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Synthesis and evaluation of *N*-alkyl-β-D-glucosylamines on the growth of two wood fungi, *Coriolus versicolor* and *Poria placenta*

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ABSTRACT

Various glucosylamines were synthesized from glucose and different alkyl amine compounds. These amino compounds are β-D-glucopyranosylamine (GPA), *N*-ethyl-β-D-glucopyranosylamine (EtGPA), *N*-butyl-β-D-glucopyranosylamine (BuGPA), *N*-hexyl-β-D-glucopyranosylamine (HeGPA), *N*-octyl-β-D-glucopyranosylamine (OcGPA), *N*-dodecyl-β-D-glucopyranosylamine (DoGPA), *N*-(2-hydroxyethyl)-β-D-glucopyranosylamine (HEtGPA) and *N*,*N*-di(2-hydroxyethyl)-β-D-glucopyranosylamine (DHEtGPA). They were tested for their antifungal activity against the growth of *Coriolus versicolor* and *Poria placenta*. An improvement of the antifungal activity with the increase of alkyl chain length was observed. DoGPA exhibited the best antifungal activity against both strains. It completely inhibited the fungal growth at 0.01×10^{-3} mol mL^{-1} and 0.0075×10^{-3} mol mL^{-1} for *C. versicolor* and *P. placenta*, respectively. For other glucosylamines higher concentrations were needed for complete inhibition of fungi.

1. Introduction

Wood-decaying fungi degrade the principal components of wood and decrease its mechanical properties. To avoid the biodegradation process, various compounds have been used for treating wood. The treatment concerns not only reactions made with wood components, but it also includes its impregnation with various biocides.[1–3] These techniques have been used and have shown satisfying results overall. However, many of the chemicals used have a high toxicity profile and have been removed from the market. Thus, an interest in developing new bioactive agents with low toxicities and with acceptable impact on the environment is increasing. A way to achieve this goal is to use renewable materials in the synthesis process. Slight modifications of carbohydrates made by introducing some pharmacophoric groups can help to obtain desirable biocides. Recent studies have shown that amino-containing carbohydrate compounds exhibited antifungal activities and have indicated promising results.[4,5]

Interest in the study of glucosylamines has grown over the years due to their widespread uses. These compounds not only constitute starting materials for the synthesis of different amino bioactive compounds,[6,7] but also can be used without any modification in various other applications. According to Stan et al.,[8] *N*-alkyl glucosylamines can be used as gelators for various organic liquids. Furthermore, Fabbro and Grabowski,[9] Greenberg et al.,[10] and Legler et al.,[11,12] reported that these compounds constitute good inhibitors of glycosidase activities. For this reason, it seems interesting to determine if these compounds possess an antifungal activity on wood fungi. Moreover, the synthesis and the evaluation of the biological activities of different *N*-alkyl glucosylamines were of interest since the association of the amino compounds with a carbohydrate not only facilitates the interaction with microorganisms but also increases the compound's solubility in water, and enhances the biological activity as well.[4,13–15] These compounds are advantageous since they are easily synthesized from renewable resources such as sugars.

Different glucosylamine derivatives were synthesized in this study and tested for their antifungal activity against the growth of two basiodiomycetes, one a white-rot fungus, *C. versicolor* and the other a brown-rot fungus, *Poria placenta*. The effect of the alkyl group length on the antifungal activities was also investigated.

2. Results and discussion

2.1. Synthesis and identification of glucosylamines

Eight glucosylamine derivatives were synthesized as previously reported (Scheme 1 and Table 1).[16–22] In the past, GPA, OcGPA and DoGPA were partially characterized by NMR spectroscopy. In this study, we fully characterized all synthesized glucosylamines using both FTIR and NMR spectroscopy. The IR data given in this paper were not previously reported for these compounds. In addition, HEtGPA and EtGPA were characterized by HRMS. GPA was characterized by FTIR spectroscopy by the N–H bending vibration bands observed around 1619 cm^{-1} (Fig. 1). This band was detectable from

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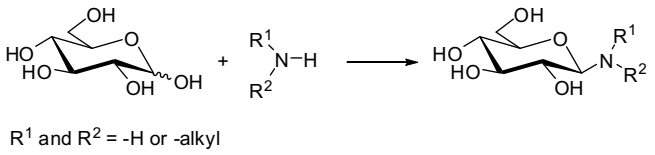

Scheme 1. Synthesis of glucosylamines.

secondary amines around 1510 cm^{-1}.[23] In addition, GPA showed two other particular signal bands in the form of double vibrations at 3333 and 3344 cm^{-1}, which indicated an N–H stretching vibration with respect to the free amino group. The primary substituted glucosylamines and GPA indicated other N–H out-of-plane vibration bands between 875 and 890 cm^{-1}. DHEtGPA did not show the vibration bands either at 1619 cm^{-1} or at 875–890 cm^{-1}. Both observations strongly supported a total substitution of the all N-hydrogen atoms in the molecule. All synthesized glucosylamines showed bands around 1099–1074 cm^{-1}. These bands were attributed to the bending vibrations of C–N. The attribution of the characteristic bands in IR spectroscopy was monitored by the IR spectrum of D-glucose (Glu), the compound used as the starting material. Both ^{1}H NMR and ^{13}C NMR spectroscopy confirmed the data obtained from FTIR spectroscopy. Formation of glucosylamines was confirmed in the first time by the H-1 signals found at lower chemical shifts compared to those of glucose. According to Blasko et al.,[24] the H-1α and H-1β of glucose are found around 5.23 and 4.65 ppm, respectively. In this study, the H-1β signals of glucosylamines were between 4.15 and 3.82 ppm, which confirms substitution of the OH group by amino groups. The stark absence of H-1α signals in the ^{1}H NMR spectra, which may be found together with H-1β in the ^{1}H NMR spectrum of glucose, also indicate the formation of glucosylamines. In comparison to GPA, the anomeric hydrogens (H-1) of *N*-alkyl-β-D-glucopyranosylamines were shifted at lower chemical shifts due to the effect of electron-donor groups fixed to the amino group of GPA. The H-1 signal of compounds dissolved in D_2O was found at 4.15, 4.05, 4.06, 4.13 and 4.03 ppm for GPA, EtGPA, HEtGPA, DHEtGPA and BuGPA, respectively, while those of HeGPA, OcGPA and DoGPA dissolved in

Table 1
Synthesized glucosylamines and their physical properties

R^1	R^2	Compounds	Yield (%)	Mp (°C)	R_f
H	H	GPA	98.3, lit.[17] 100	108–110, lit.[16] 128–129	0.35[a]
H	Ethyl	EtGPA	87.4	96.8–97.2	0.63[a]
H	Butyl	BuGPA	84.9	87–91, lit.[16] 88–90	0.60[a]
H	Hexyl	HeGPA	91.4	94–95, lit.[16] 93–95	0.47[b]
H	Octyl	OcGPA	97.3 lit.[21] 86	98–100, lit.[16] 102	0.58[b]
H	Dodecyl	DoGPA	72.3 lit.[21] 60	107–109, lit.[16] 105.5	0.69[b]
H	2-Hydroxyethyl	HEtGPA	58.6, lit.[18] 91	114.8–115, lit.[22] 115–116	0.67[a]
2-Hydroxyethyl	2-Hydroxyethyl	DHEtGPA	91.3, lit.[18] 58	118–119, lit.[18] 128	0.69[a]

Eluent.
[a] 8:2 MeOH–CH_2Cl_2.
[b] 2:8 MeOH–CH_2Cl_2.

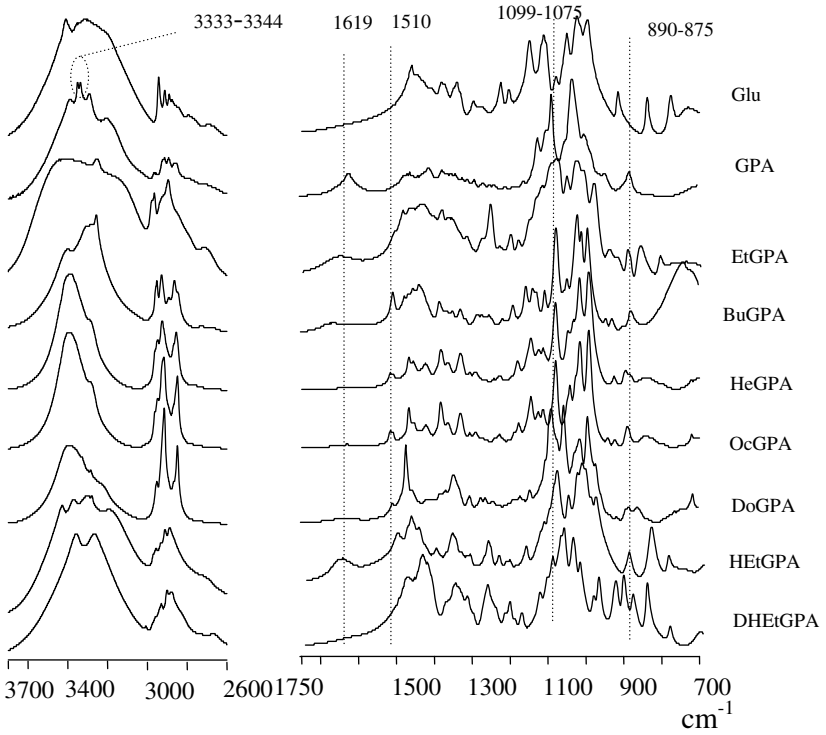

Figure 1. FTIR spectroscopy of different glucosylamines.

CD_3OD were found at 3.82, 3.83 and 3.83 ppm, respectively. The high values obtained for $J_{1,2}$ between H-1 and H-2 (8.3–9.6 Hz) indicated that the synthesized compounds are in the form of β isomers. In addition, disappearance of the C-1 signal at 96.05 ppm, which indicates α-glucose in the ^{13}C NMR spectrum,[25] confirmed the formation of glucosylamines.

Two glucosylamines such as EtGPA and HEtGPA were not fully characterized in the past.[18,22] Both compounds are now analyzed by high-resolution mass spectroscopy (HRMS). Peaks of $(M+H)^+$ were found with *m*/*z* 224.1 and 208.1 for HEtGPA and EtGPA, respectively. Other significant peaks of $(M+Na)^+$ with *m*/*z* 246.09 (67.7%), 206 (15%) and *m*/*z* 230.09 (11.7%) were found for HEtGPA and EtGPA, respectively. A peak with *m*/*z* 206 derived from HEtGPA indicated the loss of H_2O from an alkyl chain. These results completed the FTIR and ^1H NMR data and confirmed the structure of these compounds.

2.2. Antifungal activity of glucosylamines

The antifungal activity of glucosylamines on *C. versicolor* and *P. placenta* was determined by the radial growth method. The percentage of inhibition of each glucosylamine at different concentrations was determined after nine days of incubation, and results are grouped in Tables 2 and 3. Tebuconazole (TB), currently used in wood treatment, was selected as a positive control fungicide.

The antifungal activity increased with the concentration of glucosylamines. For *C. versicolor* (Table 2), concentrations from 0.075×10^{-3} to 0.15×10^{-3} mol mL^{-1} significantly delayed the growth of fungi (p <0.05). For example, the antifungal activity of GPA increased from 50% to 82% when concentrations of 0.075×10^{-3} and 0.15×10^{-3} mol mL^{-1} were used, respectively. The same observations were carried out for other tested glucosylamines. However, at concentrations lower than 0.075×10^{-3} mol mL^{-1}, GPA, EtGPA, BuGPA, HeGPA, OcGPA, HEtGPA and DHEtGPA were not significantly effective. The sensitivity of *P. placenta* (Table 3) at different compound concentrations was quite similar to that observed with *C. versicolor*. Fungus was sensitive to almost all glucosylamines from 0.025×10^{-3} mol mL^{-1} to 0.075×10^{-3} mol mL^{-1}. At lower concentrations the antifungal activity of compounds tested against *P. placenta* rapidly decreased. These results confirmed the importance of biocide concentrations in the treatment of wood decay fungi.

The same results showed the effect of alkyl chain length on the antifungal activity. The percentage values of inhibition from GPA and all *N*-alkyl glucosylamines were significantly different. At 0.1×10^{-3} mol mL^{-1}, the activity of BuGPA against *C. versicolor* was 1.7 times higher than that from GPA. Moreover, DoGPA was found to be the most effective inhibitor against *C. versicolor* under our conditions. The effect of alkyl chain length on antifungal activity was also observed with *P. placenta*. At 0.05×10^{-3} mol mL^{-1}, GPA profoundly affected the growth with an inhibition of 81%, while BuGPA significantly (p <0.05) depressed the growth of *P. placenta* with an inhibition of 96%. The difference between HeGPA, OcGPA and DoGPA was observed at 0.025 mol mL^{-1} where HeGPA and OcGPA significantly reduced (p <0.05) the growth of microorganisms with the percentages of inhibition close to 96% and 98%, respectively, while DoGPA completely inhibited the fungal growth. The higher effectiveness of DoGPA was confirmed at lower concentrations of 0.0025×10^{-3} and 0.0075×10^{-3} mol mL^{-1} where it delayed the growth of the fungal strain with 94% and 100% inhibition, respectively.

Furthermore, HEtGPA and DHEtGPA were tested to evaluate the effectiveness of a hydroxyl group in comparison with EtGPA. At the concentration of 0.075×10^{-3} mol mL^{-1}, HEtGPA and DHEtGPA reached an inhibition of *C. versicolor* about of 59% and 55%, respectively, while that from EtGPA was only 32%. No growth was observed in the case of these compounds at 0.15×10^{-3} mol mL^{-1}. The effectiveness of the hydroxyl group was also evaluated against *P. placenta*. At the concentration of 0.075×10^{-3} mol mL^{-1}, HEtGPA and DHEtGPA completely inhibited the growth of *P. placenta*, contrary to EtGPA, which delayed the growth at 59%.

The antifungal activity assessment with tebuconazole indicated that this commercial biocide completely inhibited both strains at 0.0025 mol mL^{-1}.[26,27] At the same concentration DoGPA significantly delayed the mycelium growth. Results from this study showed that, using the present experimental conditions, *C. versicolor* was more resistant to the synthesized glucosylamines than *P. placenta*, the reason for which was not determined in this study. However, according to the literature, both wood fungi exhibited their activity by different decay mechanisms. On one hand, *C.*

Table 2
Inhibition of the growth of *C. versicolor* after nine days of incubation with different concentrations of glucosylamines and the corresponding free amines (values in parentheses)

	Percentage of inhibition ± SEM								
mol/mL 10^{-3}	GPA	EtGPA	BuGPA	HeGPA	OcGPA	DoGPA	HEtGPA	DHEtGPA	TB
0.0025	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	61.2 ± 3.1	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0
0.01	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	14.5 ± 0.4	100.0 ± 0.0 (66.3 ± 2)	0.0 ± 0.0	0.0 ± 0.0	—
0.075	49.8 ± 3.1	32.2 ± 4.9	76.9 ± 2.5	80.4 ± 0.4	86.3 ± 0.4	100.0 ± 0.0	58.8 ± 1.0	54.9 ± 2.8	—
0.1	50.6 ± 0.0	37.7 ± 5.1	87.5 ± 0.3	100.0 ± 0.0 (55.4 ± 1.8)	100.0 ± 0.0 (59.6 ± 1.4)	100.0 ± 0.0	62.7 ± 0.8	65.9 ± 1.8	—
0.15	82.4 ± 0.0	98.8 ± 0.8	100.0 ± 0.0 (23.1 ± 1.8)	—	—	—	100.0 ± 0.0 (19.2 ± 2.4)	100.0 ± 0.0 (26.7 ± 1.3)	—

Table 3
Inhibition of the growth of *P. placenta* after nine days of incubation with different concentrations of glucosylamines

	Percentage of inhibition ± SEM								
mol/mL 10^{-3}	GPA	EtGPA	BuGPA	HeGPA	OcGPA	DoGPA	HEtGPA	DHEtGPA	TB
0.0025	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	93.7 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	100 ± 0.0
0.0075	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	—
0.025	40.0 ± 3.5	15.3 ± 1.4	78.4 ± 3.5	96.1 ± 2.0	98.0 ± 1.4	100.0 ± 0.0	17.3 ± 2.8	29.8 ± 0.4	—
0.05	81.6 ± 2.2	26.3 ± 4.1	95.7 ± 1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	29.4 ± 4.2	45.5 ± 2.0	—
0.075	100.0 ± 0.0	58.8 ± 4.4	100.0 ± 0.0	—	—	—	100.0 ± 0.0	100.0 ± 0.0	—

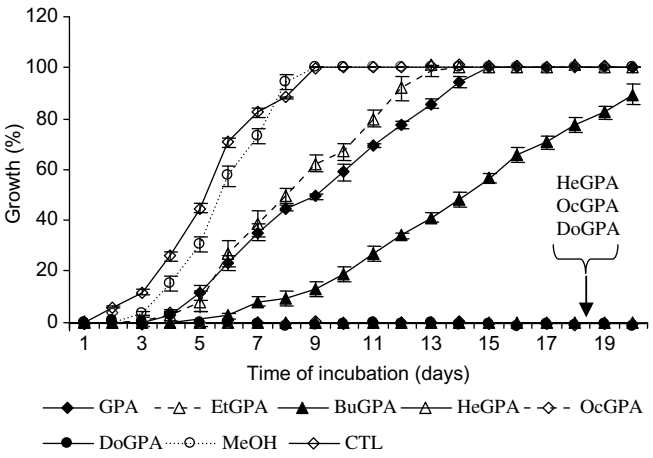

Figure 2. Effect of the length of alkyl chains on the kinetic growth of *C. versicolor* at the concentration of 0.1×10^{-3} mol mL^{-1}.

versicolor, a white-rot fungus, is able to produce laccases, which are highly effective in lignin degradation, while *P. placenta*, a brown-rot fungus, mainly acts with a nonenzymatic mechanism.[28,29] On the other hand, it is known that *C. versicolor* is highly effective in effecting the decomposition of hardwood components, while *P. placenta* is more active in the case of softwood components.[30,31] These reports can help one to understand the difference in sensitivity between *P. placenta* and *C. versicolor*.

According to the nature and concentration of glucosylamines, the kinetic growth of fungi was differently affected by these compounds. To visualize this, the radial growth of mycelium was followed daily. The behaviour of the fungal strains towards these inhibitors at the chosen concentrations is represented in Figures 2 and 3 for *C. versicolor* and in Figures 4 and 5 for *P. placenta*. A plot of kinetic growth of *C. versicolor* is represented in Figure 2 at a concentration of 0.1×10^{-3} mol mL^{-1} to determine the impact of both alkyl and hydroxyl groups on the kinetic growth of fungi. GPA, BuGPA and EtGPA affected the lag phase of mold by delaying the growth of *C. versicolor* of approximately two days compared to the control experiment, whereas HeGPA, OcGPA and DoGPA completely cancelled the growth of fungus. The same behaviour was observed for *P. placenta*, with an increase of the lag phase by the bioactive agents from one to six days (Fig. 4). At a concentration of 0.075×10^{-3} mol mL^{-1}, EtGPA and HEtGPA delayed the lag phase of *P. placenta* for four and 12 days, respectively, while DHEtGPA completely inhibited the growth of fungus until the twentieth day of the experiment (Fig. 5).

Amino compounds, especially amino sugars, are widely distributed in living matter where they play different biological roles.[9–11,32] Since these findings were available, glucosylamines were generally used to synthesize different amino compounds with different biological and physiological activities like mono-

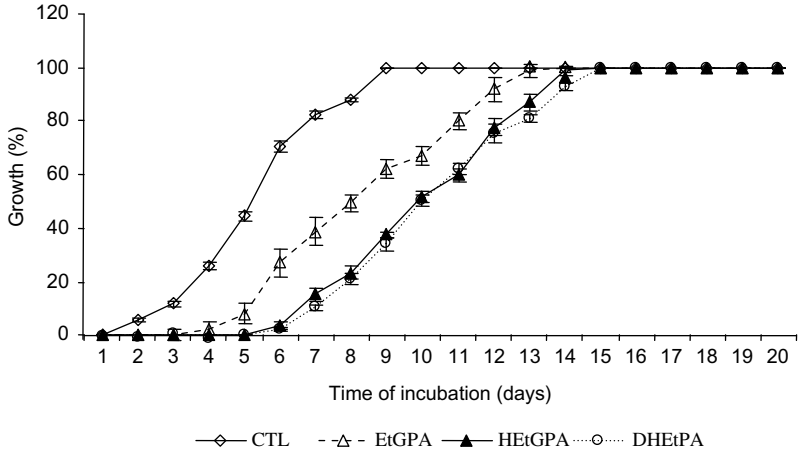

Figure 3. Comparative effect of EtGPA, HEtGPA and DHEtGPA on the kinetic growth of *C. versicolor* at the concentration of 0.1×10^{-3} mol mL^{-1}.

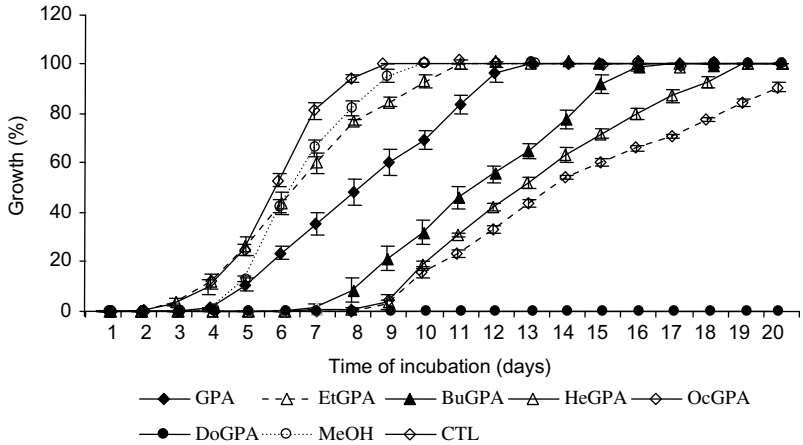

Figure 4. Effect of alkyl chains length on the kinetic growth of *Poria placenta* at a concentration of 0.025×10^{-3} mol mL^{-1}.

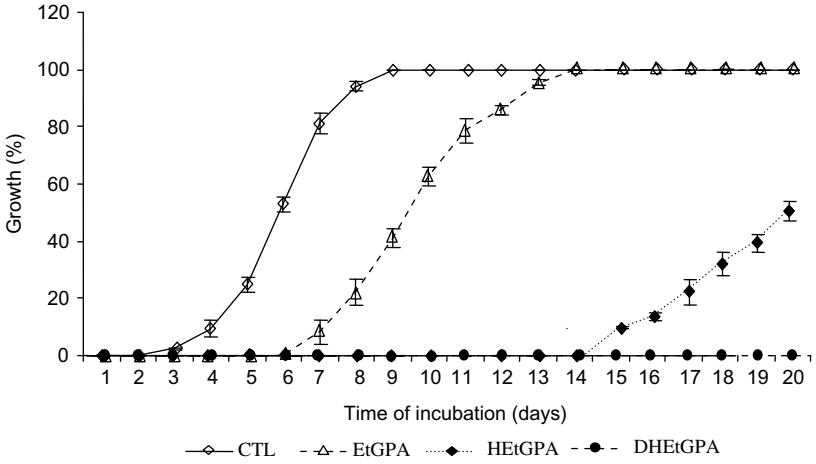

Figure 5. Comparative effect of EtGPA, HEtGPA and DHEtGPA on the kinetic growth of *P. placenta* at a concentration of 0.075×10^{-3} mol mL^{-1}.

cyclic β-lactams, glucopeptidic substances, and β-D-glucosylamidines.[33–35] However, only a few studies on their own importance as unmodified compounds, in this field, have been carried out. According to Fabbro and Grabowski,[9] Greenberg et al.[10] and Legler[11] these compounds are good inhibitors of glucosidases. Although their mechanisms of action are not well known, it is proposed that these amino compounds exhibit their biological activity as their ammonium forms.[2] However, according to Legler and Finken,[12] gluconamidines, analogues of glucosylamines, were more easily protonated than the corresponding glucosylamines under assay conditions, but they exhibited quite similar activities to those of the glucosylamines. Furthermore, other studies showed that a large variety of glucosidases was more inhibited by the non-protonated form of inhibitors.[12] These reports could be taken as evidence that glucosylamines can act with more than one mechanism. According to Kapferer,[32] glucosidase inhibitors are characterized by their irreversible binding to enzymes by a covalent bond. Furthermore, Krasikov et al.[36] showed that glucosidases exhibited their activities of α-glucosidic bond hydrolysis by nucleophilic a double-displacement mechanism, which includes catalytic attack by an acid. In this way, glucosylamines may act by the formation of hydrogen bonds with acid and then can block the enzymatic activities. We used *C. versicolor* and *P. placenta* because of their wide use in the antifungal evaluation of inhibitors and in the study of wood durability.[37–43] Results of this study showed that the antifungal activity of glucosylamines directly increases with the length of alkyl chain. This could be due to an increase in the hydrophobic character of the molecules with increasing alkyl chain length. DoGPA is more hydrophobic than the other tested glucosylamines, and this can indicate its high efficiency. The impact of alkyl chain on the biological activity of molecules has been reported in other research reports.[10,12,44–46] According to Bradley et al.,[47] the modification of carbohydrates with hydrophobic groups increases their affinity for the receptors. In addition, Kubo et al.[48] reported the beneficial effect of hydrophobic alkyl chains of alcohols on antifungal activity against *Saccharomyces cerevisiae*. Furthermore, the surfactant character of a long alkyl chain can also explain these results. Indeed, the ability of surfactant compounds to inhibit fungi and their mechanism of action has been reported.[49] These previous reports may confirm our results from HeGPA, OcGPA and DoGPA. The antifungal activity noted from GPA to BuGPA may be explained by the biochemical mechanisms proposed by others, like cell-surface recognition processes and an enhancement of transport through cell membranes, all introduced by glucosyl groups onto amines. Determination of the antifungal activity of such amines showed that hexylamine, octylamine, and dodecylamine exhibited less activity than the corresponding glucosylamines. Those with alkyl chains of C_2 to C_4 did not significantly alter the development of fungi. These results indicate the benefit of the contribution of the glucosyl group in the activity studied, which probably was mediated by small molecules from GPA to BuGPA to inhibit the growth of fungi. An additive contribution of pyranosyl groups on the biological activity of amines has been reported in other research.[45] In this study, HEtGPA was found to be more active than EtGPA. The introduction of a hydroxyl group enhanced antifungal activity of EtGPA. However, there was no significant difference between the antifungal activities of HEtGPA and DHEtGPA in spite of the second introduction of 2-hydroxylethyl group to HEtGPA. The introduction of hydroxyl group to the ethyl chain should enhance the recognition affinity of biocides to the microorganism and thus enhance the biological activity. A limited contribution of the second hydroxyl group to antifungal activity may be due to the chemical instability of DHEtGPA in solution, a fact that was verified during our study (result not shown).

In conclusion, the efficiency shown by *N*-alkyl-β-D-glucosylamines and in particular DoGPA on the growth of both *C. versicolor* and *P. placenta*, could be exploited in further research with respect to new biocide development from renewable resources.

3. Experimental

3.1. General methods

Glucose, ethylamine, butylamine, hexylamine, octylamine, dodecylamine, ethanolamine, diethanolamine, *N*,*N*-diisopropylethylamine and 2-propanol were purchased from Sigma–Aldrich. Solvents such as MeOH, chloroform, diethyl ether and acetone were purchased from BDH Prolabo and were used without further purification.

β-D-Glucopyranosylamine and different *N*-alkyl-β-D-glucopyranosylamines were synthesized and characterized by FTIR, ^{1}H NMR, ^{13}C NMR spectroscopy and their melting points. For EtGPA and HEtGPA, further analysis was done with HRMS. The synthesis was monitored by thin-layer chromatography (TLC) on aluminum plates (Silica Gel 60 F254, E. Merck) and developed with a solution of 0.5% potassium permanganate in 0.1 M aq NaOH. Different systems of solvents were used as eluents. The melting points were determined using an Electrothermal 9100 Digital Melting Point apparatus IA 9100. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 MHz and at 75 MHz, respectively, on a Bruker Avance 300 spectrometer. Chemical shifts are given in ppm, and the

assignments of ^{1}H NMR and ^{13}C NMR were made by COSY, HMBC or HMQC NMR spectroscopy. FTIR spectra were recorded on Perkin–Elmer Paragon 1000 PC spectrophotometer from a 300-mg KBr pellet disk containing 3 mg of compound. The KBr pellet disks were formed from an Edwards High Vaccum Pump Es 50. Spectra were recorded between 400 and 4000 cm^{-1} using 50 scans at a resolution of 4.0 cm^{-1}. HRMS analysis was conducted on a Qq-TOF tandem mass spectrometer.

3.2. Synthesis method for β-D-glucopyranosylamine (GPA)

The synthesis was done from D-glucose through β-D-glucopyranosyl ammonium carbamate using the method of Likhosherstov et al.[17] FTIR: ν_{max} 3389, 3344–3333, 2963-2857, 1619, 1457–1371, 1085, 1029, 875, and 640–559 cm^{-1}; ^{1}H NMR data (D_2O): δ_H 4.15 (d, 1H, $J_{1,2}$ 8.9 Hz, H_β-1), 3.95 (dd, 1H, $J_{5,6a}$ 2 Hz, $J_{6a,6b}$12 Hz, H-6a), 3.76 (dd, 1H, $J_{5,6b}$ 5.7 Hz, H-6b), 3.52 (t, 1H, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 8.8 Hz, H-3), 3.48–3.38 (m, 2H, H-4 and H-5), 3.23 (t, 1H, H-2); ^{13}C NMR δ_C (D_2O), 85.02 (C-1) 76.8 (C-5), 76.5 (C-3), 74.2(C-2), 69.8 (C-4), 60.8 (C-6).

3.3. Synthesis methods for of *N*-alkyl and *N*,*N*-dialkyl glucosylamines

3.3.1. *N*-Ethyl-β-D-glucopyranosylamine (EtGPA)

D-Glucose (3.0 g, 0.017 mol) and ethylamine (16.6 mL, 2 M in THF, 0.033 mol) were mixed and refluxed for 24 h. The solvent was evaporated, and 15 mL of Et_2O was added. The solid thus obtained was filtered and washed three times with Et_2O and dried. FTIR: ν_{max} 3406, 3249, 2969–2840, 1513, 1485–1362, 1082, 1026, 877, and 632–509 cm^{-1}; ^{1}H NMR (D_2O): δ_H 4.05 (d, 1H, $J_{1,2}$ 9,6 Hz, H_β-1), 3.94 (dd, 1H, $J_{5,6a}$ 2.1 Hz, $J_{6a,6b}$ 12.6 Hz, H-6a), 3.76 (dd, 1H, $J_{5,6b}$ 5.4 Hz, H-6b), 3.51 (t, 1H, $J_{2,3}$ 9.0 Hz, $J_{3,4}$ 9.0 Hz, H-3), 3.41(ddd, 1H, $J_{4,5}$ 9 Hz, H-5), 3.39 (t, 1H, H-4), 3.25 (t, 1H, H-2), 3.01–2.66 (mm, 2H, N–CH_2-), 1.13 (t, 3H, –CH_3); ^{13}C NMR (D_2O): δ_C 89.59 (C-1), 77.24 (d, C-3), 76.16 (C-5), 73.37 (C-2); 70.28 (d,C-4), 61.27 (C-6), 39.68 (–CH_2–), 14.20 (–CH_3); TOFMS (positive-ion): *m*/*z* 208 $(M+H)^+$ (100%), 230 $(M+Na)^+$ (11%).

3.3.2. *N*-(2-Hydroxyethyl)-β-D-glucopyranosylamine (HEtGPA)

The modified method of Mohammad and Olcott[22] was used to synthesize this product. To a suspension of D-glucose (3.0 g, 0.017 mol) in 6 mL of MeOH was added 95% ethanolamine (1.16 mL, 0.018 mol). The mixture was refluxed for 7 h at 50 °C. The solvent was removed under reduced pressure, and a viscous yellow liquid was obtained. This residue was triturated three times with 6 mL of Et_2O and left for 4 days to solidify in 10 mL of the same solvent. The solid was filtered and abundantly washed with $CHCl_3$ followed with Et_2O. The white solid obtained was dried to give the desired compound. FTIR ν_{max} 3428, 3372, 3282, 3198, 2958-2890, 1510, 1474–1267, 1090, 1020, 880, and 637–517 cm^{-1}; ^{1}H NMR (D_2O): δ_H 4.06 (d, 1H, $J_{1,2}$ 9.1 Hz, H_β-1), 3.94 (dd, 1H, $J_{6a,6b}$ 12 Hz, $J_{5,6a}$ 1.9 Hz, H-6a), 3.75 (dd, 1H, $J_{5,6b}$ 6.8 Hz, H-6b), 3.69 (t, 2H, –CH_2OH), 3.50 (t, 1H, H-3), 3.41 (ddd, 1H, H-5), 3.38 (t, 1H, H-4), 3.23 (t, 1H, $J_{2,3}$ 9.0 Hz, H-2), 3.07–2.84 (mm, 2H, N–CH_2–); ^{13}C NMR (D_2O): δ_C 90.06 (C-1), 76.97–76.93 (C-3, C-5), 73.21 (C-2), 70.10 (C-4), 61.36 (C-6), 61.10 (–CH_2–OH), 47.16 (–NH–CH_2–), +TOF-MS: *m*/*z* 206 (15%), 224 $(M+H)^+$ (100%), 246 (M+Na) (67%).

3.4. Synthesis of other analogues

The methods previously reported[16,18,19,22] were slightly modified and used to synthesize DHEtGPA, BuGPA, HeGPA, OcGPA and DoGPA. Reactions carried out between D-glucose and different alkylamines such as diethanolamine, butylamine, hexylamine, octylamine and dodecylamine in MeOH or EtOH gave DHEtGPA, BuGPA, HeGPA, OcGPA and DoGPA, respectively. All reactions were conducted at 50 °C. Their physicochemical characteristics are listed in the following:

3.4.1. *N*,*N*-Di(2-hydroxyethyl)-β-D-glucopyranosylamine (DHEtGPA)

FTIR ν_{max} 3355, 3266, 2929–2873, 1476-1211, 1099, 1068, 1040, 973, 931, 909, and 713–506 cm^{-1}; ^{1}H NMR (D_2O): δ_H 4.15–4.12 (dd, 1H, $J_{1,2}$ 8.7 Hz, H_β-1), 3.95 (dd, 1H, $J_{5,6a}$ 1.5 Hz, H-6a), 3.77 (dd, 1H, $J_{5,6b}$ 4.7 Hz, H-6b), 3.73–3.64 (m, 4H, –$2CH_2$OH), 3.56–3.39 (m, 4H, H-3, H-4, H-5, H-2), 3.10–2.94 (m, 4H, 2N–CH_2–); ^{13}C NMR (D_2O): δ_C 93.59 (C-1), 77.2 (C-2), 77.08 (C-3), 70.15 (C-5), 70.09 (C-4), 61.13 (C-6), 59.95 (–$2CH_2$OH), 51.22 (–$(CH_2)_2$N–).

3.4.2. *N*-Butyl-β-D-glucopyranosylamine (BuGPA)

FTIR ν_{max} 3406, 3299, 3260, 2958–2863, 1507, 1474–1325, 1074, 1018–993, 875, and 735–570 cm^{-1}; ^{1}H NMR (D_2O): δ_H 4.03 (d, 1H, $J_{1,2}$ 9.2 Hz, H_β-1), 3.95 (dd, 1H, $J_{5,6a}$ 1.8 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 3.77 (dd, 1H, $J_{5,6b}$ 5.3 Hz, H-6b), 3.51 (t, 1H, H-3), 3.41 (ddd, 1H, H-5), 3.39 (t, 1H, $J_{3,4}$ 9.1 Hz, H-4), 3.22 (t, 1H, $J_{2,3}$ 9.8 Hz, H-2), 2.95–2.64 (mm, 2H, N–CH_2–), 1.56–1.32 (mm, 4H, –CH_2–CH_2–), 0.95–0.90 (t, 3H, –CH_3); ^{13}C NMR (D_2O): δ_C 89.89 (C-1); 77.15 (d, C-3), 76.16 (C-5), 73.29 (C-2); 70.20 (d, C-4), 61.18 (C-6), 45.04 (–$NHCH_2$–), 31.36 (–CH_2–), 20.08 (–CH_2–), 13.49 (–CH_3).

3.4.3. *N*-Hexyl-β-D-glucopyranosylamine (HeGPA)

FTIR ν_{max} 3394, 3383, 3283, 2946–2851, 1513, 1466–1289, 1076, 1018–993, 875, and 716–534 cm^{-1}; ^{1}H NMR (CD_3OD): δ_H 3.85–3.81 (dd, 1H, $J_{5,6a}$ 2.3 Hz, $J_{6a,6b}$ 11.9 Hz, H-6), 3.82–3.80 (d, 1H, $J_{1,2}$ 9.0 Hz, H_β-1), 3.67–3.61 (dd, 1H, $J_{5,6b}$ 5.5 Hz, H-6b), 3.36–3.23 (m, 3H, H-3, H-4, H-5), 3.05 (t, 1H, H-2), 2.94–2.57 (m, 2H, $NHCH_2$–), 1.50–1.44 (br, 2H, $NHCH_2CH_2$–), 1.31 (s, 6H, $(CH_2)_3$), 0.90 (t, 3H, –CH_3); ^{13}C NMR (CD_3OD): δ_C 91.87 (C-1), 78.96 (C-3), 74.98 (C-5), 71.88 (C-2), 62.97 (C-4), 47.23 (C-6), 32.93 (NCH_2), 31.04–23.72 ($4CH_2$), 14.38 (–CH_3).

3.4.4. *N*-Octyl-β-D-glucopyranosylamine (OcGPA)

FTIR ν_{max} 3395, 3384, 3288, 2952–2851, 1513, 1465–1230, 1082, 1043–990, 878, and 718–531 cm^{-1}; ^{1}H NMR (CD_3OD): δ_H 3.85–3.81 (dd, 1H, $J_{6a,6b}$ 11.7 Hz, $J_{5,6a}$ 2.2 Hz, H-6), 3.83–3.80 (d, 1H, $J_{1,2}$ 8.3 Hz, H_β-1), 3.68–3.61 (dd, 1H, $J_{5,6b}$ 5.6 Hz, H-6b), 3.37–3.22 (m, 3H, H-3, H-4, H-5), 3.05 (t, 1H, H-2), 2.94–2.57 (m, 2H, $NHCH_2$–), 1.50–1.47 (br, 2H, $NHCH_2CH_2$–), 1.31 (s, 10H, $(CH_2)_5$), 0.90 (t, 3H, –CH_3); ^{13}C NMR (CD_3OD): δ_C 91.87 (C-1), 78.96 (C-3, C-5), 74.97 (C-2), 71.87 (C-4), 62.97 (C-6), 47.24 (NCH_2), 33.02–23.72 ($6CH_2$), 14.29 (–CH_3).

3.4.5. *N*-Dodecyl-β-D-glucopyranosylamine (DoGPA)

FTIR ν_{max} 3395, 3288, 2958–2846, 1510, 1468–1342, 1088, 1051–996, 878, and 716–529 cm^{-1}; ^{1}H NMR (CD_3OD + one drop of acetone-d_6): δ_H 3.87–3.82 (dd, 1H, $J_{5,6a}$ 2.3 Hz, $J_{6a,6b}$ 11.7 Hz, H-6a), 3.83 (d, 1H, $J_{1,2}$ 9.1 Hz, H_β-1), 3.68–3.62 (dd, 1H, $J_{5,6b}$ 5.5 Hz, H-6b), 3.38–3.25 (m, 3H, H-3, H-4, H-5), 3.06 (t, 1H, $J_{2,3}$ 8.5 Hz, H-2), 2.95–2.86 (m, 2H, $NHCH_2$–), 1.51 (br, 2H, $NHCH_2CH_2$–), 1.30 (s, 18H, $(CH_2)_9$), 0.89 (t, 3H, –CH_3); ^{13}C NMR (CD_3OD + one drop of acetone-d_6): δ_C 91.68 (C-1), 78.79 (C-3, C-5), 74.80 (C-2), 71.69 (C-4), 62.79 (C-6), 47.07 (C-1′), 32.93 (C-10′), 30.91 (C-2′), 30.59 (C-5′-C-8′), 30.31 (C-4′, C-9′), 28.28 (C-3′), 23.58 (C-11′), 14.29 (–CH_3).

3.5. Antifungal activity assessment

3.5.1. Microorganisms

Two wood fungi, *Coriolus versicolor* (CTB 863-A) and *P. placenta* (FPRL 280) were used. They were obtained from CIRAD fôret

(MontPellier, France). The target fungal strains were recovered from malt agar culture and incubated at 25 °C and 75% relative humidity (RH) for 15 days. The mycelium was then suspended in 10 mL of sterilized physiological solution and vigorously shaken. The suspension thus obtained was used for the test experiments.

3.5.2. Radial growth assays

The antifungal activities of glucosylamines were evaluated on malt agar medium amended with different concentrations of potential bioactive agents. Plates were inoculated at the middle with one drop of strain suspension. Control experiments, without any growth inhibitor, were conducted in parallel. The potential bioactive agents HeGPA, OcGPA and DoGPA were incorporated in the culture medium using MeOH as solvent. In parallel, control experiments with MeOH without any glucosylamines were also tested. Prior to the fungal inoculation, the evaporation of MeOH in Petri dishes was observed after 40 min under a laminar flow. The dishes were incubated at 75% relative humidity and 25 °C, and the fungal colony diameter was measured daily for 20 days. The percentage of inhibition from different glucosylamines was calculated after 9 days of incubation when the mycelium in the control experiments completely covered the dishes. It was expressed as an average diameter and calculated using the following equation:

$$\text{Percentage of inhibition} = \frac{\text{CD} - D}{\text{CD}} \times 100$$

where CD is the control diameter, D is the test diameter.

Each test and control experiments consisted of three replicates, and each bioactive agent was tested three times. Graphics of the kinetic growth of each fungus was drawn by different diameter means from the growth measured daily during 20 days of incubation.

3.5.3. Analysis of the results

Results from the antifungal assays were statistically analyzed using Student's t-test. Standard error means (SEM) were calculated as follows:

$$\text{SEM} = \frac{\sigma}{\sqrt{n}}$$

where n is the size of sample ($n = 3$), σ is the standard deviation.

The probability (p) was determined from the critical t-value (t) between results from different experiments after 9 days of incubation.

$$t = \frac{M1 - M2}{\text{SD}}.$$

where $M1$ is the control mean, $M2$ is the test experiment mean, and SD is the standard deviation between means.

The degree of freedom used to determine this probability was equal to 4 in our experiment.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.07.005.

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