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SYNTHESIS AND ANTIMICROBIAL STUDIES OF SOME NOVEL BENZOYLATED N- GLUCOSYL THIOBIURETS

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ABSTRACT

Benzoylated sugar derivatives bearing N and S linked functionalities are known for their various biological activities. These compounds are also known for their physiological and pharmaceutical importance and show wide applications in industry and in many other ways. A series of new 1-tetra-O-benzoyl-β-D-glucosyl-5-aryl-4-thiobiurets have been synthesized by the interaction of tetra-O-benzoyl-β-D-glucosyl isocyanate and aryl thiocarbamides. These compounds were screened for their *in vitro* antibacterial and antifungal activity against *E. coli, S. aureus, P.vulgaris, B. cereus, P. aeruginosa, A. niger* and *C. albicans* respectively. The identities of these newly synthesized compounds are established on the basis of usual chemical transformations and IR, ^{1}H NMR, and Mass spectral studies.

Keywords: Glucosyl isocyanate, Aryl thiocarbamides, Glucosyl thiobiurets, Antibacterial, Antifungal activities.

INTRODUCTION

Carbohydrates are most abundant class of organic compounds found in living organisms. Urea, thiourea and their derivatives not only show strong antimicrobial activity but are versatile reagent in organic synthesis. A number of thiourea derivatives have been reported to exhibit marked antibacterial[1], herbicidal and fungicidal[2] activities. Sugar thioureas[3] has synthetic applications in neoglycoconjugate synthetic strategies[4], including neoglycoproteins[5], glycoclusters[6] and pseudooligosaccharides[7].

Thiobiurets are also important derivatives of thiourea which may increase the biological activity of thioureas. Thiobiuret derivative are effective fungicides, bactericides, herbicides and also have demonstrated effective growth regulating activity[8]. Some thiobiuret derivatives also showed analgesic[9], anticonvulsant and hypnotic activity[10]. Glucosyl isocyanate is important synthetic intermediates in carbohydrate chemistry. In the view of the above applications here we synthesis several 1-tetra-O-benzoyl-β-D-glucosyl-5-aryl-4-thiobiurets (III_{a-g}) for the first time by interaction of tetra-O-benzoyl-β-D-glucosyl isocyanate (I) and aryl thiocarbamides. Here we prepared the required glucosyl isocyanate by employing the classical Fischer's method[11] by the reaction of tetra-O-benzoyl-α-D-glucosyl bromide with lead cyanate instead of silver[11] or potassium[12] thiocyanate. 1-aryl thiocarbamides were prepared by interaction of ammonium thiocyanate and amines hydrochlorides[13]. These compounds were screened for their *in vitro* antibacterial and antifungal activity against *E. coli, S. aureus, P.vulgaris, B. cereus, P.aeruginosa, A. niger* and *C. albicans* respectively by cup plate agar diffusion method[14-16].

EXPERIMENTAL

General Methods

Melting points were recorded on electro thermal melting point apparatus and have been left uncorrected. Specific rotations $[\alpha]_D^{32}$ were measured on Equip-Tronics digital polarimeter model no. EQ 800 at 32^0C in $CHCl_3$. IR spectra were recorded on Perkin-Elmer RXI-FTIR Spectrometer. ^{1}H NMR spectra were obtained on a Brucker DRX-300 (300MHz FT NMR) NMR spectrometer in $CDCl_3$ solution with TMS as an internal reference. The mass spectra were recorded on a Jeol SX -102 FAB mass spectrometer. Thin Layer Chromatography [TLC] was performed in E. Merck per coated silica gel plates

and detected by exposure under short UV light. The compounds described in this paper were first time synthesized by the multistep reaction protocol.

Synthesis of Tetra-O-benzoyl-β-D-glucosyl isocyanate (I)

Tetra-O-benzoyl-β-D-glucosyl isocyanate (I) was prepared for first time by the condensation of tetra-O-benzoyl-α-D- glucosyl bromide (0.004M, 2.64g) and lead cyanate (0.004M, 1.16g) in boiling xylene medium (30ml) for 3hr with frequent shaking. After the removal of lead bromide, the xylene filtrate was triturated with petroleum ether (60-80^{0}C) when tetra-O-benzoyl-β-D-glucosyl isocyanate (I) was precipitated out. It was purified by dissolving it in a minimum quantity of chloroform and reprecipitating with petroleum ether (60-80^{0}C) to afford a pale yellow solid. The purity of the product was checked by TLC.

Synthesis of 1-tetra-O-benzoyl–β-D–glucosyl-5-aryl-4-thiobiurets (III_{a-g})

Condensation of tetra-O-benzoyl-β-D-glucosyl isocyanate (I) (0.004*M,* 2.48 g) and 1-aryl thiocarbamides (II_{a-g}) (0.004*M*) in benzene (20 ml) was carried out on boiling water bath for 3hr. The solvent was distilled off and the sticky residue was obtained which was triturated with petroleum ether (60-80^{o}C) to afford the title compounds (III_{a-g}). The products were crystallized from ethanol-water system (3:1). The purity of the products were checked by TLC. The % yield, m.p., optical rotation, elemental analysis and R_f values which are shown in Table-1.

I) Tetra-O-benzoyl-β-D-glucosyl isocyanate

Yield 83.33% m.p. 110-115^{0}C , $[\alpha]_D^{32}$ = +141.36^0 [c,1in $CHCl_3$], IR(KBr): ν 2954 cm^{-1} (Ali C-H), ν 2339 cm^{-1} (N=C=O), ν 1741 cm^{-1} (C=O), ν 1490 cm^{-1} (C=N), ν 1178 cm^{-1} (C-O) and ν 858cm^{-1} (D-glucosyl ring deformation); ^{1}HNMR ($CDCl_3$, ppm): δ 4.2-6.34 (m, 5H, pyranosyl ring); 4.48-4.6 (s, 2H, CH_2-pyranosyl ring); 7.2-7.3 (s, 20H, $4COC_6H_5$); Mass: m/z 621(M^+), 579, 351, 322, 245, 153, 138 , 487, 307, 105. Anal. Calcd for: $C_{35}H_{27}O_{10}N$, Found C, 67.58; H, 4.30; N, 2.20 %; Required C, 67.63; H, 4.34; N, 2.25%;

IIIa) 1-tetra-O-benzoyl–β-D–glucosyl-5-phenyl-4-thiobiuret

IR (KBr): ν 3423 cm^{-1} (N-H), ν 3066 cm^{-1} (Ar-H), ν 1730 cm^{-1} (C=O), ν 1268 cm^{-1} (C-N), ν 1173 cm^{-1} (C-O), ν 1601 cm^{-1} (Ar-C=C), ν 1096 cm^{-1} (C=S) and ν 858cm^{-1} (charecterstic of D-glucosyl ring deformation); ν 708 cm^{-1} (monosubstituted ring); ^{1}HNMR($CDCl_3$, δ ppm) 7.78–7.98 (m,5H,Ar-H), 7.75 (s,1H,Ar-N-H) and 6.3 (s, 2H, N-H); 4.2-6.34 (m, 5H, pyranosyl ring); 4.54 (s, 2H, CH_2-pyranosyl ring); 7.26-8.38 (m, 20H, $4COC_6H_5$); Mass m/z :773, 608, 579, 351, 322, 245, 153, 487, 138,105. Anal.Calcd for $C_{42}H_{35}O_{10}N_3S$: Found: C, 65.18; H, 4.48; N, 5.42; S, 4.10%. Required: C, 65.20; H, 4.52; N, 5.43; S, 4.13%.

IIIb) 1-tetra-O-benzoyl–β-D–glucosyl-5-*o*-tolyl-4-thiobiuret

IR (KBr): ν 3435 cm^{-1} (N-H), ν 3067 cm^{-1} (Ar-H), ν 1729 cm^{-1} (C=O), ν 1267 cm^{-1} (C-N), ν 1172 cm^{-1} (C-O), ν 1605 cm^{-1} (Ar-C=C), ν 1096 cm^{-1} (C=S) and ν 857cm^{-1} (charecterstic of D-glucosyl ring deformation), ν 756 cm-1 (1,2-disubstituted ring); ^{1}HNMR($CDCl_3$, δ ppm): 7.52–7.95 (m,4H,Ar-H), 7.64-7.76(s,1H,Ar-N-H) and 6.21-5.9 (s, 2H, N-H);1.86 (s,3H,CH_3) 4.24-6.35(m, 5H, pyranosyl ring); 4.48-4.6(s, 2H, CH_2-pyranosyl ring); 7.1-8.35 (m, 20H, $4COC_6H_5$); Mass m/z :787, 608, 579, 351, 322, 245, 153, 487, 138,105. Anal.Calcd for $C_{43}H_{37}O_{10}N_3S$: Found: C, 64.02; H, 4.68; N, 5.30; S, 4.05%. Required: C, 64.04; H, 4.70; N, 5.33; S, 4.06%.

IIIc) 1-tetra-O-benzoyl–β-D–glucosyl-5-*m*-tolyl-4-thiobiuret

IR (KBr): ν 3433 cm^{-1} (N-H), ν 3068 cm^{-1} (Ar-H), ν 1728 cm^{-1} (C=O), ν 1267 cm^{-1} (C-N), ν 1172 cm^{-1} (C-O), ν 1606 cm^{-1} (Ar-C=C), ν 1098 cm^{-1} (C=S) and ν 858cm^{-1} (charecterstic of D-glucosyl ring deformation), ν 735 and 770 cm^{-1} (1,3-disubstituted ring); ^{1}HNMR($CDCl_3$, δ ppm): 7.54–7.92 (m,4H,Ar-H), 7.65-7.76 (s,1H,Ar-N-H) and 6.15-5.8 (s, 2H, N-H);1.72 (s,3H,CH_3), 4.24-6.35(m, 5H, pyranosyl ring); 4.48-4.6 (s, 2H, CH_2-pyranosyl ring); 7.1-8.2 (m, 20H, $4COC_6H_5$); Mass m/z :787, 608, 579, 351, 322, 245, 153, 487, 138,105. Anal. Calcd for $C_{43}H_{37}O_{10}N_3S$: Found: C, 64.01; H, 4.67; N, 5.32; S, 4.02%. Required: C; 64.04, H; 4.70, N; 5.33, S; 4.06%.

IIId) 1-tetra-O-benzoyl–β-D–glucosyl-5-*p*-tolyl-4-thiobiuret

IR (KBr): ν 3435 cm^{-1} (N-H), ν 3067 cm^{-1} (Ar-H), ν 1729 cm^{-1} (C=O), ν 1267 cm^{-1} (C-N), ν 1172 cm^{-1} (C-O), ν 1605 cm^{-1} (Ar-C=C), ν 1096 cm^{-1} (C=S) and ν 857cm^{-1} (charecterstic of D-glucosyl ring deformation), ν 803 (1,4-disubstituted benzene); ^{1}HNMR($CDCl_3$,ppm): δ 7.52–7.95 (m,4H,Ar-H), 7.64-7.76 (s,1H,Ar-N-H) and 6.21-5.9 (s, 2H, N-H); 1.76(s,3H,CH_3); 4.24-6.35(m, 5H, pyranosyl ring); 4.48-4.6(s, 2H, CH_2-pyranosyl ring); 7.1-8.35 (m, 20H, $4COC_6H_5$); Mass m/z :787, 608, 579, 351, 322, 245, 153, 487, 138,105. Anal.Calcd for $C_{43}H_{37}O_{10}N_3S$: Found: C; 64.03, H; 4.69, N; 5.32, S; 4.04%. Required: C, 64.04; H, 4.70; N, 5.33; S, 4.06%.

IIIe) 1-tetra-O-benzoyl–β-D–glucosyl-5-*o*-Cl-phenyl-4-thiobiuret

IR (KBr): ν 3415 cm^{-1} (N-H), ν 3068 cm^{-1} (Ar-H), ν 1727 cm^{-1} (C=O), ν 1269 cm^{-1} (C-N), ν 1174 cm^{-1} (C-O), ν 1604 cm^{-1} (Ar-C=C), ν 1095 cm^{-1} (C=S) and ν 857cm^{-1} (charecterstic of D-glucosyl ring deformation); ν 756 cm-1 (1,2-disubstituted ring); ^{1}HNMR($CDCl_3$, δ ppm): 7.78–7.98 (m,4H,Ar-H), 7.69-7.78(s,1H,Ar-N-H) and 6.3-6.01 (s, 2H, N-H); 4.2-6.34(m, 5H, pyranosyl ring); 4.48-4.6(s, 2H, CH_2-pyranosyl ring); 7.26-8.38 (m, 20H, $4COC_6H_5$); Mass m/z :808, 608, 579, 351, 322, 245, 153, 487, 138,105 . Anal.Calcd for $C_{42}H_{35}O_{10}N_3SCl$: Found: C, 62.35; H, 4.30; N, 5.17; S, 3.94%. Required: C, 62.37; H, 4.33; N, 5.19; S, 3.96%.

IIIf) 1-tetra-O-benzoyl–β-D–glucosyl-*m*-Cl-phenyl-4-thiobiuret

IR (KBr): ν 3423 cm^{-1} (N-H), ν 3066 cm^{-1} (Ar-H), ν 1730 cm^{-1} (C=O), ν 1268 cm^{-1} (C-N), ν 1173 cm^{-1} (C-O), ν 1601 cm^{-1} (Ar-C=C), ν 1096 cm^{-1} (C=S), ν 858cm^{-1} (charecterstic of D-glucosyl ring deformation); ν 735 cm^{-1} and ν 770 cm^{-1}(1,3 disubstituted ring); ^{1}HNMR($CDCl_3$, δ ppm): 7.78–7.98 (m,5H,Ar-H), 7.69-7.78(s,1H,Ar-N-H) and 6.3-6.01 (s, 2H, N-H); 4.2-6.34(m, 5H, pyranosyl ring); 4.48-4.6(s, 2H, CH_2-pyranosyl ring); 7.26-8.38 (m, 20H, $4COC_6H_5$); Mass m/z :808, 608, 579, 351, 322, 245, 153, 487, 138,105. Anal.Calcd for $C_{42}H_{35}O_{10}N_3SCl$: Found: C, 62.31; H, 4.31; N, 5.16; S, 3.93%. Required: C, 62.37; H, 4.33; N, 5.19; S, 3.96%.

IIIg) 1-tetra-O-benzoyl–β-D–glucosyl-5-*p*-Cl-phenyl-4-thiobiuret

IR (KBr): ν 3423 cm^{-1} (N-H), ν 3066 cm^{-1} (Ar-H), ν 1730 cm^{-1} (C=O), ν 1268 cm^{-1} (C-N), ν 1173 cm^{-1} (C-O), ν 1601 cm^{-1} (Ar-C=C), ν 1096 cm^{-1} (C=S), ν 858cm^{-1} (charecterstic of D-glucosyl ring deformation) and ν 809 cm^{-1} (1,4-disubstituted benzene); ^{1}HNMR($CDCl_3$,ppm): δ 7.78–7.98 (m,5H,Ar-H), 7.69-7.78 (s,1H,Ar-N-H) and 6.3-6.01 (s, 2H, N-H); 4.2-6.34 (m, 5H, pyranosyl ring); 4.48-4.6 (s, 2H, CH_2-pyranosyl ring); 7.26-8.38 (m, 20H, $4COC_6H_5$); Mass m/z :808, 608, 579, 351, 322, 245, 153, 487, 138,105. Anal.Calcd for $C_{42}H_{35}O_{10}N_3SCl$: Found: C, 62.35; H, 4.32; N, 5.18; S, 3.95%. Required: C, 62.37; H, 4.33; N, 5.19; S, 3.96%.

RESULTS AND DISCUSSION

1-tetra-O-benzoyl-β-D-glucosyl-5-aryl-4-thiobiurets (III_{a-g}) (Scheme-II) were prepared by the reaction of tetra-O-benzoyl-β-D-glucosyl isocyanate (I) and aryl thiocarbamides in a benzene medium for 3hr while monitoring the reaction by TLC. After condensation the solvent was distilled off and sticky residue was triturated with petroleum ether (60-80^0) to afford white solid. It was crystallized by ethanol-water.

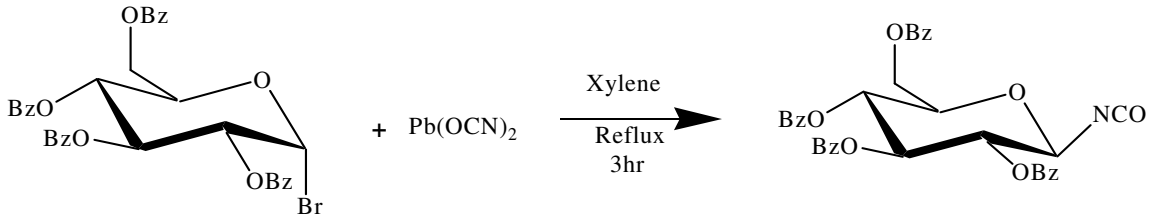
The required tetra-O-benzoyl-β-D-glucosyl isocyanate (I) was prepared by the reaction of tetra-O-benzoyl-α-D-glucosyl bromide with lead cyanate (scheme-I).The characterization of products (III_{a-g}) were established by IR, ^{1}HNMR, and Mass spectral studies.

The IR spectra[17-20, 23] of the compounds showed strong characteristic absorption of β-D-Glucopyranosyl ring deformation in the range of ν 860-848 cm^{-1}. The absorption bands for (R-N=C=O), (N-H), (C=O), (C-N), (C-O), and (C=S) stretch have appeared in the region ν 2273-2000 cm^{-1}, ν 3500-3100 cm^{-1}, ν 1750-1715 cm^{-1} , ν 1350-1210 cm^{-1}, ν 1300-1050 cm^{-1} ν 1200-1050 cm^{-1} respectively.

^{1}HNMR spectrum[17e,19-21] of the products shows signals due to glucosyl protons at δ 6.4-4.3 ppm, resonance signals for aromatic protons at δ 8.42-7.01 ppm and N-H protons δ 8.5-5.01 ppm. Mass spectra[21, 24] exhibited molecular ion peak along with characteristic fragments of tetra-O-benzoyl-β-D-glucosyl at *m/z*, 579, 351, 322, 245, 153, 487, 138, 105.

Table -1: 1-Tetra-O-benzoyl–β-D–glucosyl-5-aryl-4-thiobiurets III_{a-g}
Reactants:(i) Tetra-O-benzoyl-β-D-glucosyl isocyanate (I) (0.004M); (ii) Aryl thiocarbamides (IIa-g) (0.004M)

S. No.	Product (IIIa-g)	Reactants (IIa-g)	Yield (%)	M.P. (^oC)	$[\alpha]_D^{32}$ (c,1,in $CHCl_3$)	Found (Required) S	N	R_f (Hexane:Et OAc) (1:1)
1	IIIa	Phenyl-thiocarbamide	89.3	131	+87	4.10(4.13)	5.42(5.43)	0.77
2	IIIb	*o*-Tolyl thiocarbamide	85.1	137	+105.7	4.05(4.06)	5.30(5.33)	0.74
3	IIIc	*m*-Tolyl thiocarbamide	69.3	138	+98.85	4.02(4.06)	5.32(5.33)	0.66
4	IIId	*p*- Tolyl thiocarbamide	86.8	134	+133.2	4.04(4.06)	5.32(5.33)	0.86
5	IIIe	*o*-Cl Phenyl thiocarbamide	73.0	129	+187.6	3.94(3.96)	5.17(5.19)	0.83
6	IIIf	*m*-Cl Phenyl thiocarbamide	66.6	139	+194.1	3.93(3.96)	5.16(5.19)	0.69
7	IIIg	*p*-Cl Phenyl thiocarbamide	87.7	148	+160.3	3.95(3.96)	5.18(5.19)	0.80



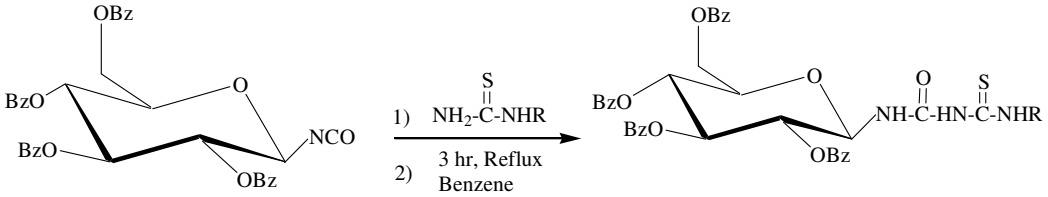
Tetra-O-benzoyl-α-D-glucosyl bromide

Lead cyanate

Tetra-O-benzoyl-β-D-glucosyl isocyanate

I

Scheme-1



I

(III_{a-g})

Scheme-2

Where, Bz- COC_6H_5
R = (a) phenyl, (b) *o*-tolyl, (c) *m*-tolyl,(d) *p*-tolyl,(e) *o*-Cl-phenyl, (f) *m*-Cl-phenyl, (g) *p*-Cl-phenyl.

Antimicrobial activity

1. Antibacterial activity

All the compounds were screened for their *in vitro* antibacterial activities against various pathogenic bacteria such as *E. coli, S. aureus, P.vulgaris, B. cereus, P. aeruginosa* by cup-plate method at concentration 100 µg/ml in DMSO by using standard Gentamycine (25 µg/ml) for bacteria. Amongst the compounds tested for antibacterial activity, compounds III_a, III_d, III_g, were highly active and compounds III_b, & III_e were moderately active while compound III_c, III_f is less active (Table-2)

2. Antifungal activity

Inhibition zone diameter in mm[a]

Compounds	Bacteria					Fungi	
	E. coli	*P. vulgaris*	*S. aureus*	*P. aeruginosa*	*B. cereus*	*A. niger*	*C. albicans*
IIIa	19	18	19	18	18	15	16
IIIb	17	14	17	12	16	14	15
IIIc	--	13	10	--	12	18	19
IIId	19	18	19	17	19	14	14
IIIe	16	14	16	14	16	15	15
IIIf	--	--	11	--	13	16	17
IIIg	19	17	19	16	19	20	20
Gentamycine	19	19	19	19	19	-	-
Nystatin	-	-	-	-	-	20	20

All the compounds were screened for their *in vitro* antifungal activities against *A. niger* and *C. albicans* by cup-plate method at a concentration 100 µg/ml in DMSO by using standard Nystatin as standard drug. Compounds III_c, III_f, III_g exhibited against A. niger and other were moderately active against fungi (Table-2).

Table-2: Antibacterial and Antifungal Activities of compounds IIIa-g

*Note: No activity was observed, a, Values are the average of three readings , Bore size 6mm

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