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Artigo

SYNTHESIS AND ANTIFUNGAL ACTIVITY OF PALMITIC ACID-BASED NEOGLYCOLIPIDS RELATED TO PAPULACANDIN D

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A series of six new palmitic acid-based neoglycolipids related to Papulacandin D were synthesized in five steps, resulting in good yields, and they were evaluated against *Candida* spp. All twelve synthetic intermediates were also evaluated. The synthesis involved the initial glycosylation of two phenols (4-hydroxy-3-methoxybenzaldehyde and 3-hydroxybenzaldehyde) via their reaction with peracetylated glucosyl bromide. This was followed by deacetylation with potassium methoxide/metanol solution and the protection of two hydroxyls (C4 and C6 positions) of the saccharide unit as benzilidene acetals (**10**–**11**). The next step involved the acylation of the acetal derivatives with palmitic acid, thereby affording a mixture of two isomers mono-acylated at the C2 and C3 positions and a di-acylated product (**12**–**17**). After being isolated, each compound was subjected to the removal of the acetal protecting group to yield the papulacandin D analogues **18**–**23**. Three compounds showed low antifungal activity against two species: *C. albicans* (compounds **7** and **23**) and *C. tropicalis* (compound **17**) at 200 μg mL^{-1}.

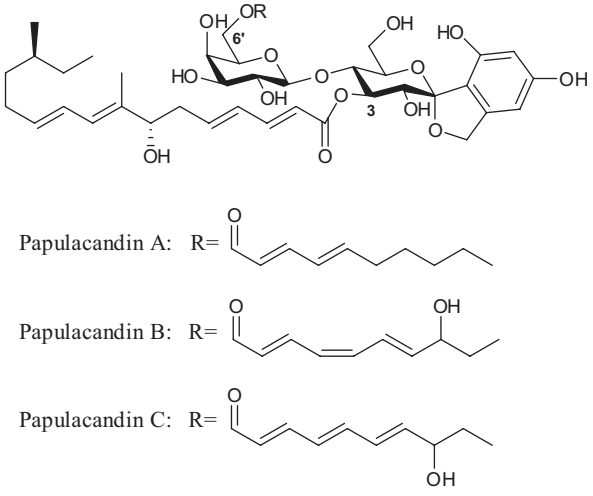
Keywords: papulacandin D; neoglycolipids; palmitic acid; analogues; antifungal activity.

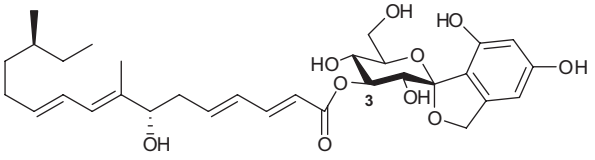
INTRODUCTION

Fungal infections represent a serious health problem. Since the late 1960s, when antibiotic therapy was already developed, accounting for a reduction in deaths by bacterial infections, a drastic rise in fungal diseases was noticed, which today accounts for a large number of deaths worldwide.[1,2] The increasing incidence of opportunistic fungal infections has some factors such as the growing number of immunodeficient patients (patients on anti-tumor therapy, transplanted, AIDS carriers or diabetics) or people experiencing some kind of stress.[3] There is a need for new antifungal therapeutic agents, since treatment failure is very common in immunocompromised patients, with high incidence of mortality.

The papulacandins are a group of naturally occurring glycolipids, isolated from the fermentation broths of *Papularia sphaerosperma*.[4] The papulacandins A, B and C are spiroglycosides derived from the disaccharide lactose, acylated at positions 3 and 6' with residues of unsaturated fatty acids containing some chiral centers. The papulacandin D is a monosaccharide spiroglucoside derived from glucose acylated at position 3 with the same fatty acid residue present in this position of papulacandins A, B and C (Figure 1).

The papulacandins show potent fungicidal activity against different strains of *Candida* sp. (MIC range of 0.1-12.5 μg mL^{-1}).[4] The success of these substances in the treatment of fungal infections caused by *Pneumocystis carinii*, a species that reaches immunocompromised patients with high mortality, has been reported.[5] However,





Papulacandin D

Figure 1. *Chemical structure of the papulacandins*

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these compounds are not active against other pathogenic fungi like *Aspergillus* sp., causing lung infections of difficult treatment.[4] Another limitation of papulacandins is their lower activity *in vivo* than *in vitro*, due to strong binding to plasma proteins.[6]

Literature reports the enzyme (1,3)-β-D-glucan synthase as the molecular target of papulacandins.[5,7] Since these compounds exert their action by inhibiting the (1,3)-β-D-glucans synthesis, major structural components of the fungal cell wall absent in mammalian cells, the papulacandins have become an important chemical class to be explored for the design and discovery of analogues or derivatives which may be useful against fungal infections *in vitro* and *in vivo*.

Recently, Kaaden and coworkers synthesized four papulacandin D analogues (Figure 2) modified in side chain, and evaluated their antifungal activity against *Candida albicans*. The compounds **1** and **4** were inactive while compounds **2** and **3** showed some activity. The papulacandin D derivative **2**, containing a palmitoyl acyl residue as side chain, showed MIC of 88 μg mL^{-1}, representing the most active compound of the series.[8]

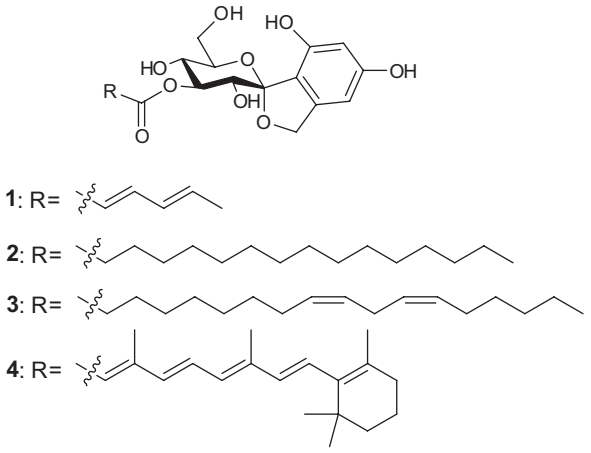


Figure 2. *Papulacandins derivatives*

Considering these findings, we describe herein the synthesis of simplified papulacandin D analogues, bearing a palmitoyl residue as side chain, since it is present in the most active compound synthesized and evaluated by Kaaden and coworkers.[8] The spiroketal ring of papulacandin D was replaced by aromatic glycosides derived from 4-hydroxy-3-methoxybenzaldehyde or 3-hydroxybenzaldehyde, which are very cheap and more accessible synthetically (Figure 3). Since SAR studies on papulacandin B showed that modifications on the aglycone had, in general, little influence on activity[9] and that no study has been carried out on Papulacandin D, we though these modifications could shed some light about the influence of the aglycone in this particular series.

RESULTS AND DISCUSSION

Chemistry

The synthetic route (Scheme 1) involved the reaction of 4-hydroxy-3-methoxybenzaldehyde or 3-hydroxybenzaldehyde with peracetylated glucosyl bromide **5** in the presence of lithium hydroxide, according to published procedure.[10] This is one of the most used methods for this purpose and was employed in the preparation of peracetylated glycosides **6** and **7** in 51% and 61% yields, respectively. Then, the peracetylated glucosides **6** and **7** were deacetylated using potassium hydroxide/methanol solution,[11] affording **8** and **9** in quantitative yields.

Interestingly, the compound **9** was obtained as a possible oligomer as observed at ^{1}H and ^{13}C NMR spectra, which did not prevent the next planned derivative (**11**) was obtained accordingly. The next step consisted on the selective protection of C4 and C6 hydroxyl groups of the saccharide unit as benzylidene acetal. This protecting group was chosen due to its stability and easy removal in mild conditions.[12] Thus, the reaction of glucosides **8** or **9** with benzaldehyde and zinc chloride afforded the acetals **10** and **11** in 72% and 60% yields, respectively. When **10** was reacted with 1.2 equivalent of palmitic acid and *N*,*N*'-dicyclohexylcarbodiimide in dichloromethane, in the presence of catalytic amount of 4-dimethylaminopyridine, a mixture of three compounds was obtained. This result is not unexpected, in view of the difficulties for the regioselectivity acylation of β-glucosides.[13] Besides, for structure-activity relationship studies it is interesting to evaluate the antifungal activity of each component of the mixture. Thus, the mixture was submitted to flash column chromatography (hexane/ethyl acetate, 9:1) which allowed for the isolation of 3-*O*-palmitoyl derivative **12** (31% yield), 2-*O*-palmitoyl derivative **13** (27% yield) and 2,3-di-*O*-palmitoyl derivative **14** (13% yield). Each isolated compound was characterized by 1D and 2D ^{1}H and ^{13}C NMR experiments. The most important information from these experiments is the downfield shift of H-2 and/or H-3 of the saccharide moiety upon esterification, as it is well stablished in the literature.[14] Finally, the

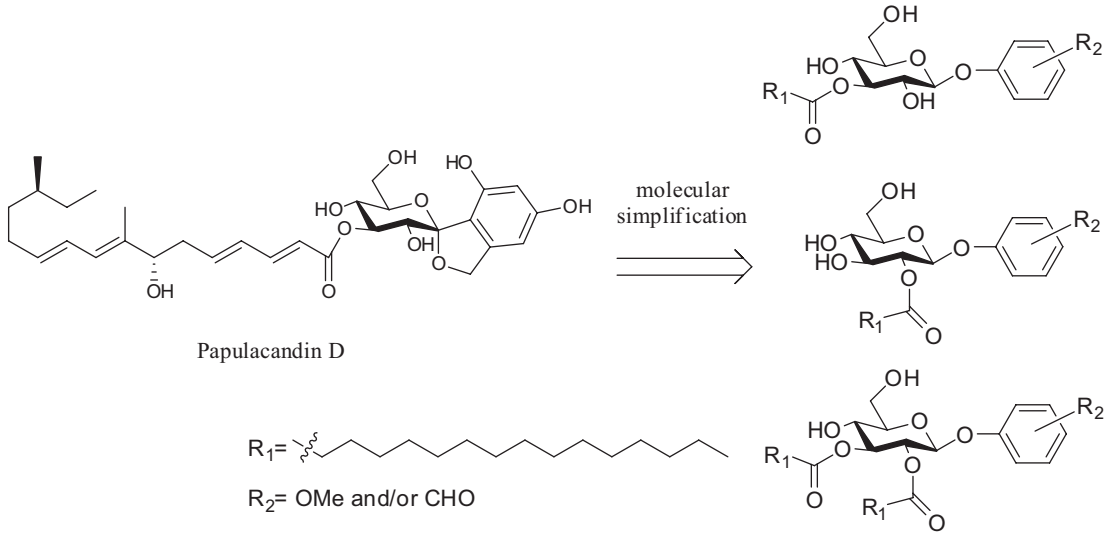
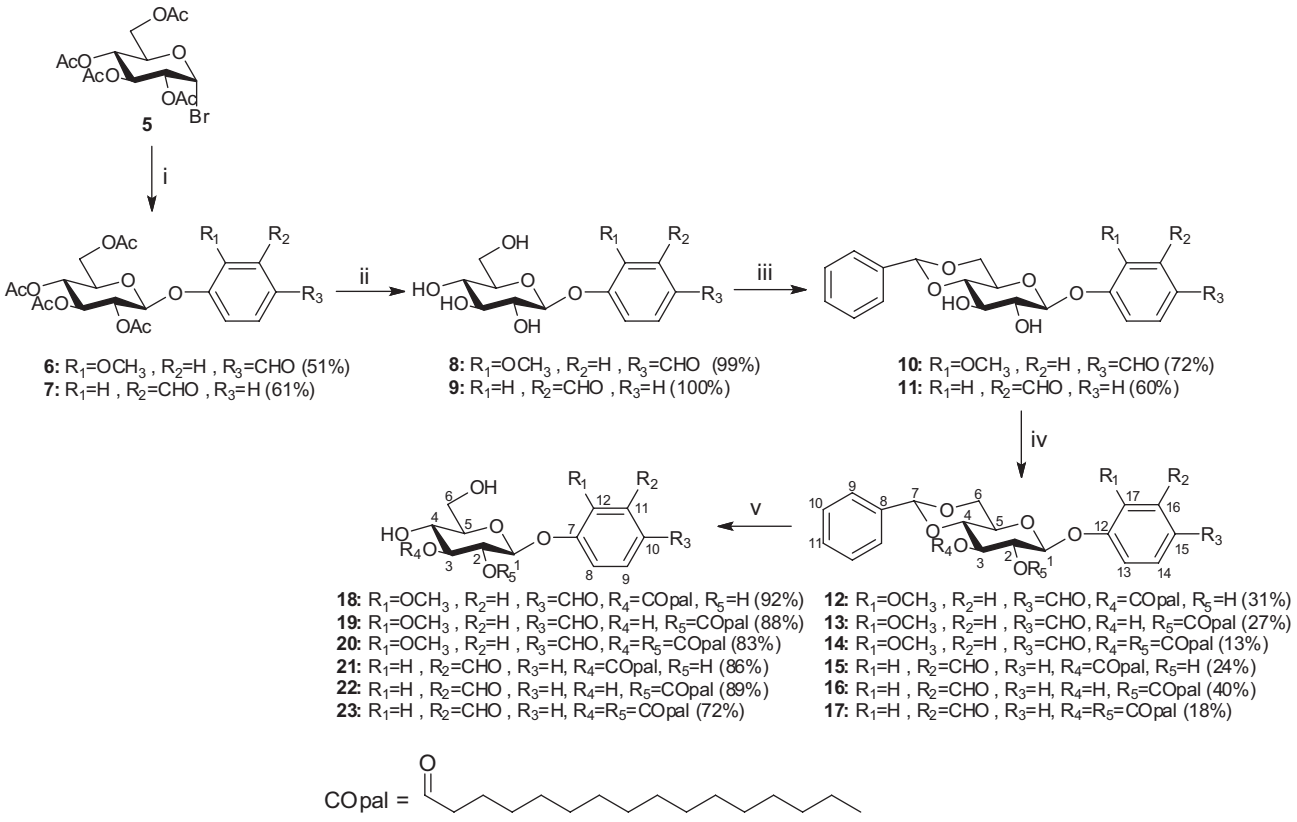


Figure 3. *Simplified analogues of Papulacandin D*



Scheme 1. *Synthesis of Papulacandin D analogues*

removal of the acetal protecting group was carried out in the presence of HCl in acetone, affording the papulacandin D analogues **18**-**20** in 92%, 88% and 83% yields, respectively.[15]

Under the same conditions as described for **10**, acylation of **11** also afforded a three-component mixture which was purified by flash chromatography to furnish 3-*O*-palmitoyl derivative **15** (24% yield), 2-*O*-palmitoyl derivative **16** (40% yield) and 2,3-di-*O*-palmitoyl derivative **17** (18% yield). Removal of 4,6-*O*-benzylidene protecting group afforded the corresponding deprotected neoglycolipids **21**-**23** in 86%, 89% and 72% yields, respectively.

Besides the 1D and 2D NMR experiments involving the protected acylated glycosides, as described above, the unprotected neoglycolipids **18**-**23** were also submitted to HMBC experiments. The results for compounds **18**-**20** are shown below (Figure 4). Thus, through correlation observed between H-3, H-2 or H-3 and H-2 protons (carbohydrate) with carbonyl carbon of acetyl group (palmitoyl side chain) we could unequivocally identify each isomer (acylated at positions 2 or 3) and the 2,3-di-acylated analogue.

In vitro assays

Compounds **6**-**23** were evaluated against *Candida albicans*, *C. krusei*, *C. parapsilosis* and *C. glabrata* according to published protocol.[16] Most compounds were inactive and three displayed low antifungal activity, two against *Candida albicans* (compounds **7** and **23**) and one against *Candida tropicalis* (compound **17**) (Table 1).

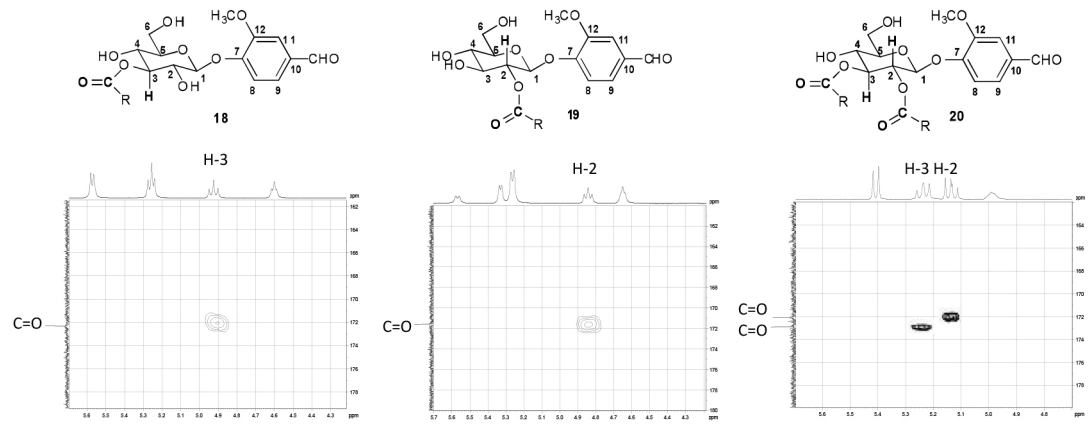


Figure 4. *Correlations between H-3, H-2 or H-3 and H-2 by HMBC*

Table 1. Minimal inhibitory concentrations (μg mL^{-1}) of synthesized compounds against *Candida* spp.

Compounds	MIC (μg mL^{-1})			
	C. albicans	*C. krusei*	*C. parapsilosis*	*C. tropicalis*
6	-[a]	-[a]	-[a]	-[a]
7	200	-[a]	-[a]	-[a]
8	-[a]	-[a]	-[a]	-[a]
9	-[a]	-[a]	-[a]	-[a]
10	-[a]	-[a]	-[a]	-[a]
11	-[a]	-[a]	-[a]	-[a]
12	-[a]	-[a]	-[a]	-[a]
13	-[a]	-[a]	-[a]	-[a]
14	-[a]	-[a]	-[a]	-[a]
15	-[a]	-[a]	-[a]	-[a]
16	-[a]	-[a]	-[a]	-[a]
17	-[a]	-[a]	-[a]	200
18	-[a]	-[a]	-[a]	-[a]
19	-[a]	-[a]	-[a]	-[a]
20	-[a]	-[a]	-[a]	-[a]
21	-[a]	-[a]	-[a]	-[a]
22	-[a]	-[a]	-[a]	-[a]
23	200	-[a]	-[a]	-[a]
Flu	2.0	32.0	1.0	2.0

-[a]: No significant activity. Flu: Fluconazole.

Compounds **18** and **21**, close analogues of the papulacandin D were inactive, while papulacandin D derivative **2** showed MIC of 88 μg mL^{-1}, as related by Kaaden and coworkers, indicating that classical aryl glycosides can't replace the spiro glucoside.[8] Nevertheless, it remains to verify if a classical glucoside with a 2,4-dihydroxy-substituted aglycone which resembles the natural product more closely, could be active. The palmitoyl side chain was not essential to the active compound **7**, devoid of such a chain, and this may suggest this compound may have had its antifungal activity by another mechanism of action, as this chain is essential for activity of the papulacandins. All the active compounds bear a 3-formylphenyl aglycone while no compound bearing the 4-formyl-2-methoxyphenyl aglycone displayed activity, indicating that a 3-formyl group, which is more reactive than that at position 4, possibly have a contribution on activity. A hydrophilic/lipophilic balance also seems to be important, since not all compounds bearing the 3-formylphenyl aglycone were active.

CONCLUSION

Six new neoglycolipids related to papulacandin D were synthesized along with isomeric 2-acylated and 2,3-diacylated compounds. These compounds as well as synthetic intermediates were evaluated against four *Candida* species. The closely related papaulacandin D analogues **18** and **21** were inactive, but two 2,3-diacylated (compounds **17** and **23**) and one peracetylated (compound **7**) showed low activity against *Candida albicans* (**7** and **23**) or *Candida tropicalis* (**17**), with a MIC of 200 μg mL^{-1}. All the active compounds are derived from 3-hydroxybenzaldehyde and represent a starting point for the design of new antifungal candidates.

SUPPLEMENTARY MATERIAL

^{1}H and ^{13}C NMR spectra of all synthesized compounds are found in the supplementary material and have free access at http://quimicanova.sbq.org.br.

ACKNOWLEDGEMENTS

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EXPERIMENTAL

Physical measurements

Melting points were determined on Microquímica MOAs 301 apparatus and were uncorrected. IR spectroscopy was performed on Spectrum One Infrared Spectrometer, Perkin-Elmer. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on Bruker Avance DRX-200 (200 MHz FT NMR) and DRX-400 (400 MHz FT NMR) in deuterated chloroform or dimethylsulfoxide. The specific optical rotation $[\alpha]_D$ were measured on Perkin Elmer 341 polarimeter, at 20 °C. Reaction courses and product mixtures were monitored by thin-layer chromatography (TLC) on silica gel-G TLC plates (Merck). For chromatograph, column grade silica gel (0.040–0.063 mm mesh size) was employed.

Synthesis of the compounds

*Synthesis of peracetylated glucosides **6** and **7**: general procedure*

A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**5**, 6.15 mmol) in acetone (20 mL) was added to a solution of 4-hydroxy-3-methoxybenzaldehyde or 3-hydroxybenzaldehyde (18 mmol) em 1.0 mol L^{-1} lithium hydroxide (10 mL) and the solution stirred for 2 hours at room temperature and monitored by TLC. After removal of acetone, the resulting suspension was extracted with dichloromethane (3 x 50 mL). The crude product was washed with 10% sodium hydroxide (3 x 30 mL), water and dried on anhydrous sodium sulphate. After filtration and removal of the solvent under reduced pressure, the crude product was recrystallized from isopropyl alcohol, affording compounds **6** and **7**.

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

This compound was obtained as white crystals; yield: 51%; m.p. 139.1-139.9 °C; $[\alpha]_D$ -41.0º (*c* 0.63, $CHCl_3$); IR (υ/cm^{-1}): 2944 (sp^3 C-H), 1752 (ester C=O), 1694 (aldehyde C=O), 1591, 1510, 1475 (Ar. C=C). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 9.8 (1H, s, C<u>H</u>O), 7.4 (1H, dd, J_4= 1.7 Hz e J_3= 8.6 Hz, H-9), 7.2 (1H, s, H-11), 7.2 (1H, d, J_3= 8,6 Hz, H-12), 5.3-5.1 (4H, m, H-1, H-2, H-3 and H-4), 4.2 (1H, dd, J_3= 5.0 Hz e J_2= 12.2 Hz, H-6), 4,1 (1H, dd, J_3= 2.5 Hz e J_2= 12.2 Hz, H-6'), 3.8 (3H, s, OC<u>H</u>$_3$), 3.9-3.8 (1H, m, H-5), 2.0-2.0 (12H, s, OCOC<u>H</u>$_3$). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 191.4 (1C, <u>C</u>HO), 170.6-169.3 (4C, ester C=O), 151.2 (1C, C-7), 151,1 (1C, C-8), 132.9 (1C, C-10), 125.4 (1C, C-11), 118.2 (1C, C-12), 110.9 (1C, C-9), 99.8 (1C, C-1), 72.5 (1C, C-5), 72.3 (1C, C-3), 71.1 (1C, C-2), 68.7 (1C, C-4), 62.0 (1C, C-6), 56.2 (1C, OC<u>H</u>$_3$), 20.7 (4C, OCOC<u>H</u>$_3$).

*3-formylphenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**7**)*

This compound was obtained as white crystals; yield: 61%; m.p. 103.6-105.9 °C; $[\alpha]_D$ -37.5º (*c* 0.58, $CHCl_3$); IR (υ/cm^{-1}): 3065 (Ar. C-H), 2975, 2941 (sp^3 C-H), 1747 (ester C=O), 1697 (aldehyde C=O), 1592, 1483 (Ar. C=C). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 9.9 (1H, s, C<u>H</u>O), 7.5-7.2 (4H, m, H-8, H-10, H-11 and H-12), 5.3-5.1 (4H, m, H-1, H-2, H-3 and H-4), 4.2-4.1 (2H, m, H-5 and H-6), 3.9 (1H, m, H-6'), 2.1-2.0 (12H, s, OCOC<u>H</u>$_3$). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 191.1 (1C, <u>C</u>HO), 170.3-168.9 (4C, ester C=O), 157.0 (1C, C-7), 137,6 (1C, C-9), 130.5 (1C, C-11), 125.6 (1C, C-10), 123.4 (1C, C-12), 114.9 (1C, C-8), 98.3 (1C, C-1), 72.3-67.9 (4C, C-2, C-3, C-4 and C-5), 61.3 (1C, C-6), 20.36-20.32 (4C, OCOC<u>H</u>$_3$).

*Synthesis of deacetylated glucosides **8** and **9**: general procedure*

The peracetylated glucosides (1 mmol) were solubilized in a solution of KOH in MeOH (20 mL, 1.0 mol L^{-1}) and stirred at room temperature for 30 min. After the completion of the reaction, as observed by TLC, the mixture was neutralized with IRA-120 resin. The resin was filtered off and washed with methanol. The collected filtrate was concentrated in vacuum to afford glucosylated derivatives **8** and **9**.

*4-formyl-2-methoxyphenyl β-D-glucopyranoside (**8**)*

This compound was obtained as white crystals; yield: 99%; m.p. 183.0-184.6 °C; $[\alpha]_D$ -52.4º (*c* 0.61, DMSO); IR (υ/cm^{-1}): 3365 (OH), 2891 (sp^3 C-H), 1688 (aldehyde C=O), 1589, 1508, 1465 (Ar. C=C). ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 9.8 (1H, s, C<u>H</u>O), 7.5 (1H, d, J_3 = 8.2 Hz, H-9), 7.4 (1H, s, H-11), 7.2 (1H, d, J_3 = 8.2 Hz, H-8), 5.3 (1H, s, OH), 5.1-5.0 (2H, sl, OH), 5.1 (1H, d, J_3 = 7.4 Hz, H-1), 4.5 (1H, s, OH), 3.8 (3H, s, OC<u>H</u>$_3$), 3.6 (1H, m, H-6), 3.4-3.2 (5H, m, H-2, H-3, H-4, H-5 and H-6'). ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 191.6 (1C, <u>C</u>HO), 151.7 (1C, C-7), 149,3 (1C, C-8), 130.5 (1C, C-10), 125.4 (1C, C-11), 114.5 (1C, C-12), 110.5 (1C, C-9), 99.4 (1C, C-1), 77.1 (1C, C-5), 76.8 (1C, C-3), 73.1 (1C, C-2), 69.5 (1C, C-4), 60.6 (1C, C-6), 55.6 (1C, OC<u>H</u>$_3$).

*3-formylphenyl β-D-glucopyranoside (oligomer) (**9**)*

This compound was obtained as white crystals; yield: 100%; m.p. 103.6-105.9 °C; $[\alpha]_D$ -37.5º (*c* 0.19 DMSO). ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 10.0-9.8 (1H, s, C<u>H</u>O), 7.59-7.54 (3H, m, Ar-H), 7.53-7.29 (2H, m, Ar-H), 7.14-7.06 (4H, m, Ar-H), 5.06-4.22 (12H, m, OH, sugar), 3.71-3.18 (10H, m, OH, sugar). ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 192.8 (1C, <u>C</u>HO), 157.8-157.2 (3C), 156.9 (1C), 139.2 (1C), 137.6 (1C), 137.4 (1C), 130.4 (1C), 130.3 (1C), 129.2 (1C), 123.4 (1C), 123.1 (1C), 122.6 (1C), 122.5 (1C), 116.6 (1C), 116.1 (1C), 116.3 (1C), 114.9 (1C), 100.8-100.2 (4C), 94.1 (1C), 80.3-80.2 (2C), 77.0-76.4 (4C), 74.1-60.5 (1C).

*Synthesis of benzylidene acetals **10** and **11**: general procedure*

To a solution of 7.95 mmol of $ZnCl_2$ in 16 mL (0.137 mol) of benzaldehyde was added 3.18 mmol of **8** or **9**, and stirred for 1 hour at room temperature, when noticed the completion of the reaction by TLC. The mixture was poured into a beaker containing ice water and ethyl ether, and upon stirring a solid formed, which was collected by filtration and washed alternately with ice water and hexanes several times.

*4-formyl-2-methoxyphenyl 4,6-O-benzylidene-β-D-glucopyranoside (**10**)*

This compound was obtained as white crystals; yield: 72%; m.p. 191.4-193.0 °C; $[\alpha]_D$ -62.5º (*c* 0.60, DMSO); IR (υ/cm^{-1}): 3364 (OH), 2880 (sp^3 C-H), 1683 (aldehyde C=O), 1590, 1506, 1465 (Ar. C=C). ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 9.9 (1H, s, C<u>H</u>O), 7.5-7.3 (8H, m, H-9, H-9', H-10, H-10', H-11, H-13, H-14 and H-16), 5.7 (1H, d, J_3 = 5.4 Hz, OH), 5.6 (1H, s, H-7), 5.5 (1H, d, J_3 = 4.8 Hz, OH), 5.2 (1H, d, J_3 = 7.4 Hz, H-1), 4.2 (1H, m, H-6 eq.), 3.7-3.5 (5H, m, H-2, H-3, H-4, H-5 and H-6 ax.), 3.3 (3H, s, OC<u>H</u>$_3$). ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 192.9 (1C, <u>C</u>HO), 157.6 (1C, C-12), 137.6 (1C, C-13), 130.5 (1C, C-8), 128.9 (1C, C-15), 128.0-116.4 (5C, C-9, C-9', C-10, C-10' and C-11), 100.7 (1C, C-1), 100.5 (1C, C-7), 80.3-67.8 (4C, C-2, C-3, C-4 and C-5), 65.9 (1C, C-6).

*3-formylphenyl-4,6-O-benzylidene-β-D-glucopyranoside (**11**)*

This compound was obtained as white crystals; yield: 60%; m.p. 158.5-160.3 °C; $[\alpha]_D$ -10.3º (*c* 0.19, DMSO); IR (υ/cm^{-1}): 3379 (OH), 2884 (sp^3 C-H), 1686 (aldehyde C=O), 1585, 1487, 1465 (Ar. C=C). ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 9.9 (1H, s, C<u>H</u>O), 7.5-7.1 (9H, m, H-9, H-9', H-10, H-10', H-11, H-13, H-14, H-15 and H-17), 5.7 (1H, d, J_3 = 4.4 Hz, OH), 5.6 (1H, s, H-7), 5.5 (1H, d, J_3 = 3,4 Hz, OH), 5.2 (1H, d, J_3 = 7.2 Hz, H-1), 4.2 (1H, m, H-6 eq.), 3.7-3.3 (5H, m, H-2, H-3, H-4, H-5 and H-6 ax.). ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 192.9 (1C, <u>C</u>HO), 157.6 (1C, C-12), 137.6 (1C, C-14), 130.5-126.3 (6C, C-9, C-9', C-10, C-10', C-11 and C-16), 123.5 (1C, C-15), 122.6 (1C, C-17), 116.4 (1C, C-13), 100.7 (1C, C-1), 100.5 (1C, C-7), 80.3-67.8 (4C, C-2, C-3, C-4 and C-5), 65.9 (1C, C-6).

*Synthesis of acylated benzylidene acetals **12**-**17**: general procedure*

To a solution of 0.745 mmol of palmitic acid, 0.745 mmol of *N*,*N*-dicyclohexylcarbodiimide (DCC) in 40 mL of dichloromethane was added, at -5 °C, 0.621 mmol of **10** or **11** and 15 mg of dimethylaminopyridine (DMAP). The mixture was stirred at -5 °C for six hours when the solvent was concentrated and the solid (dicyclohexylurea) was collected by filtration. The filtrate was concentrated to dryness affording a mixture of three products, as observed by TLC. The mixture was submitted to column chromatography (hexane/ethyl acetate 9:1) which afforded the pure products **12** (31%), **13** (27%), **14** (13%), **15** (24%), **16** (40%) and **17** (18%).

*4-formyl-2-methoxyphenyl 3-O-palmitoyl-4,6-O-benzylidene-β-D-glucopyranoside (**12**)*

This compound was obtained as white crystals; yield: 31%; m.p. 149.3-1151.3°C; $[\alpha]_D$ -33.7º (*c* 0.29 CH_2Cl_2); IR (υ/cm^{-1}): 3323 (OH), 3036 (ar. C-H), 2926, 2850 (sp^3 C-H), 1731 (ester C=O), 1691 (aldehyde C=O), 1571, 1535, 1507, 1465 (Ar. C=C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.9 (1H, s, C<u>H</u>O), 7.4-7.2 (8H, m, H-9, H-9', H-10, H-10', H-11, H-13, H-14 and H-16), 5.5 (1H, s, H-7), 5.3 (1H, t, J_3 = 9.6 Hz, H-3), 5.0 (1H, d, J_3 = 8.0 Hz, H-1), 4.3 (1H, dd, J_{eq} = 4.0 Hz e J_{ax} = 12.0 Hz, H-6 eq.), 3.9-3.8 (4H, m, OC<u>H</u>$_3$ and H-6 eq.), 3.8 (1H, t, J_3 = 10 2 Hz, H-2), 3.7 (1H, t, J_3 = 9.4 Hz, H-4), 3.6 (1H, m, H-5), 2.4 (2H, t, J_3 = 7.6 Hz, methylene), 1.3-1.2 (28H, m, methylene), 0.8 (3H, t, J_3 = 6.8 Hz, methyl). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 190.8 (1C, <u>C</u>HO), 173.6 (1C, ester C=O), 151.0 (1C, C-12), 150.8 (1C, C-13), 136,7 (1C, C-8), 132.9 (1C, C-15), 128.2 (2C, C-10 and C-10'), 126.0 (2C, C-9 and C-9'), 129.8-118.4 (4C, C-11, C-14, C-16 and C-17), 102.9 (1C, C-1), 101.5 (1C, C-7), 78.1 (1C, C-4), 73.1 (1C, C-2), 72.9 (1C, C-3), 68.4 (1C, C-6), 66.9 (1C, C-5), 56.1 (1C, OC<u>H</u>$_3$), 34.3-22.6 (14C, methylene), 14.0 (1C, methyl).

*4-formyl-2-methoxyphenyl 2-O-palmitoyl-4,6-O-benzylidene-β-D-glucopyranoside (**13**)*

This compound was obtained as white crystals; yield: 27%; m.p. 83.6-85.2 °C; $[\alpha]_D$ +12.0º (*c* 0.16, CH_2Cl_2); IR (υ/cm^{-1}): 3326 (OH), 2918, 2850 (sp^3 C-H), 1741 (ester C=O), 1685 (aldehyde C=O), 1590, 1508, 1466 (Ar. C=C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.9 (1H, s, C<u>H</u>O), 7.5-7.1 (8H, m, H-9, H-9', H-10, H-10', H-11, H-13, H-14 and H-16), 5.5 (1H, s, H-7), 5.2 (1H, t, J_3 = 8.0 Hz, H-2), 5.2 (1H, d, J_3 = 7.6 Hz, H-1), 4.4 (1H, dd, J_{eq} = 4.8 Hz e J_{ax} = 10.4 Hz , H-6 eq.), 4.0 (1H, t, J_3 = 8.8 Hz, H-3), 3.9 (3H, s, OC<u>H</u>$_3$), 3.8 (1H, t, J_3 = 10.4 Hz, H-6 ax.), 3.7 (1H, t, J_3 = 9.4 Hz, H-4), 3.6 (1H, m, H-5), 2.4-2.3 (2H, m, methylene), 1.6 (2H, t, J_3 = 7.6 Hz, methylene) 1.3-1.2 (24H, m, methylene), 0.8 (3H, t, J_3 = 6.8 Hz, H-methyl). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 190.8 (1C, <u>C</u>HO), 172.9 (1C, ester C=O), 151.1 (1C, C-12), 150.7 (1C, C-13), 136,7 (1C, C-8), 132.5 (1C, C-15), 128.3 (2C, C-10 and C-10'), 126.5 (2C, C-9 and C-9'), 129.3-117.1 (4C, C-11, C-14, C-16 and C-17), 102.2 (1C, C-7), 99.7 (1C, C-1), 80.7 (1C, C-4), 73.3 (1C, C-2), 72.2 (1C, C-3), 68.5 (1C, C-6), 66.5 (1C, C-5), 56.0 (1C, OC<u>H</u>$_3$), 34.2-22.6 (14C, methylene), 14.1 (1C, methyl).

*4-formyl-2-methoxyphenyl 2,3-di-O-palmitoyl-4,6-O-benzylidene-β-D-glucopyranoside (**14**)*

This compound was obtained as white crystals; yield: 13%; m.p. 81.5-83.1 °C; $[\alpha]_D$ -81.6º (*c* 0.19, CH_2Cl_2); IR (υ/cm^{-1}): 2953, 2916, 2849 (sp^3 C-H), 1746 (ester C=O), 1696 (aldehyde C=O), 1593, 1507, 1467 (ar. C=C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.8 (1H, s, C<u>H</u>O), 7.4-7.1 (7H, m, H-9, H-9', H-10, H-10', H-11, H-14 and H-16), 7.1 (1H, d, J_3 = 8.8 Hz, H-13), 5.5 (1H, s, H-7), 5.4 (1H, t, J_3 = 9.2 Hz, H-3), 5.3 (1H, t, J_3 = 9.2 Hz, H-2), 5.2 (1H, d, J_3 = 7.6 Hz, H-1), 4.3 (1H, dd, J_{eq} = 4.8 Hz e J_{ax} = 10.4 Hz, H-6 eq.), 3.8 (3H, s, OC<u>H</u>$_3$), 3.8-3.7 (3H, m, H-4, H-5 and H-6 ax.), 2.3-2.2 (4H, m, methylene), 1.6-1.2 (52H, m, methylene), 0.8 (6H, t, J_3 = 6.8 Hz, methyl). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 190.8 (1C, aldehyde C=O), 172.8 (1C, ester C=O), 172.1 (1C, ester C=O), 151.0 (1C, C-12), 150.8 (1C, C-13), 136,6 (1C, C-8), 132.7 (1C, C-15), 128.2 (2C, C-10 and C-10'), 126.0 (2C, C-9 and C-9'), 129.1-110.6 (4C, C-11, C-14, C-16 and C-17), 101.5 (1C, H-7), 110.1 (1C, C-1), 78.1 (1C, C-4), 71.6 (1C, C-2), 71.1 (1C, C-3), 68.4 (1C, C-6), 66.8 (1C, C-5), 56.0 (1C, OC<u>H</u>$_3$), 34.1-24.9 (28C, methylene), 14.5 (2C, methyl).

*3-formylphenyl 3-O-palmitoyl-4,6-O-benzylidene-β-D-glucopyranoside (**15**)*

This compound was obtained as white crystals; yield: 24%; m.p. 91.4-92.6 °C; $[\alpha]_D$ +16.0º (*c* 0.10 CH_2Cl_2); IR (υ/cm^{-1}): 3554 (OH), 2921, 2852 (sp^3 C-H), 1732 (ester C=O), 1701 (aldehyde C=O), 1589, 1488 (Ar. C=C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.9 (1H, s, C<u>H</u>O), 7.5-7.2 (9H, m, H-9, H-9', H-10, H-10', H-11, H-13, H-14, H-15 and H-17), 5.5 (1H, s, H-7), 5.3 (1H, t, J_3 = 9.0 Hz, H-3), 5.1 (1H, d, J_3 = 7.6 Hz, H-1), 4.4-4.3 (1H, m, H-6 eq.), 3.9-3.6 (4H, m, H-2, H-4, H-5 and H-6 ax.), 3.4 (1H, s, OH), 2.4 (2H, t, J_3 = 7.4 Hz, methylene), 1.6 (2H, m, methylene), 1.2 (24H, m, methylene), 0.8 (3H, t, methyl). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 191.8 (1C, <u>C</u>HO), 174.4 (1C, ester C=O), 157.5 (1C, C-12), 137.9 (1C, C-8), 136,9 (1C, C-14), 136.9 (1C, ar.), 128.4 (2C, C-10 and C-10'), 126.8 (2C, C-9 and C-9'), 130.5-116.6 (5C, C-11, C-13, C-15, C-16 and C-17), 101.6 (2C, C-7 and C-1), 78.2 (1C, C-4), 73.8 (1C, C-5), 73.4 (1C, C-3), 68.6 (1C, C-6), 66.7 (1C, C-2), 34.5-22.8 (14C, methylene), 14.2 (1C, methyl).

*3-formylphenyl 2-O-palmitoyl-4,6-O-benzylidene-β-D-glucopyranoside (**16**)*

This compound was obtained as white crystals; yield: 40%; m.p. 97.2-98.6 °C; $[\alpha]_D$ +23.8º (*c* 0.23, CH_2Cl_2); IR (υ/cm^{-1}): 3439 (OH), 3071 (ar. C-H), 2917, 2850 (sp^3 C-H), 1732 (ester C=O), 1700 (aldehyde C=O), 1597, 1584, 1483 (Ar. C=C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.96 (1H, s, C<u>H</u>O), 7.5-7.2 (9H, m, H-9, H-9', H-10, H-10', H-11, H-13, H-14, H-15 and H-17), 5.5 (1H, s, H-7), 5.2-5.1 (2H, m, H-2 and H-1), 4.4 (1H, dd, J_{eq} = 4.2 Hz e J_{3ax} = 10.0 Hz, H-6 eq.), 3.9 (1H, t, J_3 = 8.4 Hz, H-3), 3.8-3.6 (3H, m, H-4, H-5 and H-6 ax.), 2.3 (2H, t, J_3 = 7.4 Hz, methylene), 1.6 (2H, q, J_3 = 7.0 Hz, methylene), 1.2 (24H, s, methylene), 0.8 (3H, t, methyl). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 191.7 (1C, <u>C</u>HO), 173.2 (1C, ester C=O), 157.5 (1C, C-12), 138,0 (1C, C-8), 136.9 (1C, C-14), 128.5 (2C, C-10 and C-10'), 126.5 (2C, C-9 and C-9'), 130.5-115.7 (5C, C-11, C-13, C-15, C-16 and C-17), 102.1 (1C, C-7), 99.3 (1C, C-1), 80.7 (1C, C-4), 73.6 (1C, C-5), 72.3 (1C, C-2), 68.6 (1C, C-6), 66.6 (1C, C-3), 34.4-22.8 (14C, methylene), 14.3 (1C, methyl).

*3-formylphenyl 2,3-di-O-palmitoyl-4,6-O-benzylidene-β-D-glucopyranoside (**17**)*

This compound was obtained as white crystals; yield: 18%; m.p. 60.3-63.1 °C; $[\alpha]_D$ +8.9º (*c* 0.22, CH_2Cl_2); IR (υ/cm^{-1}): 2910, 2850 (sp^3 C-H), 1747 (ester C=O), 1698 (aldehyde C=O), 1596, 1536, 1484, 1467 (Ar. C=C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.9 (1H, s, C<u>H</u>O), 7.6-7.2 (9H, m, H-9, H-9', H-10, H-10', H-11, H-13, H-14, H-15 and H-17), 5.5 (1H, s, H-7), 5.4 (1H, t, J_3 = 8.8 Hz, H-2), 5.3 (1H, t, J_3 = 8.8 Hz, H-3), 5.2 (1H, d, J_3 = 7.6 Hz, H-1), 4.4 (1H, dd, J_{eq} = 4.8 Hz e J_{ax} = 10.4 Hz, H-6 eq.), 3.8 (2H, m, H-4 and H-6 ax.), 3.7-3.6 (1H, m, H-5), 2.3-2.2 (4H, m, methylene), 1.5 (4H, m, methylene), 1.2 (48H, sl, methylene), 0.8 (6H, t, J_3 = 6.6 Hz, methyl). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 191.3 (1C, <u>C</u>HO), 172.7 (1C, ester C=O), 172.2 (1C, ester C=O), 157.2 (1C, C-12), 137,9 (1C, C-8), 136.6 (1C, C-14), 128.2 (2C, C-10 and C-10'), 126.1 (2C, C-9 and C-9'), 130.3-116.1 (5C, C-11, C-13, C-15, C-16 and C-17), 101.5 (1C, C-7), 99.4 (1C, C-1), 78.1 (1C, C-4), 71.6 (1C, C-2), 71.2 (1C, C-3), 68.4 (1C, C-6), 66.7 (1C, C-5), 34.1-22.6 (28C, methylene), 14.1 (2C, methyl).

*Synthesis of neoglycolipids **18**-**23**: general procedure*

To a solution of 0.389 mmol of acylated benzylidene acetals in 20 mL of acetone, was added 15 drops of HCl, at 0 ºC. The reaction mixture was stirred at room temperature and the reaction monitored by TLC. After the completion of the reaction, the mixture was neutralized with IRA 400 resin. The resin was filtered off and the filtrate was concentrated in vaccum to afford neoglycolipids **18**-**23**.

*4-formyl-2-methoxyphenyl 3-O-palmitoyl-β-D-glucopyranoside (**18**)*

This compound was obtained as white crystals; yield: 92%; m.p. 134.4-135.8 °C; $[\alpha]_D$ +83.3º (*c* 0.14 DMSO); IR (υ/cm^{-1}): 3323 (OH), 2919, 2850 (sp^3 C-H), 1733 (ester C=O), 1702 (aldehyde C=O), 1625, 1592, 1575, 1535, 1508 (Ar. C=C). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.8 (1H, s, C<u>H</u>O), 7.5 (1H, dd, J_4 = 1.8 Hz e J_3 = 8.4 Hz, H-9), 7.4 (1H, d, J_4 = 1.8 Hz, H-11), 7.3 (1H, d, J_3 = 8.4 Hz, H-8), 5.5 (1H, d, J_3 = 6.4 Hz, OH), 5.2 (2H, m, H-1 e OH), 4.9 (1H, t, J_3 = 9.4 Hz, H-3), 4.6 (1H, t, J_3 = 5.6 Hz, OH), 3.8 (3H, s, OC<u>H</u>$_3$), 3.6 (1H, dd, J_3 = 5.2 Hz e J_2 = 10.4 Hz, H-6), 3.5-3.3 (4H, m, H-2, H-4, H-5 and H-6), 2.3 (2H, t, J_3 = 7.4 Hz, methylene), 1.7-1.2 (26H, m, methylene), 0.8 (3H, t, J_3 = 6.8 Hz, methyl). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 191.4 (1C, <u>C</u>HO), 172.3 (1C, ester C=O), 151.4 (1C, C-7), 149.2 (1C, C-8), 130.6 (1C, C-10), 125.2 (1C, C-11), 114.5 (1C, C-12), 110.5 (1C, C-9), 98.9 (1C, C-1), 77.4 (1C, C-5), 76.7 (1C, C-3), 71.0 (1C, C-2), 67.2 (1C, C-4), 60.1 (1C, C-6), 55.6 (1C, OC<u>H</u>$_3$), 33.7-22.0 (14C, methylene), 13.8 (1C, methyl).

*4-formyl-2-methoxyphenyl 2-O-palmitoyl-β-D-glucopyranoside (**19**)*

This compound was obtained as white crystals; yield: 88%; m.p. 135.1-137.0 °C; $[\alpha]_D$ +27.7º (*c* 0.14 DMSO); IR (υ/cm^{-1}): 3322 (OH), 2920, 2849 (sp^3 C-H), 1718 (ester C=O), 1688 (aldehyde C=O), 1624, 1591, 1536, 1510, 1462 (Ar. C=C). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.8 (1H, s, C<u>H</u>O), 7.5 (1H, d, J_3 = 8.0 Hz, H-9), 7.4 (1H, s, H-11), 7.3 (1H, d, J_3 = 8.4 Hz, H-8), 5.4 (1H, d, J_3 = 5.2 Hz, OH), 5.3-5.2 (2H, m, H-1 and OH), 4.8 (1H, t, J_3 = 8.6 Hz, H-2), 4.6 (1H, s, OH), 3.7 (3H, s, OC<u>H</u>$_3$), 3.7-3.4 (5H, m, H-3, H-4, H-5, H-6 and H-6'), 2.2 (2H, m, methylene), 1.4-1.0 (26H, m, methylene), 0.8-0.8 (3H, m, methyl). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 191.4 (1C, <u>C</u>HO), 171.6 (1C, ester C=O), 151.3 (1C, C-7), 149.5 (1C, C-8), 131.1 (1C, C-10), 124.9 (1C, C-11), 115.5 (1C, C-12), 110.8 (1C, C-9), 97.9 (1C, C-1), 77.3 (1C, C-5), 73.7 (1C, C-2), 73.0 (1C, C-3), 69.6 (1C, C-4), 60.4 (1C, C-6), 55.7 (1C, OC<u>H</u>$_3$), 33.6-22.0 (14C, methylene), 13.8 (1C, methyl).

*4-formyl-2-methoxyphenyl 2,3-di-O-palmitoyl-β-D-glucopyranoside (**20**)*

This compound was obtained as white crystals; yield: 83%; m.p. 93.5-95.0 °C; $[\alpha]_D$ +90.0º (*c* 0.66 DMSO); IR (υ/cm^{-1}): 3433 (OH),

2955, 2849 (sp^3 C-H), 1743 (ester C=O), 1689 (aldehyde C=O), 1589, 1508, 1471 (Ar. C=C). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.9 (1H, s, CHO), 7.5 (1H, dd, J_4 = 1.8 Hz e J_3 = 8.0 Hz, H-9), 7.4 (1H, d, J_4 = 1.8 Hz, H-11), 7.4 (1H, d, J_3 = 8.0 Hz, H-8), 5.4 (1H, d, J_3 = 7.6 Hz, H-1), 5.2 (1H, t, J_3 = 9.0 Hz, H-3), 5.1 (1H, t, J_3 = 9.0 Hz, H-2), 4.9 (1H, s, OH), 4.0 (1H, s, OH), 3.8 (3H, s, OCH_3), 3.9-3.7 (4H, m, H-4, H-5, H-9 and H-6'), 2.3-2.2 (2H, m, methylene), 1.6-1.5 (2H, m, methylene), 1.3-1.2 (52H, m, methylene), 0.8 (6H, t, J_3 = 6.6 Hz, methyl). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 191.1 (1C, CHO), 172.8 (1C, ester C=O), 172.0 (1C, ester C=O), 152.2 (1C, C-7), 150,9 (1C, C-8), 132.8 (1C, C-10), 125.4 (1C, C-11), 117.1 (1C, C-12), 111.5 (1C, C-9), 99.4 (1C, C-1), 77.7 (1C, C-5), 75.5 (1C, C-3), 71.8 (1C, C-2), 68.7 (1C, C-4), 61.5 (1C, C-6), 56.2 (1C, OCH_3), 34.3-22.9 (28C, methylene), 14.1 (2C, methyl).

*3-formylphenyl 3-O-palmitoyl-β-D-glucopyranoside (**21**)*

This compound was obtained as white crystals; yield: 86%; m.p. 74.5-76.0 °C; $[\alpha]_D$ +19.8º (*c* 0.70 DMSO); IR (υ/cm^{-1}): 3404 (OH), 2919, 2850 (sp^3 C-H), 1721 (ester C=O), 1681 (aldehyde C=O), 1608, 1589, 1486, 1469 (Ar. C=C). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.9 (1H, s, CHO), 7.6-7.5 (3H, m, H-9, H-10 and H-12), 7.3 (1H, d, J_3 = 7.6 Hz, H-8), 5.5 (1H, d, J_3 = 5.6 Hz, OH), 5.2 (1H, d, J_3 = 5.6 Hz, OH), 5.1 (1H, d, J_3 = 7.6 Hz, H-1), 4.9 (1H, t, J_3 = 9.4 Hz, H-3), 4.6 (1H, s, OH), 3.7-3.6 (1H, m, H-6), 3.5-3.3 (4H, m, H-2, H-4, H-5 and H-6'), 2.3 (2H, t, J_3 = 7.2 Hz, methylene), 1.5 (2H, q, J_3 = 7.0 Hz, methylene), 1.2 (24H, m, methylene), 0.8 (3H, t, J_3 = 6.8 Hz, methyl). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 192.7 (1C, CHO), 172.2 (1C, ester C=O), 157.6 (1C, C-7), 137,5 (1C, C-9), 130.3-116.5 (3C, C-8, C-10 and C-11), 122.5 (1C, C-12), 100.0 (1C, C-1), 77.2 (1C, C-3), 76.7 (1C, C-5), 71.1 (1C, C-2), 67.4 (1C, C-4), 60.2 (1C, C-6), 33.7-22.0 (14C, methylene), 13.8 (1C, methyl).

*3-formylphenyl 2-O-palmitoyl-β-D-glucopyranoside (**22**)*

This compound was obtained as white crystals; yield: 89%; m.p. 136.0-138.0 °C; $[\alpha]_D$ +103.4º (*c* 0.11 DMSO); IR (υ/cm^{-1}): 3509 (OH), 2920, 2849 (sp^3 C-H), 1721 (ester C=O), 1703 (aldehyde C=O), 1596, 1529, 1489, 1466 (Ar. C=C). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.9 (1H, s, CHO), 7.5-7.2 (4H, m, H-8, H-9, H-10 and H-12), 5.3 (1H, s, OH), 5.2 (1H, d, J_3 = 8.0 Hz, H-1), 5.2 (1H, sl, OH), 4.8 (1H, t, J_3 = 8.8 Hz, H-2), 4.6 (1H, sl, OH), 3.7 (1H, m, H-6), 3.5-3.3 (4H, m, H-3, H-4, H-5 and H-6'), 2.2 (2H, t, J_3 = 7.2 Hz, methylene), 1.5 (2H, q, J_3 = 7.2 Hz, methylene), 1.2-1.1 (24H, m, methylene), 0.8 (3H, t, J_3 = 6.8 Hz, methyl). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 192.5 (1C, CHO), 171.8 (1C, ester C=O), 157.2 (1C, C-7), 137.5 (1C, C-9), 130.4(1C, C-10), 123.4 (1C, C-11), 122.5 (1C, C-12), 116.3 (1C, C-8), 97.8 (1C, C-1), 77.2 (1C, C-5), 73.7 (1C, C-3), 73.2 (1C, C-2), 69.6 (1C, C-4), 60.3 (1C, C-6), 33.7-22.0 (14C, methylene), 13.9 (1C, methyl).

*3-formylphenyl 2,3-di-O-palmitoyl-β-D-glucopyranoside (**23**)*

This compound was obtained as white crystals; yield: 72%; m.p. 107.0-108.0 °C; $[\alpha]_D$ +20.4º (*c* 0.58 DMSO); IR (υ/cm^{-1}): 3397 (OH), 2955, 2849 (sp^3 C-H), 1731 (ester C=O), 1687 (aldehyde C=O), 1593, 1491, 1472 (Ar. C=C). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.9 (1H, s, CHO), 7.6 (1H, d, J_3 = 7.6 Hz, H-10), 7.5-7.4 (2H, m, H-9 and H-12) 7.3 (1H, d, J_3 = 7.6 Hz, H-8), 5.5-5.2 (2H, m, H-1 and OH), 5.1 (1H, t, J_3 = 9.1 Hz, H-3), 4.9 (1H, t, J_3 = 9.1 Hz, H-2), 4.7 (1H, d, J_3 = 5.6 Hz, OH), 3.7-3.3 (4H, m, H-4, H-5, H-6 and H-6'), 2.3-0.8 (56H, m, methylene), 0.8 (6H, t, J_3 = 6.4 Hz, methyl). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 191.3 (1C, CHO), 172.6 (1C, ester C=O), 172.0 (1C, ester C=O), 152.4 (1C, C-7), 150,5 (1C, C-8), 132.4 (1C, C-10), 125.0 (1C, C-11), 117.0 (1C, C-12), 111.9 (1C, C-9), 100.2 (1C, C-1), 77.5 (1C, C-5), 75.0 (1C, C-3), 71.3 (1C, C-2), 68.2 (1C, C-4), 61.0 (1C, C-6), 34.4-22.6 (28C, methylene), 14.0 (2C, methyl).

In vitro antifungal susceptibility testing

The reference *Candida albicans* (ATCC 18804), *C. krusei* (ATCC 20298), *C. parapsilosis* (ATCC 22019) and *C. tropicalis* (ATCC 750) were obtained from the American Type Culture Collection. Susceptibility assays were performed according to the CLSI (Clinical and Laboratory for Standards Institute), broth microdilution reference method M27-A2.[16] *Candida* species were cultured on SDA (Difco Laboratories, Detroit, MI, USA) for 48 h at 35 °C. The inoculum concentration was determined by measuring the transmittance of fungal suspensions at 530 nm. The compounds **6**-**23** were primarily dissolved in dimethylsulfoxide (DMSO) and diluted in sodium bicarbonate-free RPMI 1640 medium (Sigma, St Louis, MO, USA) buffered with 165 mmol L^{-1} morpholine propanesulphonic acid (MOPS; Sigma), pH 7.0, and supplemented with 4 mmol L^{-1} L-glutamine. Fungal inoculum concentrations were adjusted to 1 x 10^3 to 5 x 10^3 cells mL^{-1}. One hundred microlitres of each test solution was distributed in sterile flat-bottom 96-well microplates (Difco) followed by the addition of 100 µL of inoculum. All the compounds were tested at a concentration range of 0.25–250 µg mL^{-1}. Serial twofold dilutions were prepared as described in CLSI document M27-A2. Microplates were incubated at 35 °C for 48 h. RPMI medium without the compounds and the solvents to be used as a control for the growth and sterility. Fluconazole (Pfizer, São Paulo, Brazil) was included as positive antifungal control. The endpoints were determined visually by comparing with the growth in the drug-free control well. The minimum inhibitory concentration (MIC) was defined as the lowest concentration that did not allow for the detection of any visual fungal growth.

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