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Antibacterial and mycelial growth inhibition of some acylated derivatives of D-glucopyranoside

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Abstract

Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside acylated derivatives (2-12) were employed as test chemicals for *in vitro* antimicrobial evaluation against four Gram-positive and six Gram-negative human pathogenic bacteria and three phytopathogenic fungi. It was revealed that a good number of tested chemicals exhibited moderate to good antimicrobial activities. It was found that these tested compounds were more effective against the phytopathogenic fungi than those of the bacterial strains. However, Methyl 4,6-*O*-benzylidene-2-*O*-(4-t-butylbenzoyl)-3-*O*-lauroyl-α-D-glucopyranoside (6) (19.5 mm) exhibited better antibacterial activity than the standard antibiotic, ampicillin (18 mm). Also, it has been observed that the mycelial growth inhibition of methyl 4,6-*O*-benzylidene-2-*O*-(4-t-butylbenzoyl)-3-*O*-(2,6-dichlorobenzoyl)-α-D-glucopyranoside (11) showed the highest inhibition (53.84%) against the *Fusarim equiseti* than that of standard antibiotic, nystatin (44.70%).

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Introduction

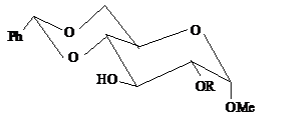
Carbohydrates are the most abundant and the most diverse biopolymers in nature. Due to their highly specific interactions with physiological receptors, they participate in many crucial biological processes. All these processes are potential targets for therapeutic intervention and carbohydrate-based drugs are rapidly being engaged by the modern biotechnology and pharmaceutical industry (Gornik *et al.*, 2006). In the last few decades, considerable works have been done in the field of antimicrobial evaluation (Singh *et al.*, 1990) of various chemical compounds. Different classes of chemical compounds have been screened for *in vitro* antimicrobial activities (Andary *et al.*, 1982; Gupta *et al.*, 1997) all over the world. Carbohydrates, especially acylated glycosides, are very important due to their effective biological activity (Andary *et al.*, 1982; Kabir *et al.*, 2007, 2009). Literature survey revealed that a wide variety of biologically active substances contain aromatic, heteroaromatic and acyl substituents (Gupta *et al.*, 1997). It is also known that the combination of two or more potent acyl substituents in a single molecular framework enhances the biological profile many fold than its parent nuclei (Kabir *et al.*, 1998). The benzene and substituted benzene nuclei play important role as common denominator for various biological activities. In the context of our studies, we observed that some acylated derivatives of D-glucose (Kabir *et al.*, 2009), D-mannose (Kabir *et al.*, 2004), L-Rahmnose (Kabir *et al.*, 2003), L-lyxose (Kabir *et al.*, 2001) and uridine (Kabir *et al.*, 1998) also exhibited effective antibacterial and antifungal activities.

In view of the above mentioned facts and in continuation of our work on synthesis of biologically important monosaccharide derivatives as potential antimicrobial agents using a fast and efficient approach. Thus, it is conceivable to develop a series of D-glucose derivatives (2-12) with the aim of investigating their antibacterial activity and mycelial growth properties.

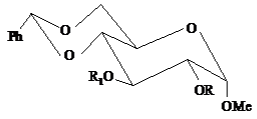
Materials and methods

Test compounds

Eleven partially protected derivatives of D-glucopyranoside (2-12) (Fig. 1) were used as test chemicals. The chemicals (2-12) were synthesized, isolated, purified and characterized in the Laboratory of Carbohydrate and Protein Chemistry, Department of Chemistry, University of Chittagong.



1 : R = H **2** : R = 4-t-butylbenzoyl



3 : R = 4-t-butylbenzoyl ; R_1 = Ac
4 : R = 4-t-butylbenzoyl ; R_1 = Pentanoyl
5 : R = 4-t-butylbenzoyl ; R_1 = Decanoyl
6 : R = 4-t-butylbenzoyl ; R_1 = Lauroyl
7 : R = 4-t-butylbenzoyl ; R_1 = 3-Cl.Bz
8 : R = 4-t-butylbenzoyl ; R_1 = 4-Cl.Bz
9 : R = 4-t-butylbenzoyl ; R_1 = 4-NO_2.BzO
10 : R = 4-t-butylbenzoyl ; R_1 = 3,5-di-NO_2.BzO
11 : R = 4-t-butylbenzoyl ; R_1 = 2,6-di-Cl.BzO
12 : R = 4-t-butylbenzoyl ; R_1 = Pivaloyl

Fig. 1. The structure of synthesized compounds (1-12).

Biological evaluation of the test compounds

The antimicrobial assay of the chemicals was done in the Microbiology Laboratory, Department of Microbiology, University of Chittagong. The tested microorganisms (bacteria and fungi) were collected from this laboratory. Nutrient Agar (NA) and Potato Dextrose Agar (PDA) were used as basal medium for antibacterial and antifungal test, respectively. In all the cases, 2% solution (w/v) of the chemicals in $CHCl_3$ was used.

Bacterial and fungal test pathogens

Compounds 2-12 were tested for their antibacterial activity against four Gram-positive and six Gram-negative human pathogenic bacterial strains, *viz.*, *Bacillus subtilis* BTCC 17, *Bacillus cereus* BTCC 19, *Bacillus megaterium* BTCC 18, *Staphylococcus aureus* ATCC 6538, *Escherichia coil* ATCC 25922, *Salmonella typhi* AE 14612, *Salmonella paratyphi* AE 14613, *Shigella dysenteriae* AE 14396, *Pseudomonas* Species CRL (ICDDR,B) and *Vibrio cholerae* AE 14748 and

three pathogenic fungi viz., *Fusarium equiseti* (Corda) Sacc., *Alternaria alternata* (Fr.) Kedissler and *Curvularia lunata* (Wakker Becdijin).

Antibacterial assay

The *in vitro* antibacterial spectrum of the newly synthesized compounds (2-12) was done by disc diffusion method (Bauer *et al*., 1966) with little modification (Miah *et al*., 1990). Sterilized paper discs of 4 mm in diameter and Petri dishes of 150 mm in diameter were used throughout the experiment. The autoclaved Mueller-Hinton agar medium, cooled to 45˚C, was poured into sterilized Petri dishes to a depth of 3 to 4 mm and after solidification of the agar medium; the plates were transferred to an incubator at 37˚C for 15 to 20 minutes to dry off the moisture that developed on the agar surface. The plates were inoculated with the standard bacterial suspensions (as McFarland 0.5 standard) followed by spread plate method and allowed to dry for three to five minutes. Dried and sterilized filter paper discs were treated separately with 50 µg dry weight/disc from 2% solution (in $CHCl_3$) of each test chemical using a micropipette, dried in air under aseptic condition and were placed at equidistance in a circle on the seeded plate. A control plate was also maintained in each case without any test chemical. These plates were kept for 4-6 hours at low temperature (4-6˚C) and the test chemicals diffused from disc to the surrounding medium by this time. The plates were then incubated at 35±2˚C for 24 hours to allow maximum growth of the organisms. The antibacterial activity of the test agent was determined by measuring the mean diameter of zone of inhibitions in millimeter. Each experiment was repeated thrice. All the results were compared with the standard antibacterial antibiotic ampicillin (20 µg/disc, BEXIMCO Pharm Bangladesh Ltd).

Mycelial growth efficacy

The antifungal functionality test of the acylated compounds (2-12) were determined by poisoned food technique (Grover & More, 1962) with some modification (Miah *et al*., 1990). Two percent solution of the test chemical (in $CHCl_3$) was mixed with sterilized melted Saburaud agar medium to obtain the desired concentration (2%) and this was poured in sterilized Petri dishes. At the center of each plate, 5 days old fungal mycelial block (4 mm in diameter) was inoculated and incubated at 27˚C. A control set was also maintained in each experiment. Linear mycelial growth of fungus was measured after 3-5 days of incubation. The percentage inhibition of radial mycelial growth of the test fungus was calculated as follows:

$$I = \left\{\frac{C-T}{C}\right\} \times 100$$

Where, I = Percentage of inhibition, C = Diameter of the fungal colony in control ($CHCl_3$), T = Diameter of the fungal colony in treatment. All the results were compared with the standard antifungal antibiotic nystatin (100 µg/ml medium, MEXIMCO Pharm Bangladesh Ltd.).

Results and discussion

In the present investigation, some acylated derivatives of D-glucopyranoside (2-12) were selected as probable test compounds and screened *in vitro* for their antibacterial and antifungal activities against ten human pathogenic bacteria and three phytopathogenic fungi. These test compounds contained a wide variety of substituents such as phenyl, acetyl, benzoyl, pentanoyl, decanoyl, lauroyl, 3-chlorobenzoyl, 4-chlorobenzoyl, 4-nitrobenzoyl, 3,5-dinitrobenzoyl and pivaloyl. These test compounds were prepared from a common precursor namely, methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (1). In fact, we deliberately incorporated the above mentioned substituents into the D-glucopyranose molecule in order to study their effectiveness against the microorganisms tested. Since we have previously observed (Kabir *et al*., 2004; Kabir *et al*., 2003) that various acylated monosaccharide derivatives showed effective biological activity, the test compounds under investigation are expected to show such activity. For comparative study, antimicrobial

activities of the standard antibiotics (ampicillin and nystatin) were also determined.

A) Effect of test chemicals on bacteria

The inhibition zone against the selected bacteria due to the effect of compounds are mentioned in Table 1 and Table 2.

Bacillus subtilis BTCC 17

The inhibition growth data (Table 1) indicated that the compound 6 was more effective than that of other chemicals such as, 5, 8 and 11 against this bacterium. The rest of compounds have no effect on this microorganism.

Table 1. Zone of inhibition observed against Gram-positive test organisms by the test chemicals

Compound no.	Diameter of inhibition zone in mm 200 µg dw/disc			
	B. subtilis	***B. cereus***	***B. megaterium***	***S. aureus***
2	NF	7	8	NF
3	NF	NF	7	7.5
4	NF	NF	NF	NF
5	7	NF	8	9
6	*12	*11.5	9	8
7	NF	NF	8	7
8	6	7.5	7.5	8
9	NF	NF	8	NF
10	NF	NF	7	12
11	9	10	8	7
12	NF	NF	8	8
****Ampicillin**	*19	*18	*16	*22

N.B: '*' = marked inhibition, '**' = standard antibiotic, 'NF' = not found, 'dw' = dry weight.

Table 2. Zone inhibition observed against Gram-negative test organisms by the test chemicals

Compound no.	Determination of inhibition zone in mm 200 µg dw/disc					
	E. coli	***S. typhi***	***S. paratyphi***	***S. dysenteriae***	***Pseudomonas* sp**	***V. cholerae***
2	*10	NF	NF	7	10	NF
3	NF	NF	NF	NF	NF	NF
4	NF	8	7	NF	NF	8
5	NF	10	7	NF	NF	9
6	*14	*14.5	*14	9	*19.5	6
7	7	NF	7.5	NF	7	NF
8	*11	6.5	NF	NF	NF	NF
9	9	NF	7	6	6.5	NF
10	NF	NF	7	NF	6.5	7
11	8	7	8.5	7	8	8
12	NF	NF	NF	NF	NF	NF
****Ampicillin**	*10	*20	*18	*22	*18	*15

N.B: '*' = marked inhibition, '**' = standard antibiotic, 'NF' = not found, 'dw' = dry weight.

Table 3. Antifungal activities of the test chemicals & nystatin

Compound no	% Inhibition of fungal mycelial growth[a] (100 µg (dw)/ml medium)		
	F. equiseti	*A. alternata*	*C. lunata*
2	NF	13.04	20.4
3	16.7	8.69	NF
4	NF	13.04	NF
5	12.3	NF	13.24
6	27.69	*41.34	15.43
7	15.87	NF	11.23
8	*30.77	8.69	23.33
9	18.46	8.69	*26.01
10	7.69	19.54	10.98
11	*53.84	21.73	*36.21
12	9.67	15.32	8.87
****Nystatin**	*44.7	*51.55	*75

N.B: '*' = marked inhibition, '**' = standard antibiotic, 'NF' = not found, 'dw' = dry weight,

[a]growth measured-radial growth in cm.

Bacillus cereus BTCC 19

It was found that the compound 6 was more effective than that of other compounds such as, 2, 8 and 11 which were somewhat less effective. The rest of the compounds such as, 3, 4, 5, 7, 9, 10 and 12 did not show any inhibition. All of these test chemicals were, however, less active against this bacterial strain than standard antibiotic, ampicillin (18 mm) in case of this bacterial strain.

Bacillus megaterium BTCC 18

We observed that the compound 6 was highly active against than that of other compounds such as, 2, 3, 5, 7, 8, 9, 10, 11 and 12. Only compound 4 was found to be inactive against this bacterium.

Staphylococcus aureus ATCC 6538

It was observed that compound 10 was reasonably active and compounds 3, 5, 6, 7, 8, 11 and 12 were less active against this bacterium. The rest of the chemicals were found to be inactive against *Staphylococcus aureus*. All of these test chemicals were less active against this bacterial strain than the standard antibiotic, Ampicillin (22 mm).

Escherichia coli ATCC 25922

It was evident from Table 2 that compounds 2, 6 and 8 were more effective than that of other chemicals such as 7, 9 and 11 which were somewhat less effective. The rest of the chemicals such as 3, 4, 5, 10 and 12 did not show any inhibition. Here again compounds 6 (14 mm) and 8 (11 mm) were showed higher inhibition than the standard antibiotic, ampicillin (10 mm).

Salmonella typhi AE 14612

Test compound 6 showed maximum inhibition (14.5 mm) as compared to that of other compounds like 4, 5, 8 and 11 and the rest did not show antibacterial functionality. All of these test compounds were, however, less active than ampicillin (20 mm) against this bacterial strain.

Salmonella paratyphi AE 146313

The screening data presented in Table 2 suggest that the compound 6 showed mild inhibition against *Salmonella paratyphi*. It was also indicated that the compound 4, 5, 7, 9, 10 and 11 were less effective against this bacteria. The rest of the chemicals such as, 2, 3, 8 and 12 have no effect on this microorganism.

Shigella dysenteriae AE 14396

The growth inhibition data indicated that the compounds 2, 6, 9 and 11 showed less active against this bacterium. The rest of the compounds 3, 4, 5, 7, 8, 10 and 12 were found to be inactive against *Shigella dysenteriae*.

Pseudomonas species CRL (ICDDR, B)

Encouragingly, in case of this bacterium, compound 6 showed higher inhibition (19.5 mm) than ampicillin (18 mm), whereas, compounds 2, 7, 9, 10 and 11 were less effective. Rest of the test compounds 3, 4, 5, 8 and 12 were unable to show any inhibition against this bacterium.

Vibrio cholerae

The inhibition zone against this microorganism due to treatment of different chemicals suggests that the test compounds 4, 5, 6, 10 and 11 showed mild inhibition against this bacterium. Chemicals 2, 3, 7, 8, 9 and 12 were found to be inactive against this bacterial strain.

From this result, it has been observed that the selectively acylated D-glucopyranoside derivatives obtained by using various acylating agent follow the order for Gram-positive organisms: 6> 11 > 8 > 5 = 12 > 10 > 7 > 2 > 3 = 9 > 4 and Gram-negative bacteria follow the order 6 > 11 > 9 > 2 > 5 > 7 > 4 > 10 > 8 > 3 = 12. Thus, we found that compounds 6, 8 and 11 showed moderate to marked inhibition against Gram-positive bacteria while compounds 6 and 11 are very active against Gram-negative bacteria. We also observed that some compounds such as 6 and 11 are active against both the Gram-positive and Gram-negative organisms. So these compounds may be targeted for future studies for their usage as broad spectrum antibiotics.

B) Effect of test compounds on mycelial growth

The results of the percentage inhibition of mycelial growth due to treatment of compounds are presented in Table 3.

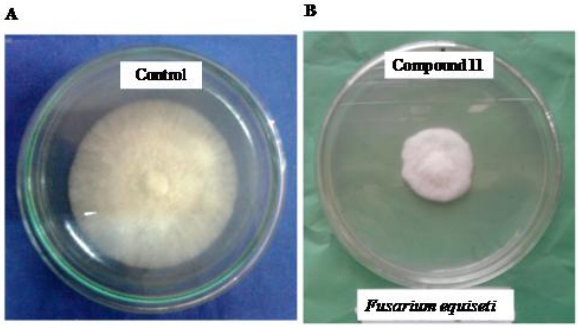


Fig. 2. % Inhibition of mycelial growth against Fusarium equiseti. A: Control and B: Compound 11.

Fusarim equiseti

The antifungal screening data as presented in Table 3 suggest that test compounds 8 (30.77%) and 11 (53.84%) (Fig. 2), display marked toxicities towards *Fusarim equiseti*. Compound 11 (53.84%) showed the highest inhibition which is more than the standard antibiotic, Nystatin (44.70%). The rest of the compounds such as, 3, 5, 6, 7, 9, 10 and 12 were less toxic to this fungus as compared to that of the standard antibiotic. We also observed that the remaining test chemicals such as, 2 and 4 were found inactive against this plant pathogenic fungus.

Alternaria alternata

It was found that the inhibition of mycelial growth of the chemical 6 (41.34%) showed very effective inhibition, though it was not as effective as the standard antibiotic, Nystatin (51.55%). We observed that compounds 5 and 7 did not show any inhibition or stimulation. Most of the acylated derivatives, however, showed moderate to poor inhibition against this plant pathogenic fungus. None of the test chemicals were found to be more active than Nystatin.

Curvularia lunata

From screening data, we found that the compounds 9 (26.01%) and 11 (36.21%) showed very effective inhibition fungus while compounds 2 (20.40%), 5 (13.24%), 6 (15.43%), 7 (11.23%), 8 (23.33%), 10 (10.98%) and 12 (8.87%)) showed moderate to poor inhibition against this plant pathogenic fungus. Whereas, the remaining test compounds were found inactive against this phytopathogen.

The overall results indicated that out of three fungi tested against the newly synthesized twelve compounds, minimum average inhibition was observed in case of *Alternaria alternata* and *Fusarium equiseti* showed maximum average inhibition than that of the other tested organisms. From the antifungal activity results, it has been observed that the acylated methyl 4,6-*O*-benzylidene-α-D-glucopyranoside derivative 11 (Fig. 2) showed the highest inhibition (53.84%) against the *Fusarim equiseti* than that of standard antibiotic Nystatin (44.70%). However, the test compounds 2-12 were found to be less active or toxic to *Alternaria alternata* and *Curvularia lunata* as compared to the standard antibiotic, nystatin. Our newly synthesized and reported chemicals (2-12) have not been tested before against the selected bacterial and fungal pathogens. This is the first report regarding the effectiveness of the selected compounds against the selected pathogens.

Conclusion

The results of the present investigation showed that some of the synthesized acylated derivatives of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside may be tested against a wide range of phytopathogenic fungi and bacteria, before sending them to pesticide producing companies for further tests. So it is hoped that the acylated derivatives of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (2-12) might show potential antiviral and anti-inflammatory activities. It is also expected that this piece of work employing carbohydrate derivatives as test compounds will help further work to the development of pesticides and medicine for plant/human disease control.

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