MICROBIAL ASSAY OF NOVEL 2-S-TETRA-O-BENZOYL-D-GLUCOPYRANOSYL-1-ARYL-5-HEPTA-O-BENZOYL-β-D-LACTOSYL-2-ISOTHIOBIURETS

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ABSTRACT
The present work aims to synthesize and screen the antifungal and antibacterial activities of a series of new 2-S-tetra-O-benzoyl-D-glucopyranosyl-1-aryl-5-hepta-O-benzoyl-β-D-lactosyl-2-isothiobiurets by the interaction of hepta-O-benzoyl-β-D-lactosyl isocyanate and 8-tetra-O-benzoyl-D-glucopyranosyl-1-aryl isothiocarbamides. The identities of these newly synthesised compounds have been established on the basis of usual chemical transformations and IR, 1HNMR and Mass spectral studies. These synthesized products were evaluated for their antimicrobial activity against some pathogenic organisms. Some of the products displayed promising activity.

Keywords: Lactosylisocyanate, glucopyranosylisothiocarbamides, lactosylisothiobiurets, antimicrobial activity.

INTRODUCTION
Carbohydrate biosynthetic pathway is often amenable to interception with synthetic unnatural substrates. Such metabolic interference can block the expression of oligosaccharides or alter the structures of sugars presented on cells. The applications of glycosidase inhibitors to agricultural and medical fields and the prospect for new therapeutic applications are reconsidered. These chemical approaches are contributing great insight into the countless biological functions of oligosaccharides. Hence, it was the thought of interest to synthesize these N-lactosides and to study their antimicrobial activity against gram +ve and gram –ve micro-organisms with the help of cup plate agar diffusion method. In present communication, we report the microbial assay of newly synthesised 2-S-tetra-O-benzoyl-D-glucopyranosyl-1-aryl-5-hepta-O-benzoyl-β-D-lactosyl-2-isothiobiurets.

EXPERIMENTAL
All the chemicals and solvents were obtained from commercial and purified using standard procedure wherever required. Melting points were taken by the open capillary method and were uncorrected. The reactions were monitored by thin layer chromatography on silica gel G plates (Merck silica-60 F254). Optical rotations [α]D 31 were measured on the Equip-Tronics EQ-800 Digital Polarimeter at 31°C in CHCl3. The structures of all the newly synthesized compounds were confirmed by IR Spectra which recorded on Perkin-Elmer spectrum RXI FTIR Spectrometer (Range: 4000-450 cm⁻¹). 1H NMR was obtained on Bruker DRX-300 NMR spectrometer operating at 300 MHz Samples were prepared in CDCl3 with TMS as an internal reference. Mass spectra were obtained on Thermo Finnegan LCQ Advantage max ion trap mass spectrometer.

General Procedure
Synthesis of 2-S-tetra-O-benzoyl-D-glucopyranosyl-1-aryl-5-hepta-O-benzoyl-β-D-lactosyl-2-isothiobiurets (3a-g)
A 0.005M of 8-tetra-O-benzoyl-D-glucopyranosyl-1-aryl isothiocarbamides(2a-g) in a 5ml of benzene was added to a 0.005M solution of hepta-O-benzoyl-β-D-lactosyl isocyanate(1) in 15ml benzene, the reaction mixture was reflux over boiling water bath for 5hr. After refluxing, the solvent was distilled off and the sticky residue obtained was triturated with petroleum ether (60-80 °C) to afford a solid (3a-g). The product was purified by chloroform petrol ether.

MICROBIAL ASSAY OF ISOTHIOBIURETS
Kedar P. Pande
RESULTS AND DISCUSSIONS

Herein, we report the synthesis of various 2-S-tetra-O-benzoyl-D-glucopyranosyl-1-aryl-5-hepta-O-benzoyl-β-D-lactosyl-2-isothiobiurets (3a-f) by interaction of hepta-O-benzoyl-β-D-lactosyl isocyanate (1) and S-tetra-O-benzoyl-D-glucopyranosyl-1-aryl isothiocarbamides (2a-g).

All products were crystallized from ethanol before recording the physical data. The purity of compound was checked by TLC. The spectral analysis 10-12 IR, 1HNMR and Mass spectra of the product were observed. Optical rotation of the product was also recorded. All the compounds have been screened for both antimicrobial and antifungal activity using cup plate agar diffusion method 13-14 by measuring the inhibition zone in mm. Amikacin (100ug/mL) was used as a standard for antibacterial activity and Fluconazole (100ug/mL) was used as a standard for antifungal activity.

2-S-tetra-O-benzoyl-D-glucopyranosyl-1-aryl-5-hepta-O-benzoyl-β-D-lactosyl-2-isothiobiurets

Where, Bz = COC₆H₅, R = (a) phenyl, (b) o-tolyl, (c) m-tolyl, (d) p-tolyl, (e) o-Cl-phenyl, (f) m-Cl-phenyl, (g) p-Cl-phenyl.

Scheme-1

Spectral Data

3a: White solid, yield: 60%, mp: 130-132°C, [α]D: -50.01° (c, 0.93, CHCl₃), Rf = 0.42 (Pet. Ether : Acetone, 3:2); IR (KBr, cm⁻¹): ν 3068 (Ar-H), 1729 (C=O), 3465 (N-H), 1653 (C=N), 1269 (C-N), 771 (C-S), 1101 & 1027 (Characteristic of Lactose); ¹H NMR (CDCl₃): δ 8.02-7.11 (42H, m, Ar-H), 5.21-5.15 (2H, s, NH protons), 6.28-3.89 (14H, m, lactosyl and glucosyl protons); Mass (m/z): 1825 (M⁺), 1053, 948, 932, 579, 531, 135. Anal.Calcd.: C, 67.72; H, 4.54; N, 2.30; S, 1.75. Found: C, 67.68; H, 4.52; N, 3.22; S, 1.71%.

3c: Yellow solid, yield: 53%, mp: 108-110°C, [α]D: +82.9° (c, 0.93, CHCl₃), Rf = 0.37 (Pet. Ether : Acetone, 3:2); IR (KBr, cm⁻¹): ν 3066 (Ar-H), 1729 (C=O), 3465 (N-H), 1653 (C=N), 1269 (C-N), 5.21-5.15 (2H, s, NH protons), 6.28-3.89 (14H, m, lactosyl and glucosyl protons); Mass (m/z): 1825 (M⁺), 1053, 948, 932, 579, 531, 135. Anal.Calcd.: C, 67.72; H, 4.54; N, 2.22; S, 1.71%.

3f: Light yellow solid, yield: 55%, mp: 126-127°C, [α]D: +69.28° (c, 0.93, CHCl₃), Rf = 0.48 (Pet. Ether : Acetone, 3:2); IR (KBr, cm⁻¹): ν 3067 (Ar-H), 1729 (C=O), 3448 (N-H), 1601 (C=N), 1270 (C-N), 772 (C-S), 1099 & 1025 (Characteristic of Lactose); ¹H NMR (CDCl₃): δ 8.28-7.19 (44H, m, Ar-H), 5.12-5.10 (2H, s, NH protons), 6.22-3.73 (14H, m, lactosyl and glucosyl protons); Mass (m/z): 1825 (M⁺), 1053, 948, 932, 579, 531, 135. Anal.Calcd.: C, 67.72; H, 4.54; N, 2.22; S, 1.71%.
Antimicrobial Studies

All compounds were screened for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis* in nutrient agar medium and for antifungal activity against *Candida albicans* and *Aspergillus niger* in potato dextrose agar medium. These sterilized agar media were poured into Petri dishes and allowed to solidify on the surface of the media, microbial suspensions were spread with the help of sterilized triangular loop. A stainless steel cylinder of 8 mm diameter (pre-sterilized) was used to bore the cavities. 0.1 ml portions of the test compounds in solvent were added into these wells. The drug solution was allowed to diffuse for about an hour into the medium. The plates were incubated at 37°C for 24 hr and 30°C for 48 hr for antibacterial and antifungal activities respectively. The zone of inhibition observed around the cups after respective incubation was measured. The results are presented in Table-1.

Antibacterial studies of these compounds indicated that compounds 3a and 3d were found to be active against *E. coli* and rest of were found to be moderately active. Compound 3a, 3b and 3g exhibited most significant activity against *S. aureus* and compound 3a, 3b and 3d towards *P. aeruginosa*. All other compounds exhibited low to moderate activity. The results of antifungal activities are also tabulated in Table1. Almost all compounds are most effectively active against *C. albicans* and *A. niger*.

Table-1: Antimicrobial activities of newly synthesized 2-S-tetra-O-benzoyl glucoopyranosyl-1-aryl-5-hepta-O-benzoyl-β-D-lactosyl-2-isothiobiurets (3a-g)

<table>
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<tr>
<th>Compound</th>
<th>E. coli</th>
<th>S. aureus</th>
<th>P. vulgaris</th>
<th>S. typhi</th>
<th>Ps. aeruginosa</th>
<th>K. pneumoniae</th>
<th>B. subtilis</th>
<th>C. albicans</th>
<th>A. niger</th>
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<td>17</td>
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<td>16</td>
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<td>19</td>
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<td>15</td>
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<tr>
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<td>28</td>
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</table>

**zone of inhibition in mm (15 or less) resistance, (16-20 mm) moderate and (more than 20mm) sensitive. Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Proteus vulgaris (P. vulgaris), Salmonella typhi (S. typhi), Klebsiella pneumoniae (K. pneumoniae), Pseudomonas aeruginosa (P. aeruginosa), Bacillus subtilis (B. subtilis), Candida albicans (C. albicans) and Aspergillus niger (A. niger).**

CONCLUSION

The new lactosyl isothiobiurets exhibits promising antibacterial and antifungal activities against the organism tested. The method adopted in this investigation is simple efficient inexpensive and is useful in synthesizing pharmacologically important molecules.
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REFERENCES

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