dichloromethane/methyl alcohol 91:9); mp 208.5-210.1 °C; [α]_D -27.2° (*c* 0.44 MeOH); IR (ATR) ν / cm^{-1} 2979 (NH.HCl amidine), 1744 (C=O ester), 1669 (C=N); ^1H NMR (400 MHz, DMSO- d_6) δ 13.85 (br s, 1H, NH benzimidazole), 9.62 (br s, 3H, H amidine), 8.01 (s, 1H, H-18), 7.98 (s, 1H, H-11), 7.87 (d, *J* 8.0 Hz, 1H, H-15), 7.77 (d, *J* 8.0 Hz, 1H, H-16), 7.55 (d, *J* 8.4 Hz, 1H, H-9), 7.29 (d, *J* 8.4 Hz, 1H, H-8), 5.43 (d, *J* 6.8 Hz, 1H, H-1), 5.35 (s, 1H, H-4), 5.31-5.24 (m, 2H, H-2 and H-3), 4.45-4.41 (m, 1H, H-5), 4.18-4.12 (m, 3H, H-6, H-6' and H-21), 3.91 (s, 3H, OCH₃), 2.17-1.96 (4s, 12H, COCH₃), 1.31 (d, 6H, H-22, *J* 6.4 Hz); ^{13}C NMR (100 MHz, DMSO- d_6) δ 169.9-169.0, 162.4, 153.8, 149.9, 147.5, 124.9, 122.5, 119.6, 117.8, 111.4, 98.8, 70.4, 70.0, 68.2, 67.2, 61.2, 56.1, 44.9, 21.2, 20.4-20.3; HRMS (ESI) *m/z*, calcd. for C₃₂H₃₉O₁₁N₄ [M + H]⁺: 655.2610, found: 655.2619.

2-[4-(2,3,6,2',3',4',6'-Hepta-*O*-acetyl- β -*D*-lactosyloxy)-3-methoxyphenyl]benzimidazole-5-[*N*-(isopropyl)carboxamidine] hydrochloride (**21**)

This product was obtained in 52% yield as a yellow solid after purified by chromatography (dichloromethane/methyl alcohol 93:7); mp 195.5-198.3 °C; [α]_D -25.0° (*c* 0.40 MeOH); IR (ATR) ν / cm^{-1} 3366, 3083 (NH.HCl amidine), 1742 (C=O ester), 1672 (C=N); ^1H NMR (400 MHz, DMSO- d_6) δ 13.76 (br s, 1H, H benzimidazole), 9.45 (br s, 3H, H amidine), 8.08 (s, 1H, H-18), 7.91 (s, 1H, H-11), 7.81 (d, *J* 8.4 Hz, 1H, H-9), 7.73 (br s, 1H, H-15), 7.51 (d, *J* 7.6 Hz, 1H, H-16), 7.22 (d, *J* 8.4 Hz, 1H, H-8), 5.44 (d, *J* 8.0 Hz, 1H, H-1), 5.26 (t, *J* 9.6 Hz, 1H, H-3),

5.22 (d, *J* 3.2 Hz, 1H, H-4'), 5.15 (dd, 2J 10.0 Hz, 3J 3.4 Hz, 1H, H-3'), 4.99 (t, *J* 9.0 Hz, 1H, H-2), 4.84 (t, *J* 9.0 Hz, 1H, H-2'), 4.76 (d, *J* 8.0 Hz, 1H, H-1'), 4.35 (d, 2J 10.4 Hz, 1H, H-6), 4.23-4.20 (m, 1H, H-5'), 4.14-3.97 (m, 5H, H-5, H-6', H-6'', H-6''', H-21), 3.91-3.86 (m, 1H, H-4), 3.82 (s, 3H, OCH₃), 2.04-1.88 (s, 21H, COCH₃), 1.28 (d, *J* 6.4 Hz, 6H, H-22); ^{13}C NMR (100 MHz, DMSO- d_6) δ 170.1-169.0, 162.4, 149.7, 147.6, 124.8, 119.3, 117.2, 109.4, 99.8, 97.8, 76.1, 72.1, 71.9, 70.9, 70.2, 69.6, 68.9, 67.0, 62.0, 60.8, 56.1, 44.9, 21.2, 20.6-20.2; HRMS (ESI) *m/z*, calcd. for C₄₄H₅₅O₁₉N₄ [M + H]⁺: 943.3455, found: 943.3474.

2-[4-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- β -*D*-glucopyranosyloxy)-3-methoxyphenyl]benzimidazole-5-[*N*-(isopropyl)carboxamidine] hydrochloride (**22**)

This product was obtained in 80% yield as a brown solid after purified by chromatography (dichloromethane/methyl alcohol 87:13); mp 221.3-223.0 °C; [α]_D -19.0° (*c* 0.42 MeOH); IR (ATR) ν / cm^{-1} 3296, 3098 (NH.HCl amidine), 1729 (C=O ester), 1663 (C=N); ^1H NMR (400 MHz, DMSO- d_6) δ 13.76 (br s, 1H, NH benzimidazole), 9.46 (br s, 3H, H amidine), 8.13 (d, 1H, NH amide, *J* 8.8 Hz), 8.01 (s, 1H, H-18), 7.94 (s, 1H, H-11), 7.86 (d, *J* 8.4 Hz, 1H, H-9), 7.75 (s, 1H, H-15), 7.55 (d, *J* 8.0 Hz, 1H, H-16), 7.32 (d, *J* 8.4 Hz, 1H, H-8), 5.47 (d, *J* 8.4 Hz, 1H, H-1), 5.27 (t, *J* 9.8 Hz, 1H, H-3), 4.95 (t, *J* 9.6 Hz, 1H, H-4), 4.23 (dd, 2J 12.0 Hz, 3J 4.8 Hz, 1H, H-6), 4.15-3.98 (m, 4H, H-6', H-5, H-2, H-21), 3.89 (s, 3H, OCH₃), 2.03-1.90 (3s, 9H, OCOCH₃), 1.86 (s, 3H, NHCOCH₃), 1.28 (d, *J* 6.4 Hz, 6H, H-22); ^{13}C NMR (100 MHz, DMSO- d_6) δ 169.9, 169.6-169.3, 162.4, 149.6, 147.8, 124.3, 119.7, 116.8, 97.9, 72.3, 70.9, 68.3, 61.6, 56.2, 53.3, 44.9, 22.6, 21.3, 20.5-20.3; HRMS (ESI) *m/z*, calcd. for C₃₂H₃₄O₁₀N₅ [M + H]⁺: 654.2770, found: 654.2799.

General procedure for the synthesis of deacetylated benzamides (**23-30**)

The peracetylated benzamidine (**15-22**; 0.5 mmol) was solubilized in a solution of KOH in MeOH (20 mL, 1.0 mol L⁻¹) and the solution was stirred at 0 °C for 30 min. After the completion of the reaction, as observed by TLC, the mixture was neutralized with IRA-120 resin at 0 °C. The resin was filtered off and washed with methanol. The collected filtrate was concentrated in vacuum to afford the deacetylated derivatives.

2-[4-(β -*D*-Glucopyranosyloxy)-3-methoxyphenyl]benzimidazole-5-carboxamidine (**23**)

This product was obtained in 100% yield as a brown solid; mp 214.5-216.8 °C; [α]_D -15.3° (*c* 0.52 MeOH);

IR (ATR) ν / cm^{-1} 3132 (OH), 1675 (C=N); ^1H NMR (400 MHz, DMSO- d_6) δ 13.66 (br s, 1H, NH benzimidazole), 9.31 (s, 2H, H amidine), 9.04 (s, 2H, H amidine), 8.11 (s, 1H, H-18), 7.89 (s, 1H, H-11), 7.85 (d, J 8.4 Hz, 1H, H-9), 7.74 (d, J 8.0 Hz, 1H, H-15), 7.64 (d, J 8.0 Hz, 1H, H-16), 7.24 (d, J 8.8 Hz, 1H, H-8), 5.03 (d, J 6.4 Hz, 1H, H-1), 3.82 (s, 1H, OCH₃), 3.66 (d, J 11.2 Hz, 1H, H-6), 3.60-3.17 (m, 5H, H-2, H-3, H-4, H-5 and H-6'); ^{13}C NMR (100 MHz, DMSO- d_6) δ 166.1, 149.1, 148.6, 122.7, 121.8, 121.0, 119.9, 115.2, 110.8, 99.6, 77.0, 76.8, 73.1, 69.6, 60.6, 55.8; HRMS (ESI) m/z , calcd. for C₂₁H₂₅O₇N₄ [M + H]⁺: 445.1718, found: 445.1797.

2-[4-(β -D-Galactopyranosyloxy)-3-methoxyphenyl]benzimidazole-5-carboxamide (**24**)

This product was obtained in 100% yield as a brown solid; mp 216.5-219.0 °C; $[\alpha]_D -55.0^\circ$ (c 0.40 MeOH); IR (ATR) ν / cm^{-1} 3156 (OH), 1673 (C=N); ^1H NMR (400 MHz, DMSO- d_6) δ 13.58 (br s, 1H, NH benzimidazole), 9.30 (s, 2H, H amidine), 8.99 (s, 2H, H amidine), 8.11 (br s, 1H, H-18), 7.87 (s, 1H, H-11), 7.78 (d, J 8.4 Hz, 1H, H-9), 7.73 (br s, 1H, H-15), 7.63 (d, J 8.0 Hz, 1H, H-16), 7.24 (d, J 8.8 Hz, 1H, H-8), 5.12 (br s, 1H, OH), 4.99 (d, J 7.6 Hz, 1H, H-1), 4.65 (br s, 1H, OH), 4.54 (br s, 1H, OH), 3.89 (s, 1H, OCH₃), 3.71-3.33 (m, 6H, H-2, H-3, H-4, H-5, H-6 and H-6'); ^{13}C NMR (100 MHz, DMSO- d_6) δ 166.1, 149.2, 148.6, 122.7, 121.8, 119.8, 115.2, 110.8, 100.2, 75.5, 73.5, 70.1, 68.0, 60.2, 55.8; HRMS (ESI) m/z , calcd. for C₂₁H₂₅O₇N₄ [M + H]⁺: 445.1718, found: 445.1763.

2-[4-(β -D-Lactosyloxy)-3-methoxyphenyl]benzimidazole-5-carboxamide (**25**)

This product was obtained in 100% yield as a brown solid; mp 225.6-228.0 °C; $[\alpha]_D -68.1^\circ$ (c 0.44 MeOH); IR (ATR) ν / cm^{-1} 3198 (OH), 1674 (C=N); ^1H NMR (400 MHz, DMSO- d_6) δ 9.33 (s, 2H, H amidine), 9.00 (s, 2H, H amidine), 8.13 (s, 1H, H-18), 7.91 (s, 1H, H-11), 7.82 (d, J 8.4 Hz, 1H, H-9), 7.77 (d, J 8.0 Hz, 1H, H-15), 7.66 (d, J 8.4 Hz, 1H, H-16), 7.28 (d, J 8.4 Hz, 1H, H-8), 5.41 (d, J 7.6 Hz, 1H, H-1), 4.26 (d, J 7.6 Hz, 1H, H-1'), 3.91 (s, 1H, OCH₃), 3.64-3.38 (m, 12H, H-2, H-2', H-3, H-3', H-4, H-4', H-5, H-5', H-6, H-6', H-6'' and H-6'''); ^{13}C NMR (100 MHz, DMSO- d_6) δ 166.2, 149.3, 148.7, 122.1, 120.3, 115.5, 111.2, 104.0, 99.2, 80.3-68.5, 60.5-60.2, 56.0; HRMS (ESI) m/z , calcd. for C₂₇H₃₅O₁₂N₄ [M + H]⁺: 607.2246, found: 607.2265.

2-[4-(2-Acetamido-2-deoxy- β -D-glucopyranosyloxy)-3-methoxyphenyl]benzimidazole-5-carboxamide (**26**)

This product was obtained in 100% yield as a brown solid; mp 211.7-213.7 °C; $[\alpha]_D -9.5^\circ$ (c 0.42 MeOH);

IR (ATR) ν / cm^{-1} 3241 (OH), 1681 (C=N); ^1H NMR (400 MHz, DMSO- d_6) δ 9.41 (s, 2H, H amidine), 9.10 (s, 2H, H amidine), 8.17 (s, 1H, H-18), 8.01 (s, 1H, H-11), 7.91-7.82 (m, 3H, H-9, H-15 and NH), 7.73 (d, J 8.4 Hz, 1H, H-16), 7.33 (d, J 8.8 Hz, 1H, H-8), 5.17 (d, J 8.4 Hz, 1H, H-1), 3.89 (s, 3H, OCH₃), 3.74-3.20 (m, 6H, H-2, H-3, H-4, H-5, H-6 and H-6'), 1.81 (s, 1H, NHCOCH₃); ^{13}C NMR (100 MHz, DMSO- d_6) δ 169.2, 165.8, 149.7, 123.3, 121.1, 117.2, 112.4, 99.6, 77.9, 74.5, 70.7, 61.2, 57.1, 56.1, 23.6; HRMS (ESI) m/z , calcd. for C₂₃H₂₈O₇N₅ [M + H]⁺: 486.1983, found: 486.1987.

2-[4-(β -D-Glucopyranosyloxy)-3-methoxyphenyl]benzimidazole-5-[*N*-(isopropyl)carboxamide] (**27**)

This product was obtained in 100% yield as a brown solid; mp 206.5-209.0 °C; $[\alpha]_D -63.6^\circ$ (c 0.44 MeOH); IR (ATR) ν / cm^{-1} 3123 (OH), 1669 (C=N); ^1H NMR (400 MHz, DMSO- d_6) δ 7.99 (s, 1H, H-18), 7.92 (s, 1H, H-11), 7.82 (d, J 8.8 Hz, 1H, H-9), 7.74 (d, J 8.4 Hz, 1H, H-15), 7.53 (d, J 8.4 Hz, 1H, H-16), 7.26 (d, J 8.4 Hz, 1H, H-8), 5.31 (br s, 1H, OH), 5.14 (br s, 1H, OH), 5.05 (d, J 7.2 Hz, 1H, H-1), 4.58 (br s, 1H, OH), 4.11-4.08 (m, 1H, H-21), 3.91 (s, 1H, OCH₃), 3.68 (d, J 10.8 Hz, 1H, H-6), 3.48-3.16 (m, 5H, H-2, H-3, H-4, H-5 and H-6'), 1.30 (d, J 6.4 Hz, 6H, H-22); ^{13}C NMR (100 MHz, DMSO- d_6) δ 162.2, 154.1, 148.9, 148.2, 122.8, 122.2, 121.6, 119.6, 115.0, 110.6, 99.4, 76.8, 76.6, 72.9, 69.4, 60.4, 55.6, 44.7, 21.1; HRMS (ESI) m/z , calcd. for C₂₄H₃₁O₇N₄ [M + H]⁺: 487.2187, found: 487.2229.

2-[4-(β -D-Galactopyranosyloxy)-3-methoxyphenyl]benzimidazole-5-[*N*-(isopropyl)carboxamide] (**28**)

This product was obtained in 96% yield as a brown solid; mp 203.7-205.8 °C; $[\alpha]_D -47.6^\circ$ (c 0.42 MeOH); IR (ATR) ν / cm^{-1} 3122 (OH), 1669 (C=N); ^1H NMR (400 MHz, DMSO- d_6) δ 13.72 (s, 1H, H benzimidazole), 9.51 (s, 1H, H amidine), 9.38 (s, 1H, H amidine), 8.99 (s, 1H, H amidine), 8.00 (s, 1H, H-18), 7.93 (s, 1H, H-11), 7.82 (d, J 8.4 Hz, 1H, H-9), 7.74 (br s, 1H, H-15), 7.54 (d, J 8.4 Hz, 1H, H-16), 7.27 (d, J 9.2 Hz, 1H, H-8), 5.11 (br s, 1H, OH), 5.02 (d, J 8.0 Hz, 1H, H-1), 4.68 (br s, 2H, OH), 4.10-4.09 (m, 1H, H-21), 3.98 (s, 1H, OCH₃), 3.66-3.54 (m, 6H, H-2, H-3, H-4, H-5, H-6 and H-6'), 1.31 (d, J 6.4 Hz, 6H, H-22); ^{13}C NMR (100 MHz, DMSO- d_6) δ 162.3, 149.0, 148.5, 122.6, 121.7, 119.7, 115.1, 110.7, 100.0, 75.4, 73.4, 70.0, 67.9, 60.1, 55.7, 44.8, 21.2; HRMS (ESI) m/z , calcd. for C₂₄H₃₁O₇N₄ [M + H]⁺: 487.2187, found: 487.2194.

2-[4-(β -D-Lactosyloxy)-3-methoxyphenyl]benzimidazole-5-[*N*-(isopropyl)carboxamide] (**29**)

This product was obtained in 100% yield as a yellow solid; mp 213.4-215.8 °C; $[\alpha]_D -80.0^\circ$ (c 0.40 MeOH);

IR (ATR) ν / cm^{-1} 3232 (OH), 1668 (C=N); ^1H NMR (400 MHz, DMSO- d_6) δ 9.33 (br s, 2H, H amidine), 9.00 (s, 2H, H amidine), 7.99 (s, 1H, H-18), 7.92 (s, 1H, H-11), 7.82 (d, J 8.4 Hz, 1H, H-9), 7.74 (d, J 8.4 Hz, 1H, H-15), 7.52 (d, J 8.0 Hz, 1H, H-16), 7.27 (d, J 8.4 Hz, 1H, H-8), 5.48 (br s, 1H, OH), 5.32 (d, J 7.6 Hz, 1H, H-1), 5.11 (br s, 1H, OH), 4.82-4.55 (m, 5H, OH), 4.26 (d, J 6.4 Hz, 1H, H-1'), 4.10-4.07 (m, 1H, H-21), 3.91 (s, 1H, OCH₃), 3.74-3.34 (m, 12H, H-2, H-2', H-3, H-3', H-4, H-4', H-5, H-5', H-6, H-6', H-6'' and H-6'''), 1.30 (d, J 6.0 Hz, 6H, H-22); ^{13}C NMR (100 MHz, DMSO- d_6) δ 162.4, 154.2, 149.1, 148.2, 123.1, 122.4, 121.8, 119.7, 115.1, 110.8, 103.8, 99.1, 80.1-68.1, 60.3-60.0, 55.8, 44.9, 21.3; HRMS (ESI) m/z , calcd. for C₃₀H₄₁O₁₂N₄ [M + H]⁺: 649.2715, found: 649.2742.

2-[4-(2-Acetamido-2-deoxy- β -D-glycopyranosyloxy)-3-methoxyphenyl]benzimidazole-5-[N-(isopropyl)carboxamidine] (**30**)

This product was obtained in 95% yield as a yellow solid; mp 201.3-203.5 °C; $[\alpha]_D^{25}$ -23.8° (*c* 0.42 MeOH); IR (ATR) ν / cm^{-1} 3234 (OH), 1668 (C=N); ^1H NMR (400 MHz, DMSO- d_6) δ 7.99 (s, 1H, H-18), 7.91-7.87 (m, 2H, H-11 and NH), 7.82 (d, J 8.4 Hz, 1H, H-9), 7.73 (d, J 8.4 Hz, 1H, H-15), 7.52 (d, J 8.4 Hz, 1H, H-16), 7.28 (d, J 8.4 Hz, 1H, H-8), 5.13-5.11 (m, 2H, H-1 and OH), 4.66 (br s, 1H, OH), 4.10-4.05 (m, 1H, H-21), 3.74 (s, 1H, OCH₃), 3.71-3.20 (m, 6H, H-2, H-3, H-4, H-5, H-6 and H-6'), 1.82 (s, 1H, COCH₃), 1.30 (d, J 6.0 Hz, 6H, H-22); ^{13}C NMR (100 MHz, DMSO- d_6) δ 169.2, 162.4, 154.3, 149.6, 148.8, 124.0, 122.5, 121.7, 119.9, 116.7, 116.6, 99.3, 77.4, 73.9, 70.3, 60.7, 56.3, 55.6, 44.9, 23.0, 21.4; HRMS (ESI) m/z , calcd. for C₂₆H₃₄O₇N₅ [M + H]⁺: 528.2453, found: 528.2458.

General procedure for the synthesis of benzamidines (**31-32**)

To a solution of 3,4-dimethoxybenzaldehyde (1 equiv.) in ethanol (35 mL), it was added the corresponding 3,4-diaminobenzamidine (**4** or **6**) (1 equiv.) and *p*-benzoquinone (1 equiv.). The mixture was heated at 70 °C for 4 h, when the completion of reaction was observed by TLC. The solution was concentrated to residue and the pure product was obtained by CCS.

2-(3,4-Dimethoxyphenyl)-1*H*-benzimidazole-5-carboxamidine hydrochloride (**31**)

This product was obtained in 69% yield as a brown solid; mp 170.5-173.3 °C; IR (ATR) ν / cm^{-1} 3297, 3060 (NH.HCl amidine), 1686 (C=N); ^1H NMR (400 MHz,

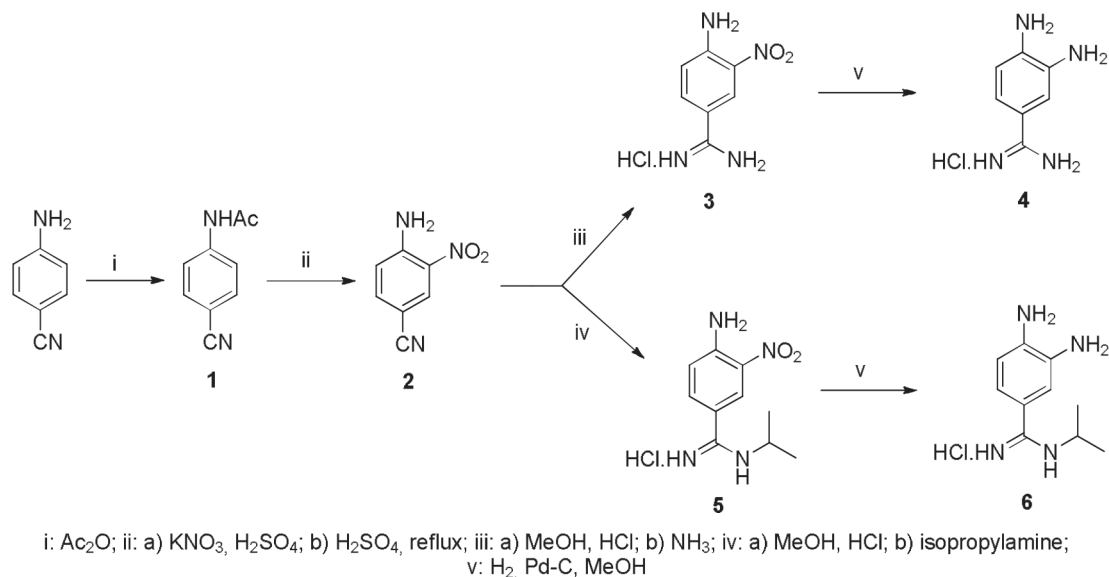
DMSO- d_6) δ 13.78 (s, 1H, NH benzimidazole), 9.39, 9.33 (s, 2H, H amidine), 9.12 (s, 2H, H amidine), 8.04 (s, 1H, H-9), 7.93-7.80 (m, 2H, H-11, H-12), 7.70-7.63 (m, 2H, H-3, H-5), 7.16 (d, J 8.4 Hz, 1H, H-6), 3.91 (s, 3H, OCH₃), 3.85 (s, 3H, OCH₃); ^{13}C NMR (100 MHz, DMSO- d_6) δ 166.2, 154.2, 150.8, 148.9, 143.3, 139.0, 121.8, 121.4, 120.9, 111.8, 111.5, 55.7, 55.6; HRMS (ESI) m/z , calcd. for C₁₆H₁₇O₂N₄ [M + H]⁺: 297.1346, found: 297.1353.

2-(3,4-Dimethoxyphenyl)-*N*-isopropyl-1*H*-benzimidazole-5-carboxamidine hydrochloride (**32**)

This product was obtained in 70% yield as a brown solid, mp 165.2-168.0 °C; IR (ATR) ν / cm^{-1} 3074 (NH.HCl amidine), 1668 (C=N); ^1H NMR (400 MHz, DMSO- d_6) δ 13.91 (s, 1H, NH benzimidazole), 9.55-9.39 (m, 2H, H amidine), 9.05 (s, 1H, H amidine), 8.06 (s, 1H, H-9), 7.96-7.87 (m, 2H, H-3, H-11), 7.69 (d, J 8.4 Hz, 1H, H-12), 7.54 (dd, 3J 19.2 Hz, 4J 8 Hz, 1H, H-5), 7.15 (d, J 8.8 Hz, 1H, H-6), 4.16-4.11 (m, 1H, H-15), 3.91 (s, 3H, OCH₃), 3.85 (s, 3H, OCH₃), 1.31 (d, J 6.4 Hz, H-16); ^{13}C NMR (100 MHz, DMSO- d_6) δ 162.4, 154.0, 150.8, 148.9, 143.2, 138.5, 122.3, 122.0, 121.6, 111.8, 111.5, 55.7, 55.6, 44.9, 21.3; HRMS (ESI) m/z , calcd. for C₁₉H₂₃O₂N₄ [M + H]⁺: 339.1816, found: 339.1833.

Antifungal and antibacterial activities evaluation

The benzamidines and starting glycosides were evaluated *in vitro* for their antibacterial and antifungal activities and the inhibitory concentrations of microbial growth were determined at 50% (IC₅₀) and 90% (IC₉₀) in $\mu\text{mol mL}^{-1}$ and compared among the microorganisms. The tests were all done in duplicates. The antifungal activity were performed according to microdilution methodology in RPMI 1640 broth supplemented with 2% glucose as document E.DEF 7.3.1.¹¹ The determination of antibacterial activity were performed according to microdilution methodology in Mueller Hinton broth adjusted with cations as described by document ISO 20776-1:2006.¹²⁻¹⁴ The stock solutions of all the compounds were prepared in DMSO 1% at final concentration and tested at concentrations from 500 to 0.98 $\mu\text{g mL}^{-1}$. The standard drug fluconazole was applied as control of fungistatic action at concentrations from 64 to 0.031 g mL^{-1} and the standard drug chloramphenicol as a control of bacteriostatic action at concentrations from 125 to 0.06 g mL^{-1} . The microplates were incubated at 35 °C for 24 h for bacteria and 37 °C and for 24 h for fungi. Results were visualized and analyzed at 530 nm in an Anthos Zenyth 200rt Microplate Reader.



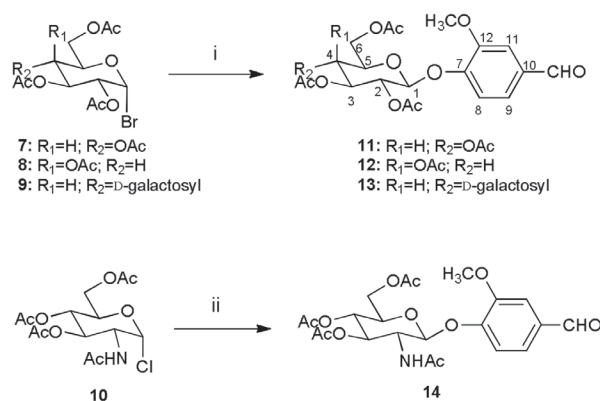
Scheme 2. Synthesis of 3,4-diaminobenzamides **4** and **6**.

Results and Discussion

Chemistry

The preparation of 3,4-diaminobenzamides (**4** and **6**) was performed in good yields from the nitrobenzimidines **3** or **5** as described by Fairley *et al.*¹⁵ and reproduced by Göker *et al.*⁹ The 4-amino-3-nitrobenzonitrile (**2**) was converted to the corresponding imidate by reaction with anhydrous methanol in the presence of HCl_(g). The reaction of this imidate with ammonia or isopropylamine afforded the nitroamidines **3** (60% yield) and **5** (63% yield), which upon catalytic hydrogenation, provided the 3,4-diaminobenzamides **4** (93% yield) and **6** (98% yield), respectively, as hydrochloride salts (Scheme 2).

The already described glycosides **11-13** were prepared from the reaction between the corresponding glycosyl bromide (**7**, **8** or **9**) with 4-hydroxy-3-methoxybenzaldehyde (vanillin) in acetone and LiOH according to the method described by Conchie *et al.*¹⁶ and reproduced by Souza *et al.*¹⁰ The reaction of **10**, prepared as described by Horton¹⁷ with vanillin in acetonitrile, in the presence of potassium carbonate and PEG 4000, afforded the glycoside **14** (Scheme 3). All glycosides were obtained in yields higher than 52% after purification as β-anomers, as confirmed by the H-1 coupling constants around 8 Hz in the corresponding ¹H NMR spectra. Besides, one observes a singlet at around 9.8 ppm of each compound corresponding to the aldehyde proton. Their infrared spectra showed bands relatives to the ester and aldehyde carbonyl groups near 1750 and 1690 cm⁻¹, respectively.

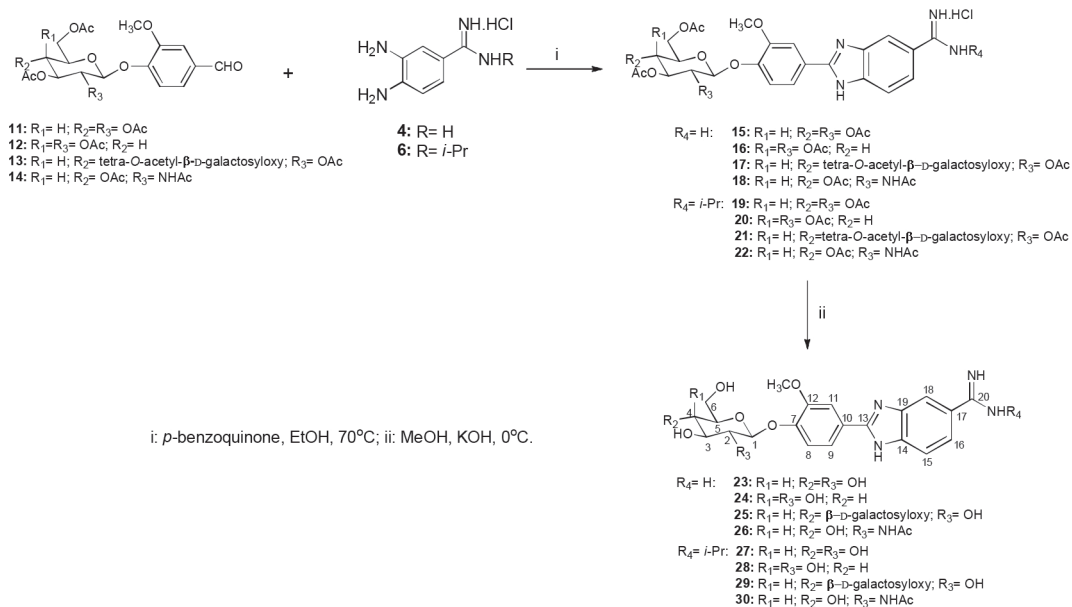


i: vanillin, LiOH, acetone; ii: vanillin, K₂CO₃, PEG 4000, acetonitrile

Scheme 3. Synthesis of glycosides **11-14**.

The reaction of 3,4-diaminobenzamides (**4** or **6**) with glycosides **11-14** furnished the peracetylated 2-aryl-5-amidinobenzimidazoles **15-22** which upon deacetylation conditions afforded the derivatives **23-30**, as depicted in Scheme 4. The derivatives **15-30** were synthesized for the first time.

There are several methods to synthesize benzimidazoles described and most of them employ the condensation of an 3,4-diaminobenzene with carboxylic acids, esters, nitriles, acyl chlorides or aldehydes in presence of oxidizing agents such as nitric acid, nitrobenzene and quinones.¹⁸ As shown is Scheme 4, in the present work the peracetylated benzimidazoles **15-22** were obtained in good yields (52-80%) from the reaction of 3,4-diaminobenzamides **4** or **6** with glycosylated aldehydes **11-14**, using *para*-benzoquinone as oxidizing agent. Deacetylation of peracetylated derivatives in methanolic solution of potassium hydroxide¹⁰ provided



Scheme 4. Synthesis of glycosylated 2-aryl-5-amidinobenzimidazoles **15-30**.

the deacetylated benzamidines **23-30** in yields higher than 95%. In the proton NMR spectra of compounds **23-26** it was observed two signals between 9.0-9.4 ppm corresponding to the three amidine protons. For derivatives **27-30** the signals of the two amidine protons are observed around 9.4 ppm. The imidazole protons of all benzamidines were registered as broad signals near 13 ppm.

The assignment of the signals to the protons in ¹H NMR spectra of the compounds was possible by using heteronuclear multiple-bond correlation (HMBC) and correlation spectroscopy (COSY) experiments. As exemplified for peracetylated benzamidine **20**, a correlation was observed between C-20 and aromatic H-16 in the HMBC experiment, which unequivocally confirmed the identity of H-16, registered as a doublet (Figure 1a). From the assignment of H-16, its correlation with H-15 can be observed in the COSY experiment (Figure 1b). The correlation between C-13 and H-11 also confirmed the identity of this aromatic proton as a singlet (Figure 1a). These observations are in agreement with the identity of the 2-aryl-5-amidinobenzimidazole system.¹⁹

Finally, for comparative purposes, the reaction of 3,4-diaminobenzamidines **4** or **6** with 3,4-dimethoxybenzaldehyde (in the conditions previously shown) afforded the corresponding derivatives devoid of the saccharide units, as shown in Scheme 5 below.

In vitro assays

All benzamidines, as well as the starting glycosides, were evaluated against different species of fungi

(*Candida albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata* and *C. parapsilosis*) and bacteria (*Escherichia coli*, *Enterococcus faecalis*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Staphylococcus aureus*) by the microdilution method and the results were estimated using the inhibitory concentration that was able to inhibit microbial growth at 50% (IC₅₀; fungistatic and bacteriostatic activities) and at 90% (IC₉₀; fungicide and bactericidal).¹¹⁻¹⁴

Regarding the antifungal potential observed for the compounds, the peracetylated glucoside **15** and galactoside **16** derivatives were active against *C. parapsilosis* at 96.4 μmol L⁻¹, suggesting that the benzamidine group is important for the activity, since the starting peracetylated glucoside **11** and galactoside **12** were inactive at the highest concentration evaluated, as shown in Table 1. In addition, the presence of the peracetylated saccharide units in **15** or **16** also contributed to the antifungal potential of these compounds, since the corresponding derivative **31** (devoid of a saccharide moiety) was also inactive against this strain. The benzamidine derivative **15** also showed a moderate activity against other *Candida* spp. evaluated at 192.8 μmol L⁻¹ (Table 1). Considering *C. glabrata*, this trend was not observed, since derivative **31** was two-fold more active than benzamidine derived from *D*-glucose (**15**). Among isopropyl benzamidines, any saccharide unit contributed negatively for antifungal activity of these derivatives, since only derivative **32** was active against *C. parapsilosis* (IC₅₀ 83.5 μmol L⁻¹) and *C. tropicalis* (IC₅₀ 167 μmol L⁻¹).

The benzamidine nucleus also contributed to the antibacterial activity of the synthesized series, and the

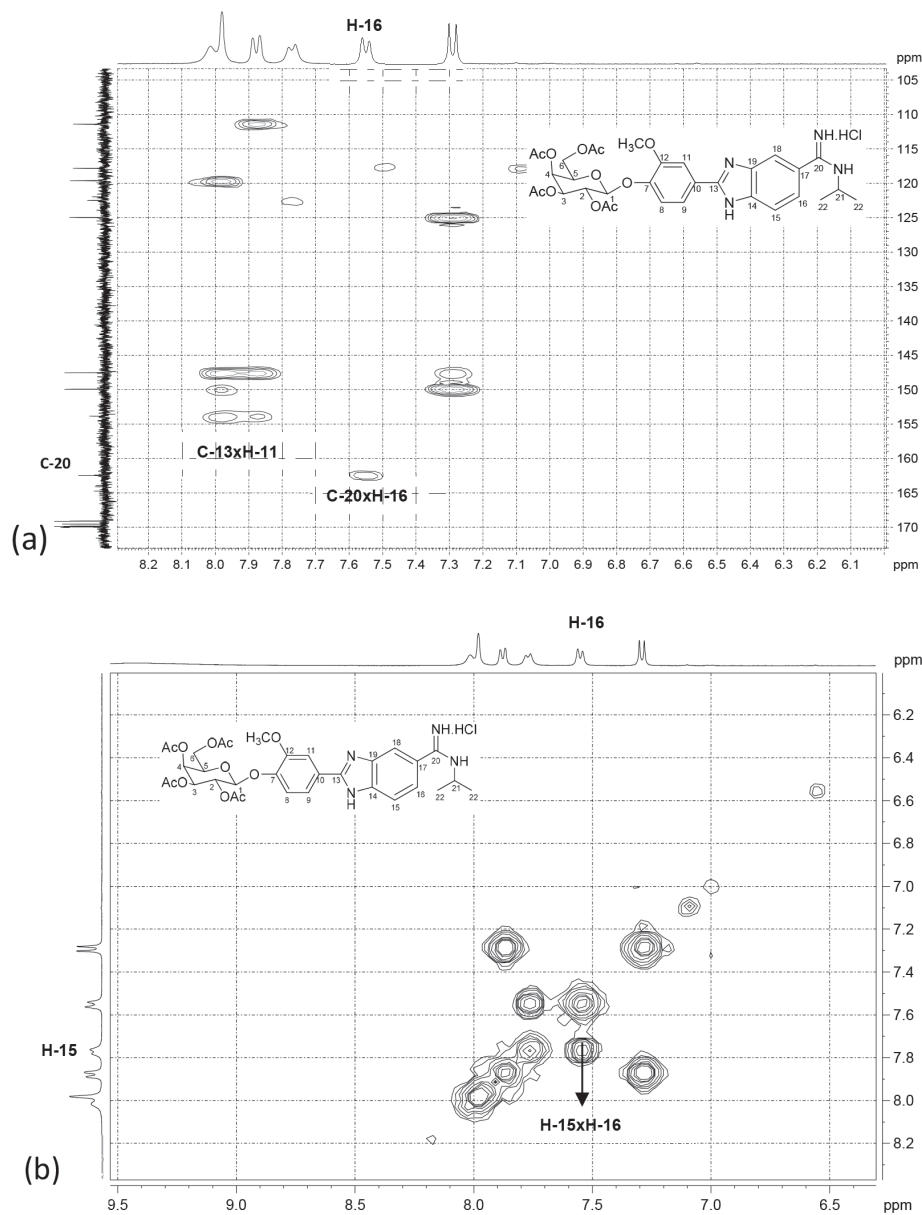
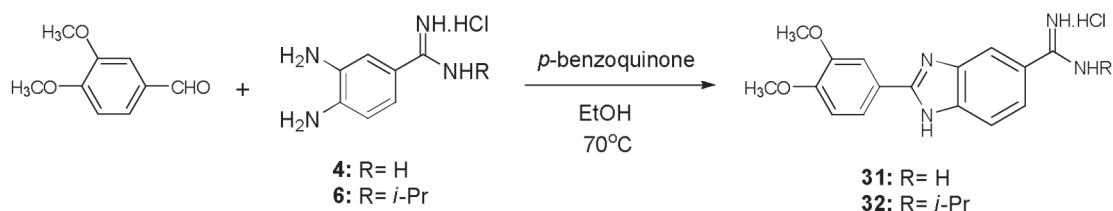


Figure 1. Correlations between H-16 and C-20 by HMBC (a) and between H-15 and H-16 by COSY (b) for compound **20**.



Scheme 5. Synthesis of 2-aryl-5-amidinobenzimidazoles **31** and **32**.

compounds that showed the best potential were peracetylated galactoside **16** (IC₅₀ 96.4 μmol L⁻¹ against *M. luteus*), *N*-acetylglucosamine glycoside **18** (IC₅₀ 96.5 μmol L⁻¹ against *E. faecalis*), glucoside **19** (IC₅₀ 90.5 μmol L⁻¹ against Gram-negative *E. coli*) and deacetylated lactoside **29** (IC₅₀ 96.4 μmol L⁻¹ against *E. faecalis*), as shown in

Table 2. Interestingly, all starting glycosides (**11-14**) were inactive against the evaluated bacterial strains, suggesting the importance of the benzimidine nucleus for the activity of these compounds. Regarding *E. coli* and *E. faecalis*, the presence of a saccharide moiety attached to the benzimidine nucleus was essential for the activity observed for the

Table 1. *In vitro* antifungal activity of the synthesized compounds

Compound	IC ₅₀ (in mass / (µg mL ⁻¹)) / (µmol L ⁻¹)				IC ₉₀ (in mass / (µg mL ⁻¹)) / (µmol L ⁻¹)					
	<i>C. albicans</i> ATCC 10231	<i>C. tropicalis</i> ATCC 750	<i>C. krusei</i> ATCC 6258	<i>C. glabrata</i> ATCC 90030	<i>C. parapsilosis</i> ATCC 22019	<i>C. albicans</i> ATCC 10231	<i>C. tropicalis</i> ATCC 750	<i>C. krusei</i> ATCC 6258	<i>C. glabrata</i> ATCC 90030	<i>C. parapsilosis</i> ATCC 22019
11	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-
15	192.8 (125)	192.8 (125)	192.8 (125)	192.8 (125)	96.4 (62.5)	-	-	-	-	192.8 (125)
16	-	-	-	-	96.4 (62.5)	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-	-	-	-
27	-	-	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-	-	-
31	-	-	-	97.0 (31.25)	-	-	-	-	-	-
32	-	167.0 (62.5)	-	-	83.5 (31.25)	-	-	-	-	-
Fluc	1.6 (0.5)	3.2 (1.0)	104.4 (16.0)	52.2 (32.0)	3.2 (1.0)	-	-	-	-	-

IC₅₀: inhibitory concentrations of microbial growth at 50%; IC₉₀: inhibitory concentrations of microbial growth at 90%; -: no significant activity; fluc: fluconazole.

Table 2. *In vitro* antibacterial activity of the synthesized compounds

Compound	IC ₅₀ (in mass / (µg mL ⁻¹)) / (µmol L ⁻¹)					IC ₉₀ (in mass / (µg mL ⁻¹)) / (µmol L ⁻¹)						
	<i>Escherichia coli</i> ATCC 25922	<i>Enterococcus faecalis</i> ATCC 51299	<i>Micrococcus luteus</i> ATCC10240	<i>Pseudomonas aeruginosa</i> ATCC 27853	<i>Salmonella typhimurium</i> ATCC 14028	<i>Staphylococcus aureus</i> ATCC 6538	<i>Escherichia coli</i> ATCC 25922	<i>Enterococcus faecalis</i> ATCC 51299	<i>Micrococcus luteus</i> ATCC10240	<i>Pseudomonas aeruginosa</i> ATCC 27853	<i>Salmonella typhimurium</i> ATCC 14028	<i>Staphylococcus aureus</i> ATCC 6538
11	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-
16	192.8 (125)	-	96.4 (62.5)	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-	-	-
18	-	96.5 (62.5)	-	-	-	-	-	-	-	-	-	-
19	90.5 (62.5)	-	-	-	-	-	-	181.0 (125)	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	127.7 (125)	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-	-	-	-	-
24	-	140.7 (62.5)	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-	-	-	-	-	-
27	-	-	-	-	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	-	-	-	-	-
29	-	96.4 (62.5)	-	-	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-	-	-	-	-
31	-	-	97.0 (31.25)	-	-	194.0 (62.5)	-	-	-	-	-	-
32	-	-	83.5 (31.25)	-	-	-	-	-	-	-	-	167.0 (62.5)
chl	3.1 (1.0)	6.2 (2.0)	1.6 (0.5)	99.0 (32.0)	3.1 (1.0)	6.2 (2.0)	12.3 (4.0)	24.7 (98.0)	3.1 (1.0)	198.0 (64.0)	6.2 (2.0)	12.3 (4.0)

IC₅₀: inhibitory concentrations of microbial growth at 50%; IC₉₀: inhibitory concentrations of microbial growth at 90%; -: no significant activity; chl: chloramphenicol.

compounds mentioned, since the derivatives having no sugar units (**31** and **32**) were inactive against these two strains. On the other hand, considering *M. luteus*, the presence of carbohydrates did not contribute to the biological activity, since benzamidines **31** and **32**, devoid of a saccharide moiety, were the most active compounds, showing antibacterial action against this species at 97.0 and 83.5 $\mu\text{mol L}^{-1}$, respectively.

In view of these findings, the derivatives **15**, **16**, **18**, **19**, **29**, **31** and **32** can be considered for further molecular modifications for design of new agents with antimicrobial potential.

Conclusions

We described herein the synthesis of a new series of glycosylated 2-aryl-5-amidinebenzimidazoles. The compounds were obtained in good yields by coupling vanillin glycosides with 3,4-diaminobenzamidines and subsequent deacetylation. Two *N*-unsubstituted amidines derived from *D*-glucose (**15**) and *D*-galactose (**16**) showed antifungal activity against *C. parapsilosis* at 96.4 $\mu\text{mol L}^{-1}$ and the non-glycosylated amidines **31** and **32** showed activity against *C. glabrata* (IC_{50} 97 $\mu\text{mol L}^{-1}$) and *C. parapsilosis* (IC_{50} 83.5 $\mu\text{mol L}^{-1}$), respectively. In addition, derivatives **16**, **31** and **32** were actives against *M. luteus* in the range of 83-97 $\mu\text{mol L}^{-1}$, derivatives **18** and **29** showed activity against *E. faecalis* at 96 $\mu\text{mol L}^{-1}$ and compound **19** inhibited growth of *E. coli* at 90.5 $\mu\text{mol L}^{-1}$.

Supplementary Information

Supplementary information (^1H , ^{13}C NMR and HRMS spectra of the synthesized compounds) is available free of charge at <http://jbc.org.br> as PDF file.

Acknowledgments

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