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Article

Synthesis of novel papulacandin D analogs and evaluation of their antifungal potential

Ana Carolina Oliveira Bretas[1], Thiago Belarmino de Souza[1], Beatriz Borelli[2], Suzana Johan[2], Ricardo José Alves[1,*]

[1] Departamento de Produtos Farmacêuticos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [2]Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Systemic fungal infections are a growing problem in contemporary medicine and few drugs are licensed for therapy of invasive fungal infections. Differences between fungi and humans, like the presence of a cell wall in fungal cells, can be explored for designing new drugs. (1,3)-β-D-glucan synthase, an enzyme that catalyzes the synthesis of (1,3)-β-D-glucan, a structural and essential component of the fungal cell wall, is absent in mammals and this makes it an excellent target for the development of new antifungal agents. Papulacandins are a family of natural antifungal agents targeting (1,3)-β-D-glucan synthase. In this study we describe the synthesis and biological evaluation of two new Papulacandin analogs as potential (1,3)-β-D-glucan synthase inhibitors.

Keywords: β-(1,3)-D-glucan synthase. Antifungal activity. Papulacandin D. Molecular simplification.

INTRODUCTION

(1,3)-β-D-glucan synthase represents an important molecular target for the development of new antifungal drugs, as this enzyme is essential for fungi and is absent in mammalian cells. This enzyme catalyzes the synthesis of (1,3)-β-D-glucan, a structural component of the fungal cell wall. The inhibition of cell wall glucan synthesis leads to leakage of essential components of the fungal cell as a result of the high osmotic pressure causing cell lysis and death of the microorganism (Kaaden, Breukink, Pieters, 2012; Denmark, Kobayashi, Regens, 2010; Tomishima *et al*., 2008; Taft, Enderlin, Selitrennikoff, 1994).

Papulacandins constitute a family of natural antifungal agent inhibitors of (1,3)-β-D-glucan synthase whose isolation and characterization were initially reported by Traxler and coworkers (Traxler, Gruner, Auden, 1977). Papulacandins were isolated from the fermentation broth of yeast *Papularia spherosperma* and there are four representatives of this family: Papulacandin A, B, C and D, as shown in Figure 1 (Traxler, Gruner, Auden, 1977; Römmele, Traxler, Wehrli, 1983). Papulacandins A, B and C contain a disaccharide lactose connected with an aromatic ring *via* C-O and C-C bonds forming a spirocyclic system, and the sugar unit is esterified at positions C-3 and C-6' with unsaturated fatty acids, being the acid residue at C-3 constituted by 16 carbon atoms while that of C-6' residue is constituted by 10 carbon atoms. Papulacandin D is the simplest representative of the family and is derivative of a D-glucose unit and is devoid of a fatty acid at C-6' (Ahmed, O'Doherty, 2005; Traxler, Tosch, Zak, 1987). The papulacandins have demonstrated potent *in vitro* antifungal activity against several pathogenic fungi: *Candida albicans*, *Candida tropicalis*, *Microsporum canis, Geotrichum lactis, Saccharomyces cerevisae* and *Pneumocystis carinii* (Denmark, Kobayashi, Regens, 2010; Barret, Pena, Willardsen, 1996; Schmatz *et al*., 1990).

The papulacandin B is the most active compound considering that both cellular growth inhibition and inhibition of glucan biosynthesis and experiments against *Candida albicans* showed minimum inhibitory concentration (MIC= 0.1 $\mu g.mL^{-1}$) compared to amphotericin B, clotrimazole, and nystatin. Papulacandin D is the simplest product obtained from the culture

***Correspondence:** R. J. Alves. Departamento de Produtos Farmacêuticos, Faculdade de Farmácia, Universidade Federal de Minas Gerais. Avenida Antônio Carlos, 6627, 31.270-901 - Belo Horizonte, MG, Brasil. Tel: +55 31 3409 6955; Fax: + 55 31 3409 6935. E-mail: ricardodylan@farmacia.ufmg.br

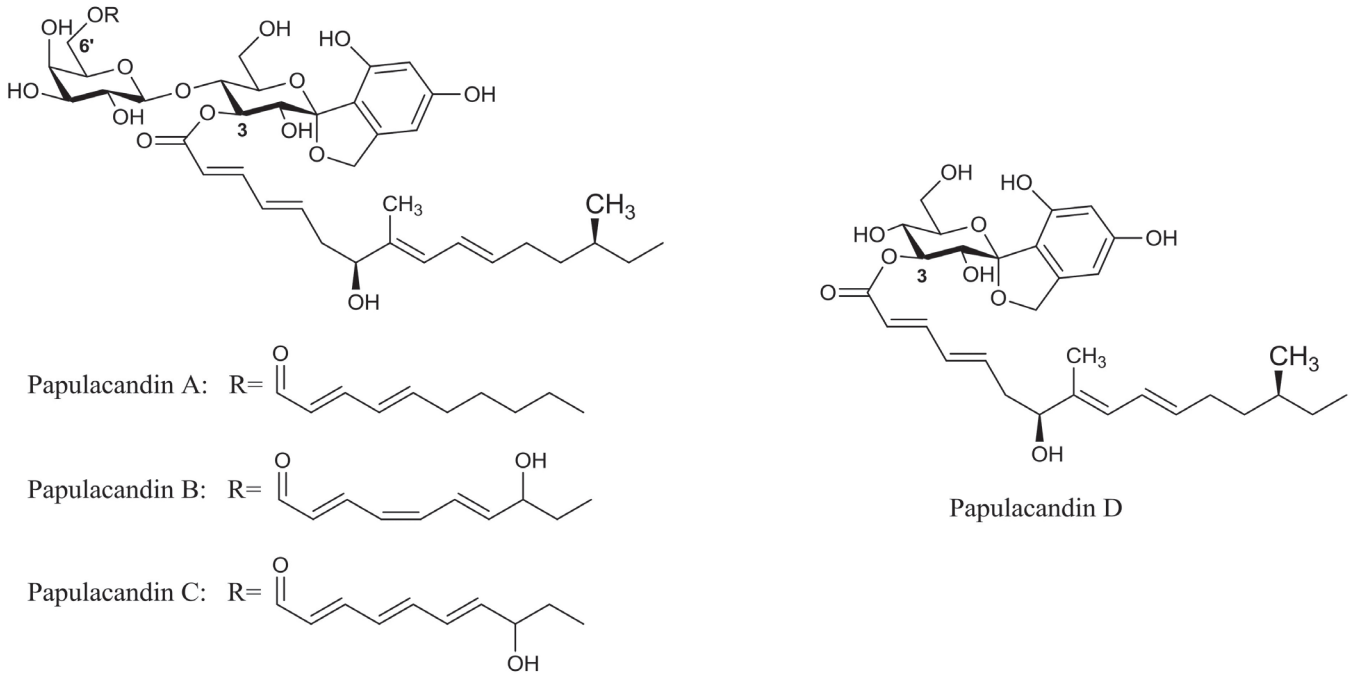


FIGURE 1 - Chemical structures of papulacandins.

broth and the less potent derivative (MIC= 1~2 μg.mL^{-1}) among papulacandins (Traxler, Gruner, Auden, 1977; Römmele, Traxler, Wehrli, 1983). However, due to its simpler structure, its synthesis or chemical manipulation has attracted the interest of researchers both due to the synthetic challenge itself and the possibility of obtaining sufficient amounts of this substance for structure-activity relationship studies in the search for new potentially active analogs. The increasing incidence of fungal resistance to existing antifungal agents and their undesirable side effects reflect the need for the development of safer and more effective antifungal drugs. Recently, our research group reported the synthesis and antifungal evaluation of papulacandin D analogs acylated at C-3 with palmitic acid. The low antifungal activity showed by these synthesized compounds can be explained by the absence of the spirocyclic nucleus or by the total saturation of the C-3 acyl chain (Souza *et al*., 2015). Therefore, based on antifungal potential of papulacandins combined with the lower structural complexity of Papulacandin D, we planned and synthesized new analogs of this compound employing the molecular simplification method and using a more complex acyl chain, containing unsaturations and different functional groups. The establishment of a short and efficient synthetic route could allow obtaining two analogs (**1** and **2**) shown in Figure 2.

The proposed papulacandin D analogs **1** and **2** are classic aromatic *O*-glycosides and their synthesis rather than spiroglycoside present in the natural papulacandins will enable the evaluation of the real importance of the spiro ring for the antifungal activity of these compounds.

On the other hand, modifications in the acyl chain were also proposed and compared to the natural prototype. The introduction of aromatic rings in the side chain would give a greater conformational restriction to this group, which may lead to increased biological activity. Besides, these modifications can contribute to reducing the difficulties related to the geometric stereoisomerism and can offer the possibility of the introduction of substituents in the aromatic ring, in order to modulate physical and chemical properties. Finally, removal of the chiral centers of the fatty acid chains eliminates difficulties encountered in the stereocontrolled synthesis of chiral compounds.

MATERIAL AND METHODS

General procedures

Chemicals were obtained from commercial sources and used without further purification, unless stated otherwise. Melting points were measured on an MQAPF 301 Apparatus and were reported uncorrected. Specific rotations were measured on an ADP 220 Bellingham + Stanley Ltd. polarimeter. NMR spectra were recorded at 400 MHz (BRUKER *AVANCE* DRX 400) or 200 MHz (BRUKER *AVANCE* DRX 200) for ^{1}H and 100 MHz or 50 MHz for ^{13}C in $CDCl_3$ or DMSO-d_6 and were reported in ppm with TMS or residual solvent for internal standard.

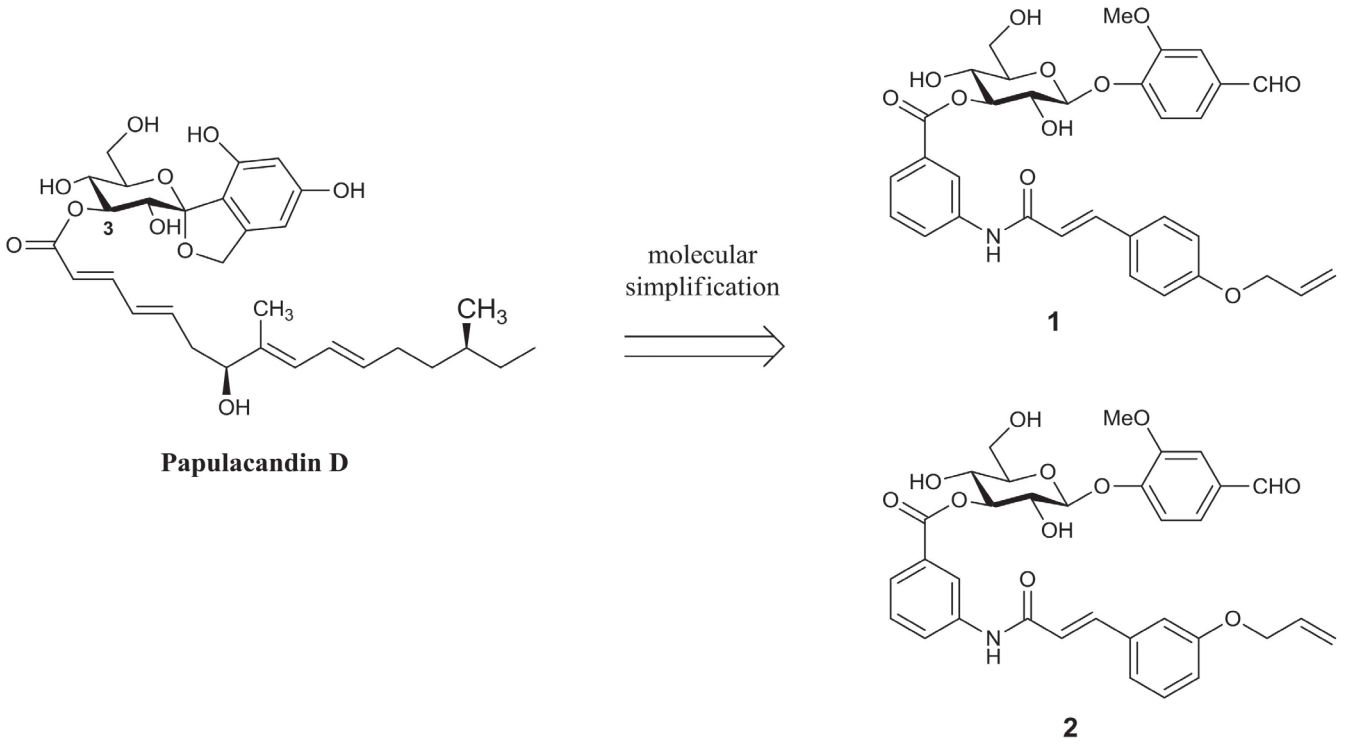


FIGURE 2 - Chemical structures of proposed new analogs of papulacandin D by molecular simplification.

Chemistry

Compounds **3** and **4** were synthesized according to the method previously described by Conchie, Levvy, and Marsh (1957) and compounds **10** and **11** were prepared following the procedure outlined by Tseng and coworkers (Tseng *et al*., 2011).

*Synthesis of 4-formyl-2-methoxyphenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**5**)*

To a solution of $LiOH.H_2O$ (1.30 g, 30 mmol) in H_2O was added an equimolar amount of 4-hidroxy-3-methoxybenzaldehyde and the mixture was stirred at room temperature for 15 min. Solution of bromide **4** (3.4 g, 10 mmol) in acetone was added dropwise. The course of the reaction was followed by TLC (Hexane/EtOAc 6:4) up until the glycosyl donor disappeared. The precipitate formed was filtered and washed exhaustively with H_2O to yield **5** (6.0 g, 54% yield) as a white solid: mp 134.3-137.8 °C (lit. (Mohri *et al*., 2003): 135-137 °C), $[\alpha]_D^{24}$ -40.0 (*c* 0.8, $CDCl_3$) (lit. (Sultana *et al*., 2006): -41.01 (*c* 0.63, $CDCl_3$)); ^{1}H NMR (200 MHz, $CDCl_3$) *δ*/ppm 9.90 (s, 1H, C<u>H</u>O), 7.42 (d, 2H, *J* 8.6 Hz, *o*C<u>H</u>-CHO), 7.24 (d, 1H, *J* 8.6 Hz, *m*C<u>H</u>-CHO), 5.34-5.09 (m, 4H, H-1, H-2, H-3 + H-4), 4.33-4.25 (m, 2H, H-6 + H-6'), 3.90- 3.82 (m, 4H, H-5 + OC<u>H</u>$_3$), 2.08, 2.05 (s, 12H, COOC<u>H</u>$_3$); ^{13}C NMR (50 MHz, $CDCl_3$) *δ*/ppm 190.9 (<u>C</u>HO), 170.5, 170.2, 169.3, 169.2 (<u>C</u>OOCH$_3$), 151.0 (<u>C</u>*ipso*), 150.9 (<u>C</u>*ipso*), 132.8 (<u>C</u>-CHO), 125.3 (*o*<u>C</u>H-CHO), 118.1 (*m*<u>C</u>H-CHO), 110.7 (*o*<u>C</u>H-CHO), 99.7 (C-1), 72.3 (C-4)•, 72.2 (C-3)•, 70.9 (C-2), 68.2 (C-5), 61.8 (C-6), 56.0 (O<u>C</u>H$_3$), 20.6, 20.5 (COO<u>C</u>H$_3$); • Interchangeable.

*Synthesis of 4-formyl-2-methoxyphenyl β-D-glucopyranoside (**6**)*

The peracetylated glucoside **5** (1.0 g, 2.1 mmol) was added to a solution of KOH (0.2 g, .3.6 mmol) in MeOH. The mixture was stirred at room temperature. After 2 h, excess Amberlite 120 IR ion exchange resin was added and the mixture was stirred for 5 min. After filtration, the solvent was evaporated to give **6** (0.64 g 98% yield) as a white solid: mp 180.3-185.7 °C (lit. (Reichel, Schickle, 1943): 185-187 °C); ^{1}H NMR (200 MHz, DMSO-d_6) *δ*/ppm 9.87 (s, 1H, C<u>H</u>O), 7.52 (dd, 1H, *J* 8.4 Hz, *J* 1.6 Hz, *o*C<u>H</u>-CHO), 7.44 (d, 1H, *J* 1.6 Hz, *o*C<u>H</u>-CHO), 7.28 (d, *J* 8.4 Hz, 1H, *m*-C<u>H</u>-CHO), 5.33 (s, 1H, O<u>H</u>), 5.10-5.05 (m, 3H, H-1 + 2xO<u>H</u>), 4.54 (s, 1H, O<u>H</u>), 3.89 (s, 3H, OC<u>H</u>$_3$), 3.67 (d, 1H, *J* 11.6 Hz, H-6), 3.48-3.18 (m, 5H, H-2, H-3, H-4, H-5 + H-6'); ^{13}C NMR (50 MHz, DMSO-d_6) *δ*/ppm 191.5 (<u>C</u>HO), 151.7 (<u>C</u>*ipso*), 149.3 (<u>C</u>*ipso*), 130.5 (<u>C</u>-CHO), 125.3 (*o*<u>C</u>H-CHO), 114.5 (*m*<u>C</u>H-CHO), 110.5 (*o*<u>C</u>H-CHO), 99.4 (C-1), 77.1 (C-4), 76.8 (C-3), 73.1 (C-2), 69.5 (C-5), 60.6 (C-6), 55.6 (O<u>C</u>H$_3$).

*Synthesis of 4-formyl-2-methoxyphenyl 4,6-O-benzylidene-β-D-glucopyranoside (**7**)*

$ZnCl_2$ (0.5 g, 3.7 mmol) was added to 5 mL of benzaldehyde and the mixture was stirred at room temperature for 15 min. Then the glycoside **6** (0.57 g, 1.8 mmol) was added. After the addition was complete the reaction mixture was stirred for 6 h and quenched with ice and petroleum ether. After vigorous shaking the glycoside **7** started to precipitate. The solid was filtrated and washed with cooled water and petroleum ether to give **7** (0.530 g, 72% yield) as a white solid: mp 176.6-179.2 °C, $[\alpha]_D^{24}$ -48.0 (*c* 1, DMSO); ^{1}H NMR (200 MHz, $CDCl_3$) *δ*/ppm9.87 (s, 1H, CHO), 7.56-7.39 (m, 8H, CH-aromatic), 5.70 (d, *J* 4.8 Hz, 1H, OH), 5.61 (s, 1H, CH-benzyl group), 5.52 (sl, 1H, OH), 5.35 (d, *J* 7.2 Hz, 1H, H-1), 4.22 (s, 1H, H-6), 3.85 (s, 3H, OCH_3), 3.69-3.38 (m, 5H, H-2, H-3, H-4, H-5 + H-6'); ^{13}C NMR (50 MHz, DMSO-d_6) *δ*/ppm 191.6 (CHO), 151.3 (C*ipso*), 149.4 (C*ipso*), 137.7 (C*ipso*), 130.8 (C-CHO), 128.9, 128.0, 126.4 (CH-aromatic), 125.2 (*o*CH-CHO), 114.7 (*m*CH-CHO), 110.7 (*o*CH-CHO), 100.7 (CH-benzyl group), 99.5 (C-1), 80.1 (C-4), 73.9 (C-3), 73.0 (C-2), 67.7 (C-6), 65.9 (C-5), 55.6 (OCH_3).

*Synthesis of 4-formyl-2-methoxyphenyl 3-O-[3-[(E-4-allyloxycinamoyl)amino]benzoyl]-4,6-O-benzylidene-β-D-glucopyranoside (**8**)*

To a solution of glycoside **7** (0.2 g, 0.5 mmol) in CH_2Cl_2 were added acid **14** (0.2 g, 0.6 mmol), 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) (0.21 g, 1.4 mmol), and 4-dimethylaminopyridine (DMAP) (0.03 g, 0.25 mmol). The reaction mixture was stirred for 24 h at room temperature. After the addition of CH_2Cl_2 (50 mL), the two layers were separated and the organic phase was washed with HCl 1M solution (3 x 20 mL) and water (3 x 20 mL). The organic phase, dried with Na_2SO4, was evaporated and the crude purified by chromatography on silica gel (CH_2Cl_2/ MeOH 99:1) to afford **8** (0.080, 23% yield) as a white solid, mp 166.0-174.2 °C, $[\alpha]_D^{24}$ +20.7 (*c* 0.58, DMSO); ^{1}H NMR (400 MHz, DMSO-d_6) *δ*/ppm 10.36 (s, 1H, NH), 9.90 (s, 1H, CHO), 8.38 (s, 1H, *o*CH-COOR), 7.98 (d, 1H, *J* 8.4 Hz, *p*CH-COOR), 7.73 (d, 1H, *J* 7.6 Hz, *o*CH-COOR), 7.59-7.34 (m, 12H, CH-aromatic), 7.04 (d, 1H, *J* 8.4 Hz, *m*CH-CHO), 6.70 (d, 1H, *J* 15.6 Hz, CH=CH), 6.10-6.03 (m, 2H, $CH=CH_2$ + OH), 5.65 (s, 1H, CH-benzyl group), 5.62 (d, 1H, *J* 7.6 Hz, H-1), 5.50-5.39 (m, 2H, H-3 + $CH=CH_2$), 5.29 (d, 1H, *J* 10.4 Hz, $CH=CH_2$), 4.63 (d, 2H, *J* 5.2 Hz, CH_2), 4.30-4.28 (m, 1H, H-6), 3.98- 3.80 (m, 7H, H-2, H-4, H-5, H-6' + OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) *δ*/ppm 195.0 (CHO), 165.0 (COOR), 164.0 (NHCO), 159.6 (C-O-allyl), 151.0 (C*ipso*), 149.3 (C*ipso*), 140.3 (CH=CH), 139.6 (C-NHCO), 137.2 (C*ipso*), 133.4 ($CH=CH_2$), 130.1 (C-CHO), 129.4, 129.1, 128.8, 128.0 (CH-aromatic), 127.2 (C-CH=CH), 125.9 (*m*CH-O-allyl) 125.0 (*o*CH-CHO), 119.9 (*o*CH-COOR), 119.3 (CH=CH), 117.6 ($CH=CH_2$), 115.1 (*o*CH-O-allyl), 114.8 (*m*CH-CHO), 110.8 (*o*CH-CHO), 100.3 (CH-benzyl group), 99.2 (C-1), 77.6 (C-4), 74.6 (C-3), 71.6 (C-2), 68.2 (CH_2), 67.6 (C-6), 65.6 (C-5), 55.7 (OCH_3).

*Synthesis of 4-formyl-2-methoxyphenyl 3-O-[3-[(E-4-allyloxycinamoyl)amino]benzoyl]-β-D-glucopyranoside (**1**)*

To a cooled to 0 °C solution of **8** (0.100 g, 0.14 mmol) in acetone (10 mL), concentrated HCl (0.5 mL) was added dropwise. The reaction mixture was stirred for 2 h. The course of the reaction was followed by TLC (CH_2Cl_2: MeOH 95:5). After completion, the solvent was evaporated and the crude purified by chromatography on silica gel (CH_2Cl_2:MeOH 95:5) to give **1** (0.061 g, 70% yield) as a white solid, mp 127.6-131.3 °C, $[\alpha]_D^{24}$ +20.7 (*c* 0.58, DMSO); ^{1}H NMR (400 MHz, DMSO-d_6) *δ*/ppm 10.36 (s, 1H, NH), 9.89 (s, 1H, CHO), 8.37 (s, 1H, *o*CH-COOR), 8.01 (d, 1H, *J* 8.0 Hz, *p*CH-COOR), 7.73 (d, 1H, *J* 7.2 Hz, *o*CH-COOR), 7.61-7.46 (m, 8H, CH=CH + CH-aromatic), 7.04 (d, 1H, *J* 8.8 Hz, *m*CH-CHO), 6.70 (d, 1H, *J* 15.6 Hz, CH=CH), 6.12-6.01 (m, 1H, $CH=CH_2$), 5.74 (d, 1H, *J* 6.0 Hz, OH), 5.42 (d, 1H, *J* 5.6 Hz, $CH=CH_2$), 5.38 (d, 1H, *J* 7.6 Hz, H-1), 5.28 (d, 1H, *J* 10.8 Hz, $CH=CH_2$), 5.21 (t, 1H, *J* 8.8 Hz, H-3), 4.63 (d, 2H, *J* 5.2 Hz, CH_2), 3.85 (s, 3H, OCH_3), 3.73-3.53 (m, 5H, H-2, H-4, H-5, H6 + H6'); ^{13}C NMR (100 MHz, DMSO-d_6) *δ*/ppm191.4 (CHO), 165.1 (COOR), 164.0 (NHCO), 159.5 (C-O-allyl), 151.3 (C*ipso*), 149.2 (C*ipso*), 140.3 (CH=CH), 139.6 (C-NHCO), 133.3 ($CH=CH_2$), 130.6 (C-CHO), 129.3, 128.9 (CH-aromatic), 125.2 (*o*CH-CHO), 123.9 (*p*CH-COOR), 123.2 (*o*CH-COOR), 119.8 (*o*CH-COOR), 119.2 (CH=CH), 117.5 ($CH=CH_2$), 115.0 (*o*CH-O-allyl), 114.5 (*m*CH-CHO), 110.5 (*o*CH-CHO), 98.8 (C-1), 78.6 (C-4), 76.6 (C-3), 71.1 (C-2), 68.2 (CH_2), 67.2 (C-5), 60.0 (C-6), 55.5 (OCH_3).

*Synthesis of 4-formyl-2-methoxyphenyl 3-O-[3-[(E-3-allyloxycinamoyl)amino]benzoyl]-β-D-glucopyranoside (**2**)*

To a solution of glycoside **7** (0.1 g, 0.25 mmol) in CH_2Cl_2 were added acid **15** (0.1 g, 0.3 mmol), 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) (0.18 g, 1.16 mmol) and 4-dimethylaminopyridine (DMAP) (0.015 g, 0.12 mmol). The reaction mixture was stirred at room temperature and the course of the reaction was followed by TLC (Hexane:EtOAc 1:1).

After the addition of CH_2Cl_2 (50 mL), the two layers were separated and organic phase was washed with HCl 1M solution (3 x 20 mL) and water (3 x 20 mL). The organic phase, dried with Na_2SO4, was evaporated. The crude was purified by chromatography on silica gel (Hexane:EtOAc 6:4) to give an inseparable mixture of **9** and by-product diester. The mixture thus obtained was submitted to deprotection reaction. To a solution of glycoside mixture (0.09 g) in acetone (10 mL) was added concentrated HCl (0.5 mL) dropwise. The reaction mixture was stirred for 2 h. The course of the reaction was followed by TLC (CH_2Cl_2: MeOH 95:5). After completion, the solvent was evaporated and the crude purified by chromatography on silica gel (CH_2Cl_2: MeOH 95:5) to give **2** (0.035 g, 22% yield) as a white solid, mp 124.7-127.8 °C, $[\alpha]_D^{24}$ +113.2 (*c* 0.16, DMSO); ^{1}H NMR (400 MHz, DMSO-d_6) *δ*/ppm 10.52 (s, 1H, NH), 9.86 (s, 1H, CHO), 8.36 (s, 1H, *o*CH-COOR), 8.00 (d, 1H, *J* 8.0 Hz, *p*CH-COOR), 7.72 (d, 1H, *J* 7.6 Hz, *o*CH-COOR), 7.49 (d, 1H, *J* 16.0 Hz,CH=CH), 7.59-6.98 (m, 8H, CH-aromatic), 6.86 (d, 1H, *J* 16.0 Hz, CH=CH), 6.06-6.02 (m, 1H, CH=CH_2), 5.72 (d, 1H, *J* 6.0 Hz, OH), 5.46 (s, 1H, OH), 5.35 (d, 1H, *J* 7.6 Hz, H-1), 5.43-5.25 (m, 2H, CH=CH_2), 5.19 (t, 1H, *J* 8.4 Hz, H-3), 4.69 (s, 1H, OH), 4.61 (d, 2H, *J* 4.8 Hz, CH_2), 3.83 (s, 3H, OCH_3), 3.69-3.48 (m, 5H, H-2, H-4, H-5, H6 + H6'); ^{13}C NMR (100 MHz, DMSO-d_6) *δ*/ppm191.5 (CHO), 165.1 (COOR), 163.8 (NHCO), 158.5 (C-O-allyl), 151.4 (C*ipso*), 149.3 (C*ipso*), 140.4 (CH=CH), 139.5 (C-NHCO), 133.6 (CH=CH_2), 130.9 (C-CHO), 130.1, 129.0 (CH-aromatic), 125.3 (*o*CH-CHO), 124.3 (*p*CH-COOR), 123.4 (*o*CH-COOR), 120.2 (*o*CH-COOR), 120.1 (CH=CH), 117.5 (CH=CH_2), 116.4 (*o*CH-O-allyl), 114.6 (*m*CH-CHO), 110.6 (*o*CH-CHO), 98.9 (C-1), 78.7 (C-4), 76.7 (C-3), 71.2 (C-2), 68.2 (CH_2), 67.3 (C-5), 60.1 (C-6), 55.7 (OCH_3).

*General procedure for synthesis of cinnamic acids **12** and **13***

To a solution of corresponding allyloxybenzaldehyde (3.30 g, 20 mmol) in pyridine (40 mL) were added malonic acid (8.50 g, 80 mmol) and piperidine (2.2 mL). The reaction mixture was warmed at 95 °C for 2 h. After completion, ice and concentrated HCl were added to pH 1. The solid which precipitated was collected by filtration and washed with water and air dried.

*(E)-4-allyloxy cinnamic acid (**12**)*: 3.60 g (86% yield),), mp 154.2-156.9 °C, ^{1}H NMR (200 MHz, DMSO-d_6) *δ*/ppm7.73 (d, 1H, *J* 15.8 Hz, CH=CH), 7.49 (d, 2H, *J* 8.4 Hz, *m*CH-O-allyl), 6.93 (d, *J* 8.4 Hz, 2H, *o*CH-O-allyl), 6.31 (d, 1H, *J* 15.8 Hz, CH=CH), 6.14-5.95 (m, 1H, CH=CH_2), 5.42 (d, 1H, *J* 17.4 Hz, CH=CH_2), 5.35 (d, *J* 10.2 Hz, 1H, CH=CH_2), 4.57 (d, *J*4.8 Hz, 2H, CH_2); ^{13}C NMR (50 MHz, DMSO-d_6) *δ*/ppm 172.3 (COOH), 160.6 (C-O-allyl), 146.4 (CH=CH), 132.6 (CH=CH_2), 130.0 (C-CH=CH + *m*CH-O-allyl), 118.0 (CH=CH_2), 115.0 (*o*CH-O-allyl), 114.8 (CH=CH), 68.8 (CH_2).

*(E)-3-allyloxy cinnamic acid (**13**)*: 3.56 g (85% yield),), mp 110.2-111.9 °C, ^{1}H NMR (200 MHz, $CDCl_3$) *δ*/ppm7.75 (d, 1H, *J* 16.0 Hz, CH=CH), 7.30 (t, 1H, *J* 7.8 Hz, *m*CH-O-allyl), 7.13 (d, *J* 7.6 Hz, 1H, *p*CH-O-allyl), 7.08 (s, 1H, *o*CH-O-allyl), 6.99-6.95 (m, 1H, *o*CH-O-allyl), 6.42 (d, 1H, *J* 16.0 Hz, CH=CH), 6.15-5.96 (m, 1H, CH=CH_2), 5.42 (d, *J*17.2 Hz, 1H, CH=CH_2), 5.30 (d, *J*10.6 Hz, 1H, CH=CH_2), 4.56 (d, 2H, *J* 5.0 Hz, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$) *δ*/ppm172.7 (COOH), 159.1 (C-O-allyl), 147.2 (CH=CH), 135.6 (C-CH=CH), 133.1 (CH=CH_2), 130.1 (*m*CH-O-allyl), 121.4 (*p*CH-O-allyl), 118.1 (CH=CH_2), 117.8 (*o*CH-O-allyl), 117.6 (*o*CH-O-allyl), 114.3 (CH=CH), 69.1 (CH_2).

*General procedure for synthesis of cinnamic derivatives **14** and **15***

To a cooled to 0 °C solution of 3-aminobenzoic acid (1.25 g, 9.1 mmol) in pyridine (20 mL) were added dropwise the appropriate cinnamic acid chloride (1.7 g, 7.6 mmol) previously dissolved in pyridine (10 mL) and triethylamine (1.5 mL, 10.5 mmol). The reaction mixture was stirred at room temperature. The course of the reaction was followed by TLC (Hexane/ EtOAc 7:3). After completion, the solvent was evaporated. EtOAc was added and the organic phase was washed with H_2O. The organic phase, dried with Na_2SO_4, was evaporated and the crude purified by flash chromatography on silica gel (Hexane/ EtOAc 7:3) to afford the amide **15**. For the amide **14** the crude was purified by recrystallization with DMSO/H_2O.

*(E)3-(4-(allyloxycinnamoyl)amino)benzoic acid (**14**)*: 1.8 g (73% yield), mp 154.2-156.9 °C, ^{1}H NMR (200 MHz, DMSO-d_6) *δ*/ppm12.95 (s; 1H, COOH), 10.30 (s, 1H, NH), 8.31 (s, 1H, *o*CH-COOH), 7.95 (d, 1H, *J* 8.0 Hz, *p*CH-COOH), 7.64 (d, 1H, *J* 7.6 Hz, *o*CH-COOH), 7.59-7.54 (m, 3H, CH=CH + *m*CH-O-allyl), 7.45 (t, 1H, *J* 8.0 Hz, *m*CH-COOH), 7.02 (d, 2H, *J* 8.4 Hz, *o*CH-O-allyl), 6.68 (d, 1H, *J*15.6 Hz, CH=CH), 6.07-6.02 (m, 1H, CH=CH_2), 5.41 (dd, 1H, *J*17.2 Hz, *J*1.6 H, CH=CH_2), 5.28 (dd, 1H, *J* 10.6 Hz, *J* 1.6 Hz, CH=CH_2), 4.63 (d, 2H, *J*5.2 Hz, CH_2); ^{13}C NMR (50 MHz, DMSO-d_6) *δ*/ppm 167.2 (COOH), 164.0 (NHCO), 159.6 (C-O-allyl), 140.2 (CH=CH), 139.6 (C-NHCO), 133.4 (CH=CH_2), 131.4 (C-COOH), 129.3 (*m*CH-COOH), 129.0 (*m*CH-

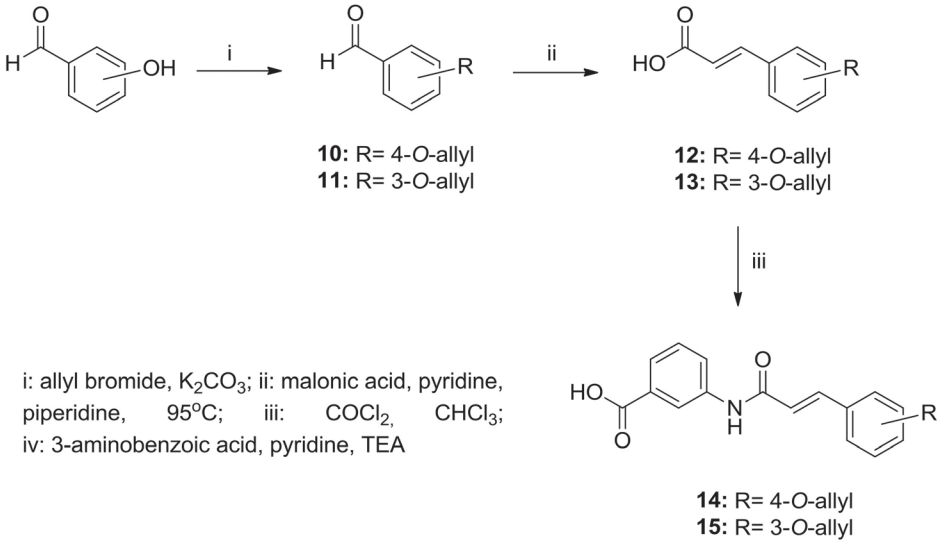
COOH), 127.3 (C-CH=CH), 123.9 (*p*CH-COOH), 123.2 (*o*CH-COOH), 119.9 (*o*CH-COOH), 119.5 (CH=CH), 117.6 (CH=CH_2), 115.1 (*o*CH-O-allyl), 68.2 (CH_2).

*(E)3-(3-(allyloxycinnamoyl)amino)benzoic acid (**15**):* 1.7 g (70% yield),), mp 199.5-202.1 °C, ^{1}H NMR (200 MHz, DMSO-d_6) δ/ppm10.41 (s, 1H, NH), 8.33 (s, 1H, *o*CH-COOH), 7.95 (d, 1H, *J* 7.8 Hz, *p*CH-COOH), 7.67-7.19 (m, 6H, CH-aromatic + CH=CH), 6.99 (d, 1H, *J* 8.0 Hz, *o*CH-O-allyl), 6.83 (d, 1H, *J* 15.6 Hz, CH=CH), 6.15-5.96 (m, 1H, CH=CH_2), 5.40 (d, 1H, *J* 17.4 Hz, CH=CH_2), 5.26 (d, 1H, *J* 10.6 Hz, CH=CH_2), 4.61 (d, 2H, *J* 4.8 Hz, CH_2); ^{13}C NMR (50 MHz, DMSO-d_6) δ 167.3 (COOH), 163.8 (NHCO), 158.6 (C-O-allyl), 140.5 (CH=CH), 139.5 (C-NHCO), 133.6 (CH=CH_2), 131.6 (C-COOH), 130.1 (*m*CH-O-allyl), 129.1 (*m*CH-COOH), 124.2 (*p*CH-COOH), 123.3 (*o*CH-COOH), 120.3 (*o*CH-COOH), 120.0 (CH=CH), 117.6 (CH=CH_2), 116.5 (*o*CH-O-allyl), 68.2 (CH_2).

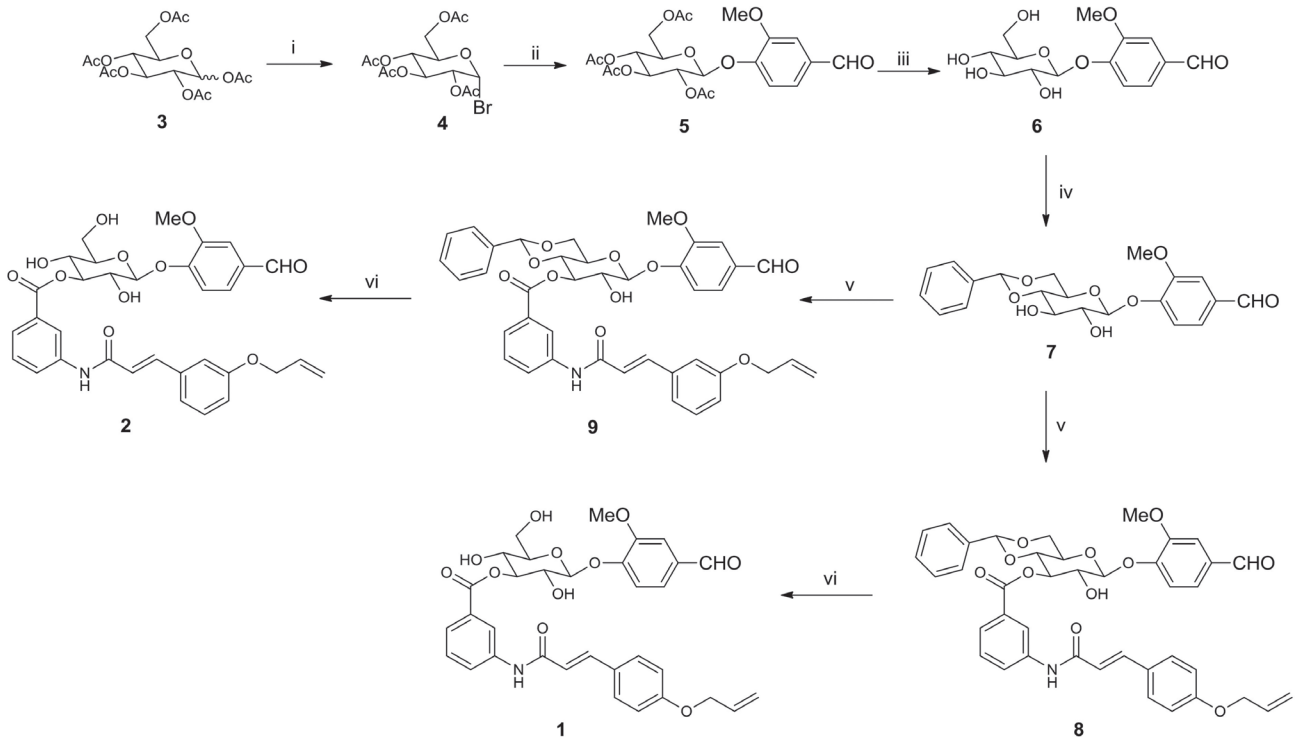
RESULTS AND DISCUSSION

Initially, the synthesis of carboxyl acid **14** and **15** was performed according to Scheme 1. The commercially available 3- and 4-hydroxybezaldehyde were alkylated with allyl bromide in the presence of K_2CO_3 (Tseng *et al*., 2011) to afford 3- and 4-allyloxybenzaldehyde **10** and **11**, respectively, in good yields. Next, Knoevenagel-Doebner reaction using the corresponding aldehydes **10** or **11**, malonic acid, pyridine and piperidine afforded the cinnamic acids **12** and **13** in 86% and 85% yield, respectively. To obtain the amides **14** and **15**, the cinnamic acids **12** and **13** were converted to the corresponding acyl chlorides, using $COCl_2$ in $CHCl_3$. The reaction of these acyl chlorides with 3-aminobenzoic acid, in pyridine, afforded the amides **14** and **15** in 73% and 70% yield, respectively.

In parallel, 2,3,4,6-tetra-acetyl-D-glucopyranose **3** was converted to known acetobromoglucose **4**, as shown in Scheme 2. Glycosylation of 4-formyl-2-methoxyphenol with bromide **4** afforded the glucosyde **5** in 54% yield after purified by recrystallization, which upon deacetylation condition was converted into derivative **6** in 98% yield. Following the proposed synthetic route, the protection of *O*-4 and *O*-6 using benzaldehyde and $ZnCl_2$ afforded the acetal **7** in 72% yield (Souza *et al*., 2015). Compound **7** was coupled with previously synthesized carboxyl acid **14** using EDAC/DMAP to give a mixture of the desired compound **8 (**obtained in 23% yield; Scheme 2**)** and the undesired 2,3-diester, which were separated by column chromatography. The obtaining of unwanted diester compound in 20% yield affected the efficiency of getting the planned derivative **8,** although this product had been isolated in sufficient amounts for the last step of the synthetic route. Finally, the 4,6-*O*-benzylidene protecting group was removed with HCl in acetone to afford the papulacandin analog **1** in 70% yield. In parallel, when the intermediary **7** reacted with carboxylic acid **15** under the same conditions described above for the preparation of **8**, an inseparable mixture of desired derivative **9** (Scheme 2) and the undesired diester was obtained. The mixture was then treated with HCl to remove the 4,6-*O*-benzylidene protecting group and the crude mixture of the deprotected products was submitted to column chromatograpy (Souza *et al*., 2015). This procedure allowed us to isolate compound **2** in 22% yield. ^{1}H NMR



SCHEME 1 - Synthesis of carboxylic acids **14** and **15**.



SCHEME 2 - Synthesis of new papulacandin analogs **1** and **2**.

analysis of new papulacandin analogs **1** and **2** revealed a large downfield shift of H-3 proton indicating that the hydroxyl group attached to C-3 was selectively esterified in these compounds. HMBC experiments ($^3J_{CH}$ correlation between the carbon of the carbonyl group of the ester chain and H-3) further proved that esterification had occurred at C-3 in both derivatives.

After characterized, compounds **1** and **2** were evaluated for growth inhibition of *Candida albicans*, *Candida tropicalis*, *Candida albicans*, *Candida krusei*, and *Paracoccidioides brasiliensis* according to published protocols (Wayne, 2002). No inhibition was observed at the highest tested concentration of 500 µg/mL. Although the compounds synthesized here have not shown any activity against the evaluated strains, the established synthetic route can be used to obtain new antifungal papulacandin analogs.

CONCLUSIONS

We reported here the synthesis, characterization, and antifungal evaluation of two new analogs of natural papulacandin D, designed by molecular simplification. Both compounds were inactive at the highest concentration evaluated (500 µg.mL-1) against all fungal species. The absence of the rigid spiroketal moiety (the analogs are classical aryl glycosides and therefore more flexible) is expected to impair the biological activity of the compounds, albeit not essential as we have demonstrated in previous work (Souza *et al.*, 2015) On the other hand, the side chain of the analogs is more rigid and less lipophilic than that of the prototype due to the presence of the aromatic rings and amide bond. It appears that the correct combination of molecular rigidity and lipophilic/hydrophilic balance is essential for activity. Although the obtained compounds displayed no antifungal activity against the evaluated fungal species, they represent a new class of papulacandin D analogs that may inspire the synthesis of novel potential antifungal derivatives using the established synthetic strategy described.

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