Design and Newly Synthesis of some 1,2-*O*-Isopropylidene-α-D-Glucofuranose Derivatives: Characterization and Antibacterial Screening Studies

Bazı 1,2-O-izopropiliden-α-D-Glucofuranose Türevlerinin Tasarım ve Yeni Sentez Metodu: Karakterizasyonu ve Antibakteriyel Tarama Çalışmaları

Research Article

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ABSTRACT

D-Glucose was converted into the 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (1) in good yield. Removal of 5,6-*O*-isopropylidene group from diacetone glucose (1) was achieved by careful hydrolysis which provided the monoacetal glucose (2). Here we reported the selective acylation of 1,2-*O*-isopropylidene-α-D-glucofuranose (2) by the direct method using a number of acylating agents furnished the corresponding 3,5,6-tri-*O*-acyl derivatives in reasonable yields. The structures of the newly synthesized compounds were ascertained by FTIR, ^{1}H-NMR spectroscopy and elemental analysis. All the synthesized compounds were employed as test chemicals for *in vitro* antibacterial functionality test against six human pathogenic bacteria. The evaluation study revealed that the tested chemicals exhibited moderate to good antibacterial activities. It was also observed that the test chemicals were more effective against Gram-positive bacteria than that of the Gram-negative microorganisms.

Key Words

D-Glucose, acylation, spectroscopy, antibacterial.

ÖZET

D-glukoz, iyi bir verimle 1,2:5,6-di-*O*-izopropiliden-α-D-gluko furanoz (1) dönüştürüldü. Diaseton glikoz (1)'dan 5,6-O-izopropiliden grubunun çıkarılması, mono asetal glikoz (2)'un elde edilmesini sağlayarak, dikkatli bir şekilde hidroliz ile elde edilmiştir. Burada karşılık gelen 3,5,6-tri-*O*-asil türevlerinin döşenmiş asile edici bir dizi ajan kullanarak uygun verimle doğrudan 1,2-*O*-izopropiliden-α-D-gluckofuranoz (2) 'nin seçici asilasyonu rapor edilmektedir. Yeni sentezlenmiş bileşiklerin yapıları FTIR, ^{1}H-NMR spektroskopisi ve element analizi ile tespit edildi. Sentezlenen tüm bileşikler altı insan patojen bakterisine karşı *in vitro* antibakteriyel işlevsellik test kimyasalı olarak kullanılmıştır. Değerlendirme çalışması, test kimyasalları iyi antibakteriyel işlevlerine orta derecede tepki verdiğini göstermiştir. Aynı zamanda, test kimyasallarının, Gram-pozitif bakterilere karşı Gram-negatif mikroorganizmalara olduğundan daha etkili olduğu görülmüştür.

Anahtar Kelimeler

D-Glikoz, asilasyon, spektroskopi, antibakteriyel.

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INTRODUCTION

Conversion of carbohydrates into newer products and isolation of various carbohydrate derivatives from natural sources are of great interest to the organic chemists. Of the carbohydrates isolated from natural sources, acyl, alkyl and glycosyl derivatives are important. Some of these have effective biological activity [1-3]. Also, some of these derivatives may have further synthetic utility as versatile intermediates in the synthesis of many important chemical compounds [2]. Selective acylation is very important in the field of carbohydrate and nucleoside chemistry because of its usefulness for the synthesis of biologically active products. Various methods for acylation of carbohydrates and nucleosides have so far been developed and employed successfully [4-6]. Of these, direct method has been found to be the most encouraging method for acylation of carbohydrates and nucleosdies [7].

In the last few decades considerable works have been done in the field biological activities by chemical compounds [8]. It must, however, be admitted that a lot of the reports on the benefits of one or the other chemicals were based on empirical knowledge. Different classes of compounds have been screened for antimicrobial activities all over the world.

It was found from the literature survey that nitrogen, sulphur and halogen containing heterocyclic compounds showed marked antimicrobial activities [9]. When heterocyclic part becomes attached to carbohydrates [10], their efficiency to inhibit bacteria or fungus sharply increased. A large number of biologically active compounds also possess aromatic, heteroaromatic and acyl substituents [10]. It is also known that if an active nucleus is linked to another active nucleus, the resulting molecule may possess greater potential for biological activity [10]. The benzene and substituted benzene neclei play important role as common denominator of various biological activities [9].

From our previous works we also observed that in many cases the combination of two or more acyl substituents in a single molecular framework enhances the biological profile manyfold than their parent nuclei [11-15]. Encouraged by our own findings and also literature reports, we synthesised a series of D-glucose derivatives deliberately incorporating a wide variety of probable biologically active components to the D-glucose moiety. Antibacterial activities of these newly synthesized compounds (Figure 1) were evaluated using a variety of bacterial strains and the results are reported here.

EXPERIMENTAL

Apparatus and chemicals

All reagents were commercially available and were used as received unless otherwise specified. Melting points were determined on an electro-thermal melting point apparatus and are uncorrected. Evaporations were carried out under reduced pressure using BUCHI rotary evaporator (Germany) with a bath temperature below 40°C. FTIR spectra were recorded on KBr disc at the Department of Chemistry, University of Chittagong, Bangladesh with an IRAffinity-1 FTIR spectrophotometer, SHIMADZU Corporation, Japan. ^{1}H-NMR spectra (400 MHz) were recorded in $CDCl_3$ using TMS as internal standard with a Bruker spectrospin spectrometer. Thin layer chromatography (t.l.c) was performed on Kieselgel GF254 and spots were detected by spraying the plates with 1% H_2SO_4 and heating the plates at 150-200°C until coloration took place. Column chromatography was performed with Merck silica gel G_{60}. Solvent system employed for t.l.c analyses was ethylacetate-hexane in different proportions.

Synthesis

1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (diacetone glucose) 1

To a solution of D-glucose (5 gm, 27.78 mmol) in dry acetone (250 mL) was stirred vigorously at room temperature when concentrated sulfuric acid (1.2 mL) was added to it. The reaction mixture was stirred at room temperature for 6 hours. Anhydrous copper(II) sulphate (15 g) was added to the reaction mixture and stirring was continued at room temperature for 8 hrs and then left overnight. The progress of the reaction was monitored by T.l.c (acetone-petroleum ether, 1:3) which indicated almost complete conversion of starting material into one product. The reaction

mixture was then neutralized by the addition of solid sodium hydrogen carbonate ($NaHCO_3$) and the inorganic materials were filtered off. The filtrate was evaporated under reduced pressure to leave a white solid, which was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was dried over (Na_2SO_4), filtered. Evaporation of the solvent under reduced pressure provided the title compound (**1**) (4 g, 54%) as a crystalline solid. The compound was sufficiently pure for use in the next stage without further purification and identification. Recrystallization from cyclohexane gave the pure diacetal (**1**), m.p.109-110°C; [16].

1,2-*O*-Isopropylidene-α-D-glucofuranose (monoacetone glucose) 2

To a solution of diacetone glucose (**1**) (3 g, 8.56 mmol) in 50% aqueous acetic acid (30 mL) was gently stirred at room temperature for 6 hrs and then kept standing at this temperature overnight. The progress of the reaction was checked by T.l.c (ethylacetate-hexane, 1:2), which showed complete conversion of the starting material to form a slower-moving product (R_f = 0.49). After completion of reaction the solvent was evaporated off under reduced pressure. The resulting syrup was taken up in chloroform and washed with saturated aqueous $NaHCO_3$ solution and finally with distilled water. The organic layer was dried (Na_2SO_4), filtered and the filtrate concentrated under reduced pressure to a syrup. Purification of the resulting syrupy residue by silica gel column chromatography with ethylacetate-hexane (1:2) as eluent furnished the monoacetal (**2**) (2 gm, 80%) as a solid mass. Recrystallization from methanol gave the title compound (**2**) as needless, m.p. 160-161°C [16].

^{1}H-NMR data (Pyr-d_5): δ_C 105.9 (C-1), 86.4 (C-2), 75.3 (C-3), 81.6 (C-4), 70.6 (C-5), 63.7 (C-6), 24.6, 27.1 (CMe_2), 111.3 (CMe_2), Lit [16].

General procedure for synthesis of D-glucofuranose derivatives 3-12

To a solution of 1,2-*O*-isopropylidene-α-D-glucofuranose (**2**) (100 mg, 0.38 mmol) in anhydrous dichloromethane (3 mL) and triethylamine (0.15 mL) was separately treated with acetic anhydride (0.17 mL, 4 molar equiv.), pentanoyl chloride (0.21 mL), hexanoyl chloride (0.25 mL), pivaloyl chloride (0.22 mL), decanoyl chloride (0.37 mL), lauroyl chloride (0.41 mL), myristoyl chloride (0.44 mL), 4-t-butylbenzoyl chloride (0.35 mL), 4-chlorobenzoyl chloride (0.24 mL) and 2,6-dichlorobenzoyl chloride (0.26 mL), respectively and stirring was continued at room temperature for 8-10 hours. The progress of the reaction was monitored by t.l.c (ethyl acetate-hexane, 1:8) which showed complete conversion of the reactant into a single faster moving product. Removal of solvent and purification by silica gel column chromatography with ethyl acetate-hexane (1:7) afforded the 3,5,6-tri-*O*-acetyl derivative (**2**), pentanoyl derivative (**3**), hexanoyl derivative (**4**), pivaloyl derivative (**5**), decanoyl derivative (**6**), lauroylate (**7**), myristoyl derivative (**8**), 4-t-butylbenzoyl derivative (**9**), 4-chlorobenzoyl derivative (**10**) and 2,6-dichlorobenzoyl derivative (**11**), respectively.

1,2-*O*-Isopropylidene-3,5,6-tri-*O*-acetyl-α-D-glucofuranose 3

Yield (110 mg, 70%) as needless, recrystallization from ethyl acetate-hexane, m.p. 116–117°C, R_f 0.51 (EtOAc:C_6H_{14}, 1:8). Anal. calcd. for $C_{15}H_{22}O_7$: C, 57.32; H, 7.04%.

Found: C, 57.39; H, 7.18%. FTIR ν1722 (-CO), 1370 (>CMe_2) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 5.85 (1H, d, J = 3.6 Hz, H-1), 5.24 (1H, d, J = 3.7 Hz, H-3), 5.11 (1H, m, H-5), 4.50 (1H, d, J = 3.7 Hz, H-2), 4.06 (3H, m, H-4, H-6a and H-6b), 2.07, 2.02, 2.00 (3×3H, 3×s, 3×MeCO-), 1.48 (3H, s, C*H*$_3$), 1.25 (3H, s, C*H*$_3$).

1,2-*O*-Isopropylidene-3,5,6-tri-*O*-pentanoyl-α-D-glucofuranose 4

Yield (124 mg, 58%) as syrup, which resisted crystallization, R_f 0.51 (EtOAc:C_6H_{14}, 1:7). Anal. calcd. for $C_{24}H_{40}O_9$: C, 61.0; H, 8.51%. Found: C, 61.09; H, 8.53%. FTIR ν 1725 (-CO), 1372 (>CMe_2) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 5.89 (1H, d, J = 3.7 Hz, H-1), 5.30 (1H, d, J = 3.7 Hz, H-3), 4.53 (1H, m, H-5), 4.56 (1H, d, J = 3.7 Hz, H-2), 4.43 (1H, m, H-6a), 4.19 (2H, m, H-4 and H-6b), 2.37 {6H, m, 3×$CH_3(CH_2)_2CH_2$CO-}, 1.62 {6H, m, 3×$CH_3CH_2CH_2CH_2$CO-}, 1.55 (6H, s, CMe_2), 1.31 {6H, m, 3×$CH_3CH_2(CH_2)_2$CO-}, 0.91 {9H, m, 3×$CH_3(CH_2)_3$CO-}.

1,2-*O*-Isopropylidene-3,5,6-tri-*O*-hexanoyl-α-D-glucofuranose 5

Yield (175 mg, 75%) as semi-solid, which resisted crystallization, R_f 0.55 (EtOAc:C_6H_{14}, 1:7). Anal. calcd. for $C_{27}H_{46}O_9$: C, 63.01; H, 9.0%. Found: C, 63.11; H, 9.02%. FTIR ν 1728 (-CO), 1380 (>CMe_2) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 100 MHz): δ_H 5.88 (1H, d, J = 3.7 Hz, H-1), 5.29 (1H, d, J = 3.7 Hz, H-3), 5.13 (1H, m, H-5), 4.52 (1H, d, J = 3.6 Hz, H-2), 4.43 (1H, dd, J = 9.6 and 5.0 Hz, H-6a), 4.17 (1H, m, H-6b), 4.01 (1H, m, H-4), 2.33 {6H, m, 3×$CH_3(CH_2)_3CH_2CO$-}, 1.62 {6H, m, 3×$(CH_3)_2CH_2CH_2CO$-}, 1.50 (3H, s, CH_3), 1.30 {15H, m, 3×$CH_3(CH_2)_2CH_2CH_2CO$-, 1×CH_3}, 0.88 {9H, m, 3×$CH_3(CH_2)_4CO$-}.

1,2-*O*-Isopropylidene-3,5,6-tri-*O*-pivaloyl-α-D-glucofuranose 6

Yield (124 mg, 80%) as homogeneous syrup, which resisted crystallization, R_f 0.52 (EtOAc:C_6H_{14}, 1:7). Anal. calcd. for $C_{24}H_{40}O_9$: C, 61.0; H, 8.51%. Found: C, 61.08; H, 8.55%. FTIR ν1728 (-CO), 1368 (>CMe_2) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 100 MHz): δ_H 5.90 (1H, d, J = 3.7 Hz, H-1), 5.09 (1H, d, J = 3.7 Hz, H-3), 4.54 (2H, m, H-5 and H-6a), 4.26 (1H, d, J = 3.7 Hz, H-2), 4.10 (1H, dd, J = 12.8 and 2.7 Hz, H-6b), 3.95 (1H, m, H-4), 1.49, 1.31 (2×3H, 2×s, CMe_2), 1.20 {27H, s, 3×$(CH_3)_3CCO$-}.

1,2-*O*-Isopropylidene-3,5,6-tri-*O*-decanoyl-α-D-glucofuranose 7

Yield (251 mg, 81%) as syrup, which resisted crystallization, R_f 0.53 (EtOAc:C_6H_{14}, 1:7). Anal. calcd. for $C_{39}H_{70}O_9$: C, 68.60; H, 10.31%. Found: C, 68.64; H, 10.33%. FTIR ν1729 (-CO), 1364 (>CMe_2) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 5.89 (1H, d, J = 3.7 Hz, H-1), 5.30 (1H, d, J = 3.7 Hz, H-3), 5.12 (1H, m, H-5), 4.54 (1H, d, J = 3.7 Hz, H-2), 4.45 (1H, dd, J = 9.6 and 2.9 Hz, H-6a), 4.17 (1H, dd, J = 12.8 and 2.7 Hz, H-6b), 4.00 (1H, dd, J = 12.2 and 5.4 Hz, H-4), 2.34 {6H, m, 3×$CH_3(CH_2)_7CH_2CO$-}, 1.62 {6H, m, 3×$CH_3(CH_2)_6CH_2CH_2CO$-}, 1.49 (3H, s, CH_3), 1.26 {39H, m, 3×$CH_3(CH_2)_6CH_2CH_2CO$-, 1×CH_3}, 0.87 {9H, m, 3×$CH_3(CH_2)_8CO$-}.

1,2-*O*-Isopropylidene-3,5,6-tri-*O*-lauroyl-α-D-glucofuranose 8

Yield (199 mg, 62%) as needles, recrystallization from ethylacetate-hexane, m.p. 62-64°C, R_f 0.51 (EtOAc:C_6H_{14}, 1:8). Anal. calcd. for $C_{45}H_{22}O_9$: C, 76.49; H, 3.13%. Found: C, 76.54; H, 3.19%. FTIR ν 1729 (-CO), 1373 (>CMe_2) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 5.90 (1H, d, J = 3.7 Hz, H-1), 5.30 (1H, d, J = 3.7 Hz, H-3), 4.53 (2H, m, H-4 and H-6a), 4.42 (1H, d, J = 3.7 Hz, H-2), 4.18 (1H, m, H-6b), 4.10 (1H, m, H-5), 2.35 {6H, m, 3×$CH_3(CH_2)_9CH_2CO$-}, 1.63 {6H, m, 3×$CH_3(CH_2)_8CH_2CH_2CO$-}, 1.51 (3H, s, CH_3), 1.25 {51H, m, 3×$CH_3(CH_2)_8CH_2CH_2CO$-, 1×CH_3}, 0.88 {9H, m, 3×$CH_3(CH_2)_{10}CO$-}.

1,2-*O*-Isopropylidene-3,5,6-tri-*O*-myristoyl-α-D-glucofuranose 9

Yield (317 mg, 82%) as crystalline solid, recrystallization from ethylacetate-hexane, m.p. 55-56°C, R_f 0.51 (EtOAc : C_6H_{14}, 1:6). Anal. calcd. for $C_{51}H_{94}O_9$: C, 71.97; H, 11.11%. Found: C, 71.99; H, 11.19%. FTIR ν 1728 (-CO), 1379 (>CMe_2) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 5.88 (1H, d, J = 3.6 Hz, H-1), 5.29 (1H, d, J = 3.7 Hz, H-2), 5.20 (1H, d, J =3.7 Hz, H-3), 4.57 (1H, m, H-5), 4.43 (1H, dd, J = 9.6 and 5.0 Hz, H-6a), 4.24 (2H, m, H-4 and H-6b), 2.28 {6H, m, 3×$CH_3(CH_2)_{11}CH_2CO$-}, 1.51 (6H, s, CMe_2), 1.24 {66H, m, 3×$CH_3(CH_2)_{11}CH_2CO$-}, 0.88 {9H, m, 3×$CH_3(CH_2)_{12}CO$-}.

1,2-*O*-Isopropylidene-3,5,6-tri-*O*-(4-t-butylbenzoyl)-α-D-glucofuranose 10

Yield (210 mg, 66%) as crystalline solid, recrystallization from ethylacetate-hexane, m.p. 80-81°C, R_f 0.53 (EtOAc:C_6H_{14}, 1:7). Anal. calcd. for $C_{42}H_{52}O_9$: C, 71.98; H, 7.46%. Found: C, 71.99; H, 7.51%. FTIR ν 1720 (-CO), 1372 (>CMe_2) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 7.97 (6H, m, Ar-H), 7.45 (6H, m, Ar-H), 6.0 (1H, d, J = 3.6 Hz, H-1), 5.58 (1H, d, J = 3.6 Hz, H-3), 4.70 (2H, m, H-4 and H-6a), 4.41 (2H, m, H-5 and H-6b), 4.06 (1H, d, J = 3.6 Hz, H-2), 1.53 (6H, s, CMe_2), 1.33 {27H, s, 3×$(CH_3)_3C$-}.

1,2-*O*-Isopropylidene-3,5,6-tri-*O*-(4-chlorobenzoyl)-α-D-glucofuranose 11

Yield (199 mg, 62%) as needles, recrystallization from ethylacetate-hexane, m.p. 165-166°C, R_f 0.52 (EtOAc : C_6H_{14}, 1:6.5). Anal. calcd. for $C_{30}H_{25}O_9Cl_3$: C, 56.64; H, 3.95%. Found: C, 56.70; H, 3.98%. FTIR ν 1729 (-CO), 1378 (>CMe_2) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 8.01 (6H, m, Ar-H), 7.40 (6H, m, Ar-H), 6.0 (1H, d, J = 3.7 Hz, H-1), 5.57 (1H, d, J = 3.6 Hz, H-3), 4.71 (2H, m, H-5 and H-6a),

4.42 (2H, m, H-4 and H-6a), 4.04 (1H, d, J = 3.7 Hz, H-2), 1.52, 1.29 (2×3H, 2×s, CMe_2).

1,2-*O*-Isopropylidene-3,5,6-tri-*O*-(2,6-dichlorobenzoyl)-α-D-glucofuranose 12

Yield (262 mg, 78%) as semi-solid which resisted crystallization, R_f 0.53 (EtOAc:C_6H_{14}, 1:7). Anal. calcd. for $C_{30}H_{22}O_9Cl_6$: C, 48.71; H, 3.00%. Found: C, 48.77; H, 3.08%. FTIR ν1727 (-CO), 1368 (>CMe_2) cm^{-1}. ^{1}H-NMR data ($CDCl_3$, 400 MHz): δ_H 7.40 (9H, m, Ar-H), 5.94 (1H, d, J = 3.7 Hz, H-1), 5.58 (1H, d, J = 3.7 Hz, H-3), 4.70 (1H, d, J = 3.7 Hz, H-2), 4.51 (2H, m, H-4 and H-6a), 4.36 (1H, m, H-6b), 4.17 (1H, m, H-5), 1.45, 1.29 (2×3H, 2×s, CMe_2).

Microbial screening studies for test bacteria

The synthesized test chemicals (**3-12**) (Figure 1) were subjected to antibacterial screening against six both Gram-positive and Gram-negative bacterial strains viz., *Bacillus cereus* BTCC 19, *Bacillus megaterium* BTCC 18, *Escherichia coli* ATCC 25922, *Salmonella typhi* AE 14612, *Salmonella paratyphi* AE 146313 and *Pseudomonas* species CRL (ICDDRB), In all cases, a 2% solution (in $CHCl_3$) of the chemicals was used.

Antibacterial activity assay

The in vitro antibacterial activities of the synthesized chemicals were detected by disc diffusion method [17,18]. Paper discs of 4 mm in diameter and glass Petri plate of 90 mm in diameter were used throughout the experiment. Paper discs were sterilized in an autoclave and dried at 100°C in an oven. Then, the discs were soaked with test chemicals at the rate of 50 μg (dry weight) per disc for antibacterial analysis. For pour plate method, one drop of bacterial suspension was taken in a sterile Petri dish and approximately 20 mL of sterilized melted NA (nutrient agar) (~45°C) was poured into the plate, and then mixed thoroughly with the direction of clock wise and anticlockwise. After solidification of the seeded NA medium, paper disc after soaking with test chemicals (2% in $CHCl_3$) were placed at the centre of the inoculated pour plate. A control plate was also maintained in each case with chloroform. Firstly, the plates were kept for 4 h at low temperature (4°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 growth of test organisms and were observed at 24 h intervals for two days. The activity was expressed in terms of inhibition zone diameter in mm. Each experiment was repeated thrice. The standard antibiotic Ampicillin from FISONS Ltd. (Bangladesh) was used as a positive control and compared with tested chemicals under identical conditions.

RESULTS AND DISCUSSION

Synthesis and characterization

In the present study, we carried out regioselective acylation of 1,2-*O*-isopropylidene-α-D-glucofuranose (**2**) using the direct method (Figure 1). A number of rarely used acylating agents were employed for this purpose. The structures of the products obtained by the direct acylation method were established by FTIR and ^{1}H-NMR spectroscopy. All these acylation products were employed as test chemicals for antibacterial screening studies against a number of human pathogenic bacteria.

In carbohydrate chemistry, selective acylation of a desired hydroxyl group is considered as an important and fundamental step, since these acyl derivatives would provide versatile intermediates for the synthesis of many important chemical compounds. Some of these acyl derivatives may also have effective biological activity. Therefore, selective acylation of carbohydrates is not only necessary for the synthesis of biologically active products but also have immense synthetic utility as versatile intermediates for the synthesis of various other chemical compounds modified at the remaining positions.

Acetylation of D-glucose was carried out using the method of [16] which involved the use of anhydrous acetone and concentrated sulphuric acid in the presence of anhydrous Copper(II) sulphate as a catalyst. Thus, treatment of D-glucose with anhydrous Copper(II) sulphate at room temperature for 18 hours furnished 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**1**) in 55% yield. This compound was identified by comparing its melting point with that of an authentic sample reported [16] in the literature. The mechanism of the reaction is likely to involve initial formation of the oxycarbonium ion (**1a**) which undergoes substitution by the sugar

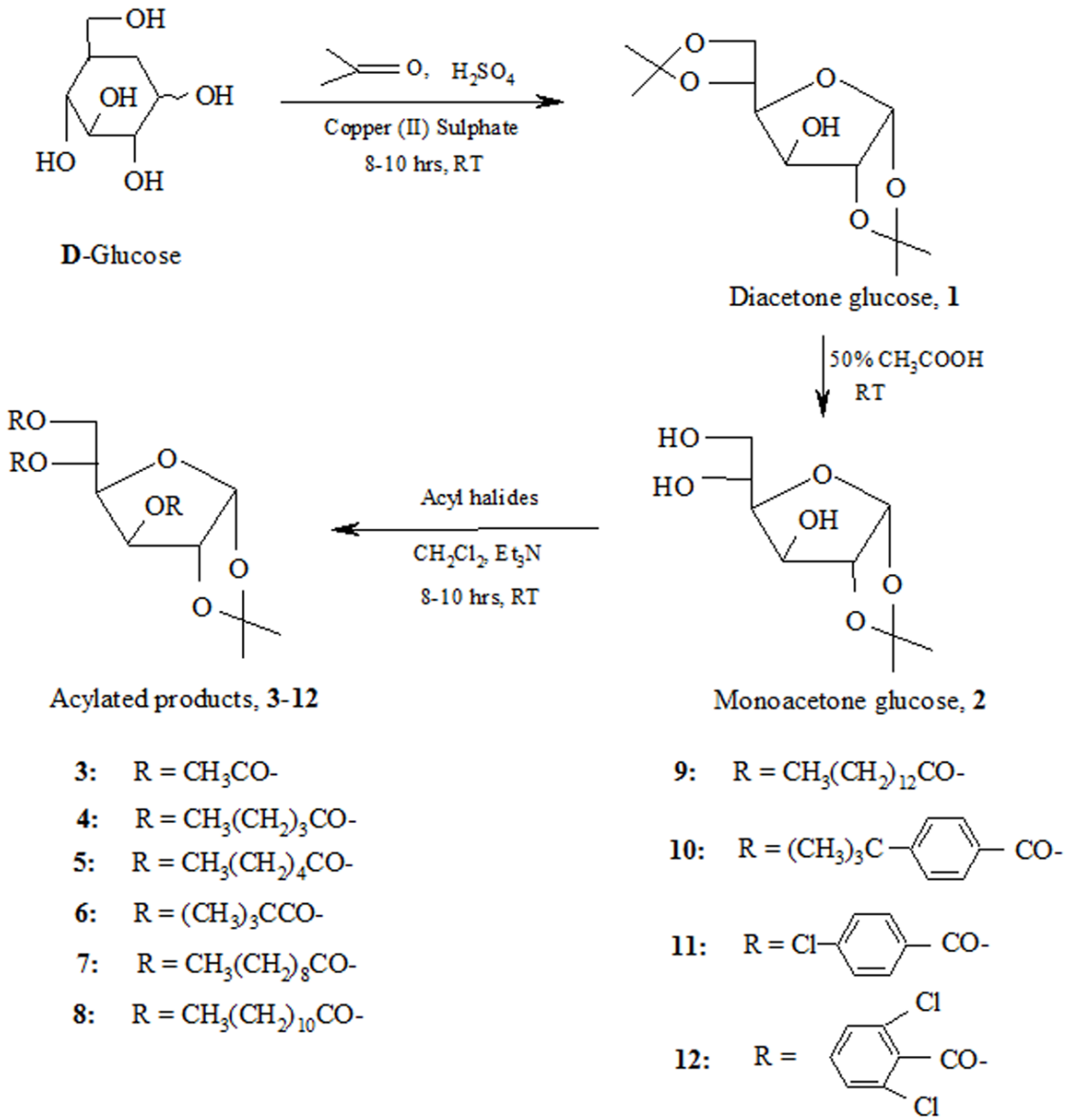

Figure 1. Reaction path of the synthesized compounds (3-12).

hydroxyl group to give the noncyclic acetal (**1b**), which eventually cyclises to give the cyclic acetal (**1c**) (Figure 2). Removal of the 5,6-*O*-isopropylidene group from diacetone (**1**) was achieved by careful hydrolysis in 50% aqueous acetic acid at room temperature for 8 hours which gave the monoacetal (**2**) in 80% yieldas a crystalline solid. However, this method was capricous and led to a variable yield of the monoacetal (**2**). The monoacetal (**2**) was indistinguisable by the usual criteria from that of an authentic sample reported [16] in the literature.

Our initial effort was treatment of the triol (**2**) with acetic anhydride in dichloromethane and triethylamine at room temperature. Conventional work-up procedure followed by silica gel column chromatographic separation, we able to isolate compound **3** in 70% yield as needless m.p. 116-117°C. The structure of the acetate derivative (**3**) was established by analyzing its FTIR and ^{1}H-NMR spectrum. The FTIR spectrum of compound **3** showed absorption bands at 1722 cm^{-1} (-CO stretching) and 1370 cm^{-1} (carbon-hydrogen stretching of >CMe$_2$),

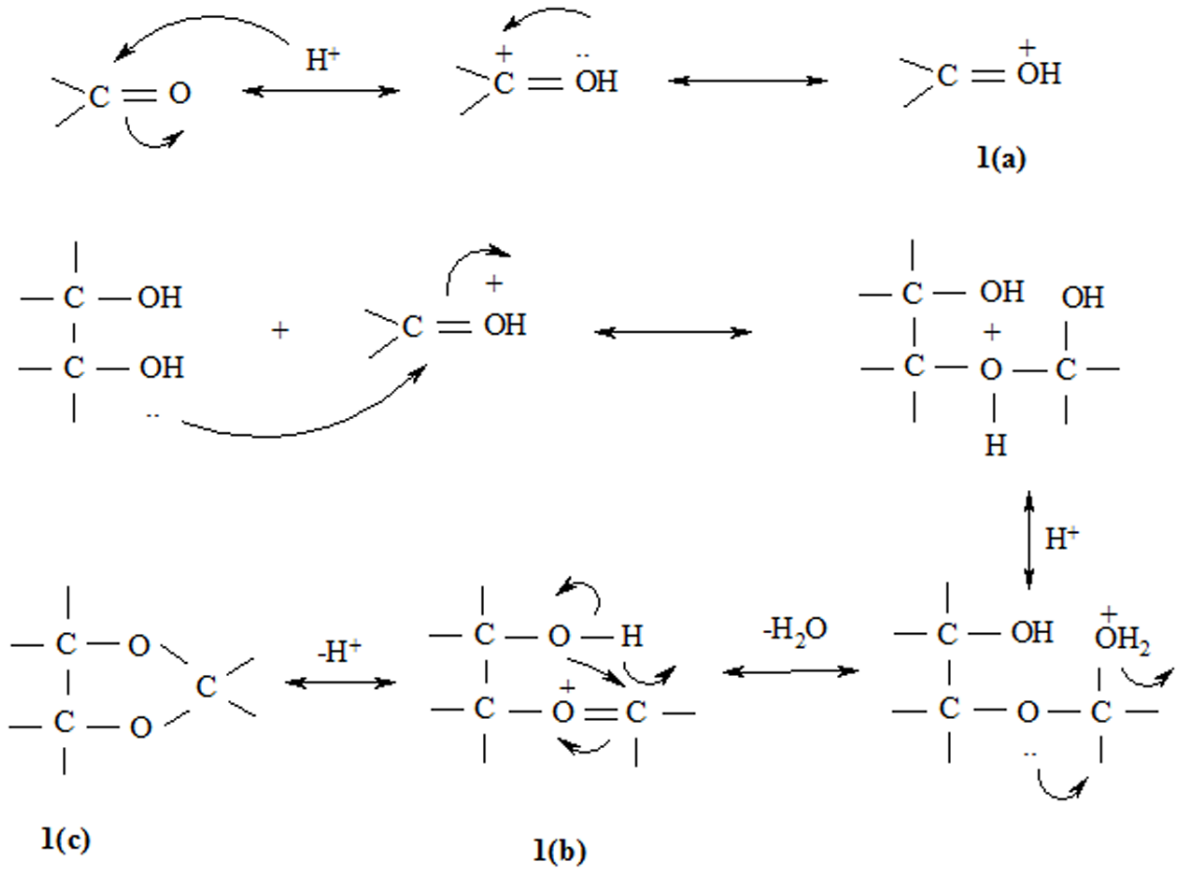

Figure 2. Formation of the cyclic acetal.

thereby suggesting the presence of carbonyl and >CMe_2 groups in the molecule. In its ^{1}H-NMR, three three-proton singlets at δ 2.07, δ 2.02 and δ 2.00 were due to the methyl protons of three acetyloxy groups. The downfield shifts of H-3, H-5 and H-6 to δ 5.24 (as d, J=3.7 Hz), 5.11 (as m) and 4.06 (as m) as compared from their usual values (~4.00 ppm) [19] indicated the attachment of the acetyl groups at positions 3, 5 and 6. The rest of the spectrum was in complete agreement with the structure accorded as 1,2-*O*-isopropylidene-3,5,6-tri-*O*-acetyl-α-D-gluco-furanose (**3**).

Acylation of compound **2** was done using excess pentanoyl chloride in dichloromethane and triethylamine followed by usual work-up and purification procedure and the pentanoyl derivative (**4**) was obtained in 58% yield as syrup. The FTIR spectrum of this compound (**4**) showed absorption bands at 1725 and 1372 cm^{-1} due to carbonyl and >CMe_2 stretchings. In its ^{1}H-NMR spectrum, three six-proton multiplet at δ 2.37 {3×$CH_3(CH_2)_2CH_2CO$-} and δ 1.62 {3×$CH_3CH_2CH_2CH_2CO$-} and δ 1.31 {3×$CH_3CH_2(CH_2)_2$ CO-} and one nine-proton multiplet at δ 0.91 {3×$CH_3(CH_2)_3CO$-} were indicative of the presence of three pentanoyl groups in the molecule. The deshielding of C-3, C-5 and C-6 protons to δ 5.30 (as d, J=3.7 Hz, H-3), δ 4.53 (as m, H-5), δ 4.43 (as m, H-6a) and 4.19 (as m, H-6b) from their usual values [19] confirmed the attachment of three pentanoyl groups at these positions. Analysis of the rest of the FTIR and ^{1}H-NMR led us to assign its structure as 1,2-*O*-isopropylidene-3,5,6-tri-*O*-pentanoyl-α-D-glucofuranose (**4**).

Reaction of compound **2** with hexanoyl chloride in CH_2Cl_2-Et_3N medium, followed by conventional aqueous work-up procedure and silica gel column chromatographic purification provided the hexanoyl derivative (**5**) in 75% yield as semi-solid mass which resisted crystallization. The FTIR spectrum displayed the following characteristic absorption bands at 1728 cm^{-1} (for –CO stretching) and 1380 cm^{-1} (for carbon-hydrogen stretching) in the molecule. The ^{1}H-NMR spectrum of compound **5** displayed two six-proton multiplet at δ 2.33 {3×$CH_3(CH_2)_3CH_2CO$-},

and δ 1.62 {3×$(CH_3)_2CH_2CH_2CO$-}, a twelve-proton multiplet at δ 1.30 and one nine-proton multiplet at δ 0,88 showing the attachment of three hexanoyl groups in the molecule. The resonance for H-3, H-5 and H-6 appeared at δ 5.29 (as d, J=3.7 Hz), δ 5.13 (as m), δ 4.43 (as dd, J=9.6 Hz and 5.0 Hz, H-6a) and δ 4.17 (as m, H-6b) which shifted downfield from their values indicating the attachment of the pentanoyl groups at positions 3, 5 and 6. Complete analysis of the FTIR and ^{1}H-NMR spectrum enabled us to assign the structure of the hexanoyl derivative as 1,2-*O*-isopropylidene-3,5,6-tri-*O*-hexanoyl-α-D-glucofuranose (**5**).

We then treated compound **2** with pivaloyl chloride in dichloromethane-triethylamine at room temperature and after usual work-up and chromatographic purification, we obtained the 3,5,6-tri-*O*-pivaloyl derivative (**6**) in 80% yield as syrup. Its FTIR spectrum showed absorption bands at 1728 cm^{-1} (-CO stretching) and 1368 cm^{-1} (>CMe_2, carbon-hydrogen stretching). In the ^{1}H-NMR spectrum of **6** a twenty seven-proton singlet at δ 1.20 {3×$(CH_3)_3CCO$-} was due to the methyl protons of pivaloyl groups which indicated the introduction of three pivaloyl groups in the molecule. The downfield shift of C-3 proton to δ 5.29 (as d, J=3.7 Hz), C-5 proton to δ 4.54 (as m), C-6 proton to δ 4.54 (as m, H-6a), and δ 4.10 (as dd, J=12.8 and 2.7 Hz, H-6b) from their usual values (~4.00 ppm) [19] showed the attachment of the pivaloyl groups at positions 3, 5 and 6. Complete analysis of the FTIR and ^{1}H-NMR spectra was consistent with the structure of the compound assigned as 1,2-*O*-isopropylidene-3,5,6-tri-*O*-pivaloyl-α-D-glucofuranose (**6**).

Encouraged by the results obtained by above acylation of the triol (**2**), we then used decanoyl chloride as the next acylating agent. Thus, the triol was treated with equimolecular amount of decanoyl chloride in dry CH_2Cl_2-$(C_2H_5)_3N$ medium, followed by conventional work-up and silica gel column chromatography, provided the decanoyl derivative (**7**) in 81% yield as clear syrup. The FTIR spectrum of this compound (**7**) displayed absorption bands at 1729, 1364 cm^{-1} due to carbonyl and carbon-hydrogen stretchings. The ^{1}H-NMR spectrum of this compound (**7**) provided the following characteristic peaks : two six-proton multiplet at δ 2.34 {3×$CH_3(CH_2)_7CH_2CO$-} and 1.62 {3×$CH_3(CH_2)_6CH_2CH_2CO$-}, a thirty six-proton multiplet at δ 1.26 {3×$CH_3(CH_2)_6CH_2CH_2CO$-} and a nine-proton multiplet at δ 0.87 {3×$CH_3(CH_2)_8CO$-} indicating the introduction of three decanoyl groups to the triol molecule. The downfield shift of H-3, H-5 and H-6 resonances to 5.30 (as d, J=3.7 Hz), 5.12 (as m), 4.45 (as dd, J=9.6 and 2.9 Hz, H-6a) and 4.17 (as dd, J=12.8 and 2.7 Hz, H-6b) as compared to the usual values (~4.00 ppm) [19], was indicative of the attachment of the three decanoyl groups at positions 3, 5 and 6. On the basis of complete analysis of the FTIR and ^{1}H-NMR spectra, the structure of this compound was accorded as 1,2-*O*-isopropylidene-3,5,6-tri-*O*-decanoyl-α-D-glucofuranose (**7**).

Our next effort was to react 1,2-*O*-isopropylidene-α-D-glucofuranose (**2**) with unimolecular amount of lauroyl chloride in dichloromethane-Et_3N at room temperature. Usual work-up and separation by silica gel column chromatography furnished the lauroyl derivative (**8**) in 62% yield as needless m.p. 62-63°C. The FTIR spectrum of compound **8** showed the following absorption bands: 1729 cm^{-1} (-CO stretching) and 1373 cm^{-1} (>CMe_2, carbon-hydrogen stretching). Its ^{1}H-NMR spectrum displayed two six-proton multiplet at 2.35 {3×$CH_3(CH_2)_9CH_2CO$-}, δ 1.63 {3×$CH_3(CH_2)_8CH_2CH_2CO$-} a fourty eight-proton multiplet at δ 1.25 {3×$CH_3(CH_2)_8CH_2CH_2CO$-} and a nine-proton multiplet at δ 0.88 {3×$CH_3(CH_2)_{10}CO$-}, thereby suggesting the presence of three lauroyl groups in the molecule. Also the C-3, C-5 and C-6 protons were deshielded considerably to δ 5.30 (as d, J=3.7 Hz, H-3), δ 4.53 (as m, H-6a), δ 4.18 (as m, H-6b) and 4.10 (as m, H-5) from their usual values (~4.00 ppm) [19], thus showing that the three lauroyl groups were introduced at positions 3, 5 and 6. On the basis of complete analysis of the FTIR and ^{1}H-NMR spectrum, the structure of the lauroyl derivative was assigned as 1,2-*O*-isopropylidene-3,5,6-tri-*O*-lauroyl-α-D-glucofuranose (**8**).

Treatment of the triol (**2**) with unimolecular amount of myristoyl chloride in dichloromethane-triethyl amine medium at room temperature, followed by removal of solvent and silica gel column chromatographic purification, compound **9** was established in 82% yield as prism, m.p 55-56°C. Its FTIR spectrum exhibited absorption bands at 1728 and 1379 cm^{-1} due to –CO stretching and > CMe_2

stretching respectively. In its ^{1}H-NMR spectrum, a six-proton multiplet at δ 2.28 {3×$CH_3(CH_2)_{11}CH_2CO$-}, a sixty-six proton multiplet at δ 1.24 {3×$CH_3(CH_2)_{11}CH_2CO$-} and a nine-proton multiplet at δ 0.88 {3×$CH_3(CH_2)_{12}CO$-} indicated the attachment of three myristoyl groups in the molecule. The downfield shift of the C-3, C-5 and C-6 protons to δ 5.20 (as d, J=3.7Hz), δ 4.57 (as m), δ 4.43 (as dd, J=9.6 and 5.0 Hz, H-6a) and δ 4.24 (as m, H-6b) from their usual values [19] and the resonances of other protons in their anticipated portions showed the attachment of myristoyl groups at positions 3, 5 and 6. By complete analysis of the FTIR and ^{1}H-NMR spectrum, the structure of the tri-*O*-myristoate was assigned as 1,2-*O*-isopropylidene-3,5,6-tri-*O*-myristoyl-α-D-glucofuranose (**9**).

We then allowed the triol (**2**) to react with 4-t-butylbenzoyl chloride in anhydrous dichloromethane-triethylamine medium and after usual work-up and chromatographic purification, it yielded 4-t-butylbenzoate (**10**) in 66% as needless, m.p. 80-81°C. The FTIR spectrum of this compound indicated absorption bands at 1720 cm^{-1} corresponding to carbonyl stretchings (-CO) and 1372 cm^{-1} to carbon-hydrogen stretching (>CMe_2). Its ^{1}H-NMR spectrum displayed the following characteristic aromatic two six-proton multiplet peaks at δ 7.97 (as m, Ar-H), δ 7.45 (as m, Ar-H) and a twenty seven-proton singlet at δ 1.33 {as s, 3Φ(CH_3)$_3$C-} which corresponded to the presence of three 4-t-butylbenzoyl groups in the molecule. The deshielding of C-3, C-5 and C-6 protons to δ 5.58 (as d, 3.6 Hz, H-3), δ 4.41 (as m, H-5), δ 4.70 (as m, H-6a) and 4.41 (as m, H-6b) from their usual values confirmed the attachment of three 4-t-butylbenzoyl group at these positions. Complete analysis of the FTIR and ^{1}H-NMR spectrum led us to establish its structure as 1,2-*O*-isopropylidene-3,5,6-tri-*O*-(4-t-butylbenzoyl)-α-D-glucofuranose (**10**).

4-Chlorobenzoylation of triol (**2**) by direct method using 4-chlorobenzoyl chloride in dry CH_2Cl_2-Et_3N and after similar work-up and purification techniques, the product (**11**) was isolated in 81% yield as prism, m.p. 165-166°C. The FTIR spectrum of this compound displayed absorption bands at 1729 and 1378 cm^{-1} corresponded to carbonyl (-CO) and carbon-hydrogen (>CMe_2) stretchings, respectively. In its ^{1}H-NMR spectrum, the two six-aromatic proton multiplet at δ 8.01 (as m, Ar-H), δ 7.40 (as m, Ar-H) are characteristic of *p*-substituted benzoyl groups. The deshielding of C-3, C-5 and C-6 protons from their usual values (~4.00 ppm) [19] and the resonance of other protons in their anticipated positions confirmed the structure of this compound as 1,2-*O*-isopropylidene-3,5,6-tri-*O*-(4-chlorobenzoyl)-α-D-glucofuranose (**11**).

Our final effort was to carry out selective 2,6-dichlorobenzoylation of compound **2** with 2,6-dichlorobenzoyl chloride is dry dichloromethane and triethylamine medium using conventional work-up and purification procedures afforded the compound **12** in 78% yield as a semi-solid which resisted crystallization. The FTIR spectrum of this compound showed the following characteristic peaks: 1727 (-CO stretching) and 1368 cm^{-1} (>CMe_2) stretching. The ^{1}H-NMR spectrum, a nine-proton multiplet at δ 7.40 (as m, Ar-H) corresponded to three 2,6-dichlorobenzoyl groups. The rest of the spectrum was consistent with the structure of the compound assigned as 1,2-*O*-isopropylidene-3,5,6-tri-*O*-(2,6-dichlorobenzoyl)-α-D-glucofuranose (**12**).

A series of acylated derivatives of monoacetone glucose (**2**) were thus prepared employing a wide variety of acylating agents containing some probable biologically prone atoms/groups. All the compounds thus prepared were employed as test chemicals for evaluating their antibacterial activities against a number of human pathogenic bacteria.

Antibacterial screening studies
Effects of test chemicals on human pathogenic bacteria

The results of *in vitro* antibacterial activity studies of the selected test chemicals (3-12) are presented in the Table 1 and Figure 3. For comparative study, biological activity of standard antibiotics (Ampicillin) was also determined. The results of the D-glucofuranose derivatives on each microorganism are discussed below.

Bacillus cereus

The growth inhibition of this Gram-positive bacterium for different chemical treatment is presented in Table 1 and Figure 3. The screening data suggested that the chemical **7** (13 mm) was more effective than that of other chemicals such

Table 1. Zone of inhibition observed against microorganisms (both Gram-positive & Gram-negative) by the test chemicals.

Compound	Diameter of inhibition zone in mm 200 ↔g dw/disc					
	B. cereus	*B. megaterium*	*E. coli*	*S. typhi*	*S. paratyphi*	*P. species*
3	NF	NF	NF	NF	8.0	NF
4	8.0	7.0	NF	NF	7.0	NF
5	NF	NF	NF	NF	NF	NF
6	NF	7.0	10.0	NF	7.0	NF
7	*13.0	9.0	8.5	*14.0	*11.0	*12.0
8	10.0	10.0	NF	*17.0	NF	10.0
9	NF	7.0	8.5	10.0	NF	NF
10	NF	7.0	NF	NF	NF	NF
11	*10.5	10.0	*11.5	10.0	10.0	10.0
12	10.0	9.0	8.5	9.0	7.5	9.0
**Ampicillin	*19.0	*16.0	*19.0	*20.0	*18.0	*16.0

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

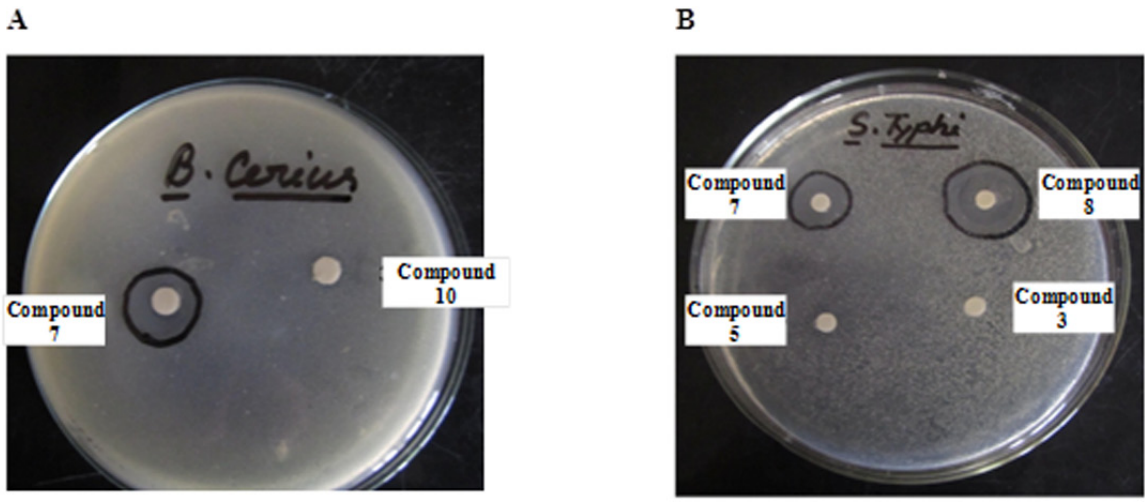

Figure 3. (A): Inhibition of zone against *Bacillus cereus* by the compounds 7 and 10. (B): Inhibition of zone against *Salmonella typhi* by the compounds 3, 5, 7 and 8.

as **4**, **8**, **11** and **12** which were somewhat less effective. The rest of the chemicals such as **3**, **5**, **6**, **9** and **10** did not show any inhibition.

Bacillus megaterium

The in *vitro* growth inhibition of this bacterium showed that compound **8** and **11** were more active against this bacterium. The compounds **4**, **6**, **7**, **9**, **10** and **12** have shown relatively poor inhibition. The inhibition zone for the rest of the chemicals were found zero. All of these test chemicals were, however, less active against this bacterial strain than standard antibiotic, Ampicillin in case of this bacterial strain.

Escherichia coli

From the inhibition data of the Gram-negative bacteria, it was found that the chemical **11** was more effective than that of other chemicals such as **6**, **7**, **9** and **12** which were somewhat less effective. The rest of the chemicals did not show any inhibition.

Salmmonella typhi

The screeing data (Table 1 and Figure 3) suggests that the test chemicals **8** (17 mm) and **7** (14 mm) were more effective against this bacterium. Chemicals **9**, **11** and **12** were less active whereas **3**-**6** and **10** were found to be inactive against this

bacterial strain. All of these test chemicals were however less active against this bacterial strain than standard antibiotics, Ampicillin (20 mm).

Salmonella paratyphi

The *in vitro* growth inhibitions of this bacterium suggested that the compound **7** showed good inhibition activity against this pathogen. The chemicals **3**, **4**, **6**, **11** and **12** were somewhat less effective. The rest of the chemicals did not show any growth inhibition against *S. paratyphi* bacterium.

***Pseudomonas* species**

Only four compounds showed good growth inhibition against this bacterium. The screening data suggested that **7**, **8**, **11** and **12** were more prone towards inhibition against this bacterium than that of other chemicals. The remaining test chemicals were found to be inactive against this micro-organism.

From the above results it was observed that the selectively acylated derivatives **7** and **11** showed marked inhibition against Gram-positive bacteria while compounds **7** and **8** were showed very active against Gram-negative bacteria. In general, it has been observed that average antibacterial results of the chemicals for Gram-positive bacteria follow the order **7** ≥ **11** > **8** > **12** > **4** > **6** = **9** = **10**, whereas Gram-negative bacteria follow the order **7** > **11** > **12** > **8** > **9** > **3** > **6** = **4**. Most tested chemicals showed mild inhibition and some were unable to show any inhibition against the tested bacteria. Antibacterial activities of our test chemicals are in accordance with the results we observed before [12,20]. We also observed that some compounds are active against both the Gram-positive and Gram-negative bacteria. So these compounds may be considered for future studies for their usage as broad spectrum antibiotics. This is the first antibacterial activity test report of our newly synthesized D-glucofuranose derivatives against the selected human pathogenic microorganisms.

CONCLUSION

The present work reports the synthesis of a new series of 1,2-*O*-isopropylidene-α-D-glucofuranose (**2**) derivatives **3**-**12**. Antibacterial activity of the newly synthesized compounds exhibited good to moderate activities against selected human pathogenic strains. The results of this investigation may create an opportunity for further evaluation of these test chemicals against other microorganisms. It is also expected that this work employing D-glucofuranose derivatives as test chemicals will help further work on the development of pesticides and/or medicines.

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