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Synthesis and biological studies of some novel benzoylated *N*-glucosyl isothiobiurets

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ABSTRACT

Sugar isocyanates are important reagents in medicinal/pharmaceutical chemistry. Some sugar isothiobiurets and isodithiobiurets show potential antimicrobial activities. Looking at the importance of these compounds we plan the synthesis of 1-aryl-5-tetra-O-benzoyl-β-D-glucosyl-2-S-benzyl-2-isothiobiurets. Several 1-aryl-5-tetra-O-benzoyl-β-D-glucosyl-2-S-benzyl-2-isothiobiurets have been synthesized by the interaction of tetra-O-benzoyl-β-D-glucosyl isocyanate with 1-aryl-S-benzyl isothiocarbamides. 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, S-benzyl isothiocarbamides, Glucosyl isothiobiurets, Antibacterial, Antifungal activities.

INTRODUCTION

The past years have witnessed an increasing interest in sugar derivatives because of their biological properties such as antiproliferative[**1**], antitubercular[**2**], cytotoxic[3], antimetastasis[4], chemotherapeutic agents[5], antimicrobial[6].

In the recent reports from our laboratory, we described the preparation of N and S-linked sugar derivatives with their biological activity[7-9]. As we know the glucosyl isothiocyanate and isocyanate[10-12] are most often used starting material for synthesis of variety of N and S-linked sugar derivatives. Non glucosidic isodithiobiurets and isothiobiurets are known to show anticonvulsant and hypnotic activities[13]. Some sugar isothiobiurets and isodithiobiurets show potential antimicrobial activities[14].

Such noteworthy and diversified pharmaceuticals values of glucosides have forced our interest on studies towards the glucose moiety. Our present invention relates to synthesis of several N-glucosides derivatives via the most powerful intermediates glucosyl isocyanate. In the view of the above applications here we synthesis several 1-aryl-5-tetra-O-benzoyl-β-D-glucosyl-2-S-benzyl-2-isothiobiurets (III_{a-g}) for the first time by interaction of tetra-O-benzoyl-β-D-glucosyl isocyanate (I) and 1-aryl-2-s-benzyl isothiocarbamides. Here we prepared the required glucosyl isocyanate by employing the classical Fischer's method by the reaction of tetra-O-benzoyl-α-D-glucosyl bromide with lead cyanate instead of silver or potassium thiocyanate. 1-aryl thiocarbamides were prepared by interaction of ammonium thiocyanate and amines hydrochlorides[15-17]. 1-aryl-2-S-benzyl-isothiocarbamides were prepared by methods described earlier[18].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[19-21].

MATERIALS AND METHODS

General Methods

Melting points were taken in open capillary tube on Mac digital melting point apparatus and are uncorrected. Specific rotations $[\alpha]_D^{32}$ was measured on Equip-Tronics Digital Polarimeter model no. EQ 800 at 32^0C in $CHCl_3$. IR spectrum was recorded on Perkin-Elmer Spectrum RXI-FTIR Spectrometer in solid phase KBr. ^{1}H NMR spectrum 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[16-17] (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 (60-80^0C) 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 (60-80^0C) to afford a pale yellow solid. The purity of the product was checked by TLC.

Synthesis of 1-aryl- 5-tetra-O-benzoyl–β-D–glucosyl-2-S-benzyl-2-isothiobiurets (III_{a-g})

Condensation of tetra-O-benzoyl-β-D-glucosyl isocyanate (I) (0.004*M*, 2.48 g) and 1-aryl-2-S-benzyl isothiocarbamides (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^oC) 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.

Table -1: Synthesis of 1-aryl- 5-tetra-O-benzoyl–β-D–glucosyl-2-S-benzyl-2-isothiobiurets (III_{a-g})

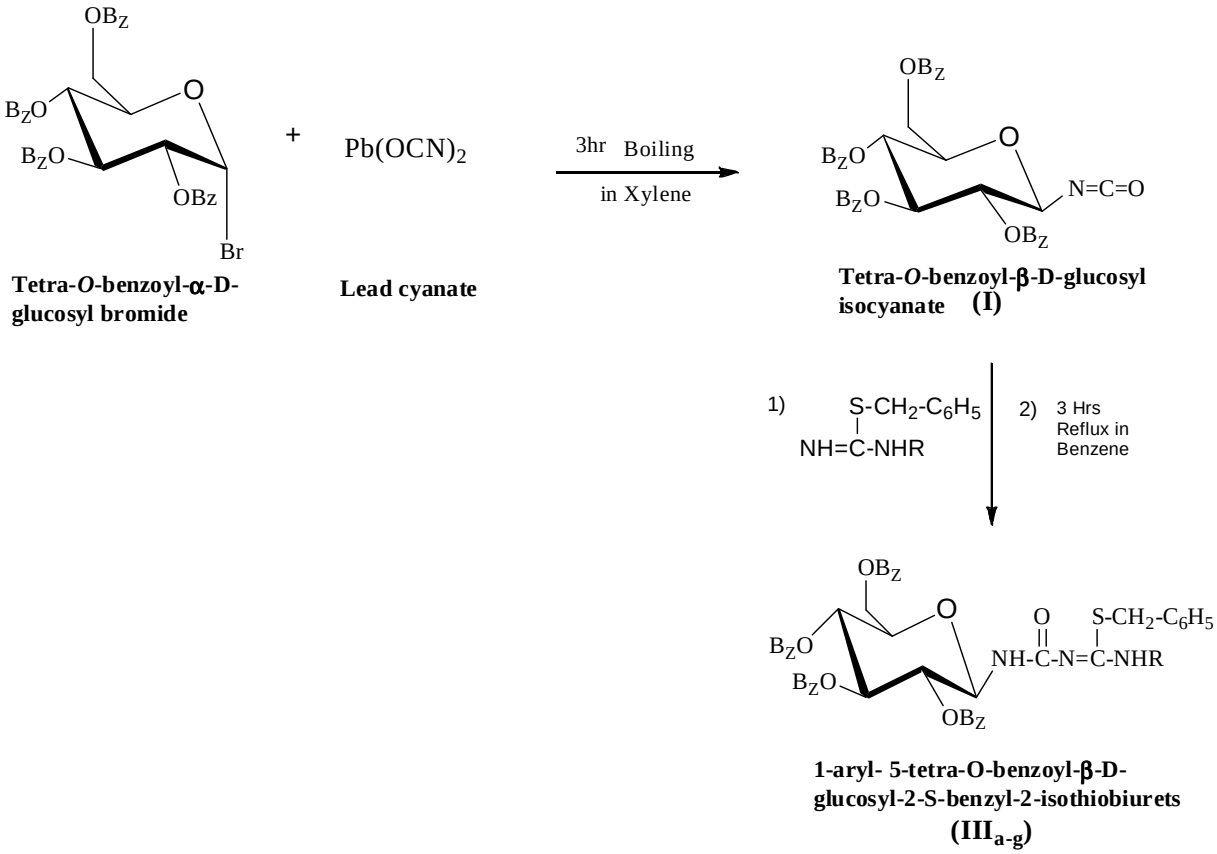
Reactants:*-i) Tetra-O-benzoyl-β-D-glucosyl isocyanate (I) (0.004M,)*
ii) 1-Aryl-2-S-benzyl isothiocarbamides (IIa-g) (0.004M)

Sr No	Product (IIIa-g)	Reactants (IIa-g)	Yield (%)	M.P. (^{o}C)	$[\alpha]_D^{32}$ (c,in$CHCl_3$)	Found (Required)		R_f (Hexane:EtOAc) (1:1)
						S	N	
1	IIIa	1-Phenyl….	88.10	117-120	+68.87 (0.784)	3.68(3.70)	4.85(4.86)	0.78
2	IIIb	1-*o*-Tolyl…	84.23	120	+151.51 (0.72)	3.61(3.64)	4.73(4.78)	0.74
3	IIIc	1-*m*-Tolyl…..	68.70	123	+160.85 (0.74)	3.61(3.64)	4.69(4.78)	0.67
4	IIId	1-*p*- Tolyl ….	87.63	126	+120.64 (0.74)	3.63(3.64)	4.76(4.78)	0.88
5	IIIe	1-*o*-Cl Phenyl…….	77.25	127-132	+93.83 (0.74)	3.54(3.56)	4.65(4.68)	0.83
6	IIIf	1-*m*-Cl Phenyl…….	71.20	121-126	-98.05 (0.76)	3.53(3.56)	4.62(4.68)	0.68
7	IIIg	1-*p*-Cl Phenyl…….	86.29	116-120	+101.78 (0.78)	3.55(3.56)	4.66(4.68)	0.84

RESULTS AND DISCUSSION

Synthesis of 1-aryl- 5-tetra-O-benzoyl–β-D–glucosyl-2-S-benzyl-2-isothiobiurets (III_{a-g}) (Scheme-I) were prepared by the reaction of tetra-O-benzoyl-β-D-glucosyl isocyanate (I) and 1-aryl-2-S-benzyl isothiocarbamides 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).



Tetra-*O*-benzoyl-α-D-glucosyl bromide

Lead cyanate

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

1-aryl- 5-tetra-O-benzoyl-β-D-glucosyl-2-S-benzyl-2-isothiobiurets (III_{a-g})

Scheme I

*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.*

The characterization of products (III_{a-g}) were established by IR, ^{1}HNMR, and Mass spectral studies. The IR spectra [22-25], [28], [29b], [30] 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), (S-CH_2 wagging) and (C-O) stretch have appeared in the region ν 2273-2000 cm^{-1}, ν 3500-3100 cm^{-1}, ν 1750-1715 cm^{-1}, ν 1450–1690 cm^{-1}, ν 1220-1270 cm^{-1}, ν 1200-1050 cm^{-1} respectively. ^{1}HNMR spectrum[22e],[24-25] 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[23], [26] 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.

Antimicrobial activity

Most of the synthesized compounds exhibited mild to moderate anti-microbial activity against the tested microorganisms. Compounds **IIIb, IIId, IIIe** and **IIIg** were found to possess significant antibacterial and antifungal activity when compared to standard drug (Gentamycine and Nystatin for antibacterial and antifungal respectively). The entire synthesized compounds exhibited mild to moderate activity are shown in Table -2.

IIIa) 1-phenyl-5-tetra-O-benzoyl–β-D–glucosyl-2-S-benzyl-2-isothiobiuret

IR (KBr, cm^{-1} ν) : 3406 (N-H), 3066 (Ar-H), 2959 (Ali-H), 1723 (C=O), 1626 (Ar-C=C), 1491 (C=N), 1270 (S-CH_2 wagging), 1094 (C-O), 853 (characteristic of D-glucosyl ring deformation); 760 (C-S), and 709 (monosubstituted ring). ^{1}HNMR (300MHz, $CDCl_3$, δ ppm): 7.67-8.18 (m, 20H, $4COC_6H_5$); 7.78–7.98 (m, 5H, N–Ar-H); 7.69-7.78 (m, 5H, S-Ar-H); 6.97 (s, 1H, N-H); 6.3 (s, 1H, N-H pyranosyl ring); 4.2-6.34 (m, 5H, pyranosyl ring); 4.48-4.6 (s, 2H, CH_2-pyranosyl ring);3.01 (s, 2H, S-CH_2); Mass m/z :863, 579, 351, 322, 245, 153,

487, 138,105. Anal.Calcd for $C_{49}H_{41}O_{10}N_3S$: Found: C, 68.10; H, 4.73; N, 4.85; S, 3.68%. Required: C, 68.13; H, 4.75; N, 4.86; S, 3.70%.

IIId) 1-*p*-tolyl-5-tetra-O-benzoyl–β-D–glucosyl-2-S-benzyl-2-isothiobiuret

IR (KBr, cm^{-1} ν) : 3331 (N-H), 3065 (Ar-H), 2960 (Ali-H), 1728 (C=O), 1600 (Ar-C=C), 1508 (C=N), 1270 (S-CH_2 wagging), 1092 (C-O) and 856 (characteristic of D-glucosyl ring deformation), 814 (1,4-disubstituted benzene), 770 (C-S), and 708 (monosubstituted ring); ^{1}HNMR (300MHz, $CDCl_3$, δ ppm): 7.36-8.17 (s, 20H, $4COC_6H_5$), 7.0–7.23 (m,5H, Ar-H), 7.28-7.34 (m,4H, N-Ar-H), 7.00 (s, 1H, N-H); 6.3 (s, 1H, N-H pyranosyl ring), 4.24-5.89 (m, 5H, pyranosyl ring); 4.38-4.56 (s, 2H, CH_2-pyranosyl ring), 2.35(s, 3H, Ar-CH_3); Mass m/z :877, 608, 579, 351, 322, 245, 153, 487, 138,105. Anal.Calcd for $C_{50}H_{43}O_{10}N_3S$: Found: C; 68.39, H; 4.89, N; 4.76, S; 4.63%. Required: C, 68.41; H, 4.90; N, 4.78; S, 3.64%.

IIIg) 1-*p*-Cl-phenyl-5-tetra-O-benzoyl–β-D–glucosyl-2-S-benzyl-2-isothiobiuret

IR (KBr, ν cm^{-1}) : 3446 (N-H), 3066 (Ar-H), 2959 (Ali-H), 1724 (C=O), 1626 (Ar-C=C), 1269 (S-CH_2 wagging), 1094 (C-O), 853 (characteristic of D-glucosyl ring deformation), 806 (1,4-disubstituted benzene), 770 (C-S), and 707 (monosubstituted ring); ^{1}HNMR (300MHz, $CDCl_3$, δ ppm): 7.46-8.15 (s, 20H, $4COC_6H_5$), 7.45–7.25 (m, 4H, Ar-H), 7.24–6.89 (m, 5H, Ar-H), 6.89 (s,1H,Ar-N-H), 6.32 (s, 1H, N-H pyranosyl ring); 4.2-5.84 (m, 5H, pyranosyl ring); 4.26 (s, 2H, CH_2-pyranosyl ring), 3.32(s, 2H, S-CH_2) ; Mass m/z :897, 608, 579, 351, 322, 245, 153, 487, 138,105. Anal.Calcd for $C_{49}H_{40}O_{10}N_3SCl$: Found: C, 65.54; H, 4.44; N, 4.66; S, 3.55%. Required: C, 65.55; H, 4.45; N, 4.68; S, 3.56%.

Antimicrobial activity

All the compounds have been screened for both; antibacterial and antifungal activity using cup plate agar diffusion method by measuring the inhibition zone in mm. The compounds were taken at a concentration of 1mg/ml using dimethyl sulphoxide as solvent. Gentamycine (100µg/ml) was used as a standard for antibacterial and Nystatin (100µg/ml) as a standard for antifungal activity. The compounds were screened for antibacterial activity against *Escherichia Coli*, *Staphalococcus aureus*, *Proteus vulgaris*, *Psudomonas aeruginosa*, *Bacillus cereus* in nutrient agar medium and for antifungal activity against *Candida albicancs* 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 8mm diameter (pre-sterilized) was used to bore the cavities 0.1mL 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^{o}c for 24h and 30^{o}c for 48h for antibacterial and antifungal activities respectively. The zone of inhibition observed around the cups after respective incubation was measured in mm and is average of three readings. The results are presented in (Table 2).

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

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

--- No activity was observed.

a, Values are the average of three readings

Thus novel benzolyted glucosyl S-benzyl-2-isothiobiurets exhibits comparable antibacterial and antifungal activities against the organism tested. The method adopted in this investigation is simple, efficient and inexpensive is useful in synthesizing pharmacologically important molecules.

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