2-*S*-hepta-*O*-benzoyl lactosyl-1-aryl-5-hepta-*O*-benzoyl-β-lactosyl-2-isothiobiurets: Synthesis and Antimicrobial Activities

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Abstract
A series of novel 2-S-hepta-O-benzoyl lactosyl-1-aryl-5-hepta-O-benzoyl-β-lactosyl-2-isothiobiurets have been synthesized by the interaction of S-hepta-O-benzoyl lactosyl-1-arylisothiocarbamides and hepta-O-benzoyl-β-D-lactosyl isocyanate. These compounds were screened for their antibacterial and antifungal activities against Escherichia coli, Proteus vulgaris, Salmonella typhimurium, Staphylococcus aureus, Pseudomonas aeruginosa and Aspergillus niger. The newly synthesized compounds have been characterized by analytical and IR, ^{1}H NMR and Mass spectral studies.

Keywords : *Arylisothiocarbamides, Lactosyl thiocyanate, Isothiobiurets.*

Introduction

A number of thiourea derivatives have been reported to exhibit antibacterial[1], herbicidal and fungicidal[2] activities. Sugar thioureas[3] has synthetic applications in neoglycoconjugate synthetic strategies[4], including neoglycoproteins[5], glycodendrimers[6], glycoclusters[7] and pseudooligosaccharides[8].

Thiobiurets (mono and di) are also important derivatives of (thio) urea which may increase the biological activity of (thio)ureas. 1-Allyl-2-thiobiuret was shown to regulate the growth of germinating wheat and cucumber seeds[9]. Oliver and co-workers[10] reported chemosterlising action of some dithiobiuret derivatives in male house flies. The mono and dithiobiuret derivative are effective fungicides, bactericides, herbicides and also have demonstrated effective growth regulating activity[11]. Some thiobiuret derivatives also showed analgesic[12], anticonvulsant and hypnotic activity[13]. Glycosyl urea and their biuret derivatives are reported as potential glycoenzyme inhibitors[14]. D. V. Mangte and S.P. Deshmukh[15] reported antibacterial and antifungal activities of per−*O*−acetylated lactosyl iso(di) thiobiurets.

In view of the advantage conferred by glycosyl thiourea synthesis that allows rapid, convenient access to a wide array of thioureido carbohydrates[16], together with the notable biological activities of nucleoside analogues, a great deal of work has focused on the development of novel glycosyl thiourea derivatives. However, there are little reports on the synthesis and bioactivity of lactosyl thiourea derivatives. In this paper, a very efficient synthetic route to novel lactosyl isothiourea derivatives and their antimicrobial activities are reported.

Experimental

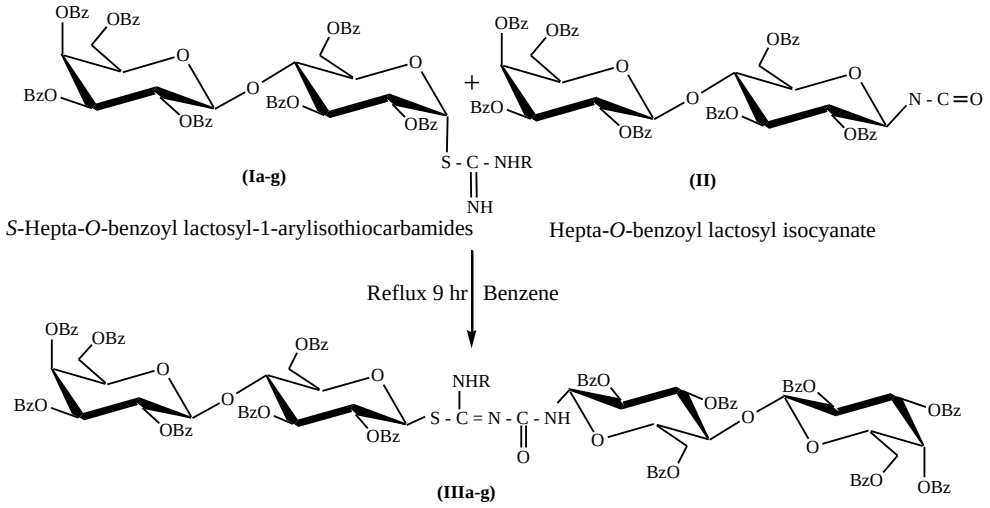
Melting points determined are uncorrected. IR Spectra were recorded on Perkin-Elmer spectrum RXI FTIR Spectrometer. ^{1}H NMR was obtained on Bruker DRX-300 MHz NMR Spectrometer. Samples were prepared in $CDCl_3$ with TMS as an internal reference. The mass spectra were obtained on Thermo Fennigan LCQ Advantage max ion trap mass spectrometer. Optical rotations $[\alpha]_D^{31}$ were measured on the Equip-Tronics EQ-800 Digital Polarimeter at 31^0C in $CHCl_3$. Thin layer chromatography was performed on silica jel G and spots were visualized by iodine vapour.

Synthesis of 2-*S*-hepta-*O*-benzoyl lactosyl-1-phenyl-5-hepta-*O*-benzoyl-β-D-lactosyl-2-isothiobiuret (**IIIa**). A mixture of *S*-hepta-*O*-benzoyl lactosyl-1-phenylisothiocarbamide (**Ia)** (1.2 g 0.001 M, in 10 mL benzene) with hepta-*O*-benzoyl-β-D-lactosyl isocyanate (**II**) (1.1 g 0.001M, in 10 mL benzene) was gently refluxed for 9 h. The progress of reaction was monitored by TLC. After completion of the reaction, benzene was distilled off. The sticky mass thus obtained was triturated with petroleum ether (60-80°C) to afford a crystalline solid (**IIIa**). It was purified by ethanol-water (1.8 g, 78.26%), m.p. 130-132°C. The physical characterization results are summarized in Table-1 (**IIIa-f**). The compounds **IIIb-f** were also prepared by similar methods (**Scheme-I)**.

Table 1. Characterization data of 2-*S*-hepta-*O*-benzoyl lactosyl-1-aryl-5-hepta-*O*-benzoyl-β-lactosyl-2-isothiobiurets (IIIa-g)

Compounds	% yield	M.P. (°C)	$[\alpha]_D^{31}$ c,1.0 in $CHCl_3$	R_f value (petroleum ether : EtOAc 6:4)	Analysis (%) : found (requires)	
					N	S
3a	78.26	130-132	+ 50.01	0.71	0.9(1.1)	5.14 (5.24)
3b	82.60	118	+ 66.4	0.97	1.08 (1.11)	4.87 (5.09)
3c	56.52	110	+ 82.9	0.84	0.98 (1.11)	4.72 (5.09)
3d	52.17	122	+ 42.32	0.67	0.88 (1.11)	4.32 (5.09)
3e	60.86	112	+ 52.14	0.79	1.07 (1.13)	5.12 (5.18)
3f	65.21	127	+ 69.28	0.81	0.98 (1.13)	4.98 (5.18)
3g	73.91	108	+ 36.59	0.91	1.08 (1.13)	4.68 (5.18)

C and H analysis was found satisfactory in all cases.



S-Hepta-*O*-benzoyl lactosyl-1-arylisothiocarbamides + Hepta-*O*-benzoyl lactosyl isocyanate

2-S-hepta-*O*-benzoyl lactosyl-1-aryl-5-hepta-*O*-benzoyl lactosyl-2-isothiobiurets

Scheme I

Where,

OBz = - COC_6H_5

R = a) phenyl b) *o*-Cl-phenyl c) *m*-Cl-phenyl d) *p*-Cl-phenyl e) *o*-tolyl f) *m*-tolyl g) *p*-tolyl

IIIa) IR (KBr, $\nu_{max,}$ cm^{-1}) : 3480 (N-H), 1729 (C=O), 1601 (C=N), 1271 (C-N), 1026 (lactosyl ring deformation), 709 (C-S) cm-1; ^{1}HNMR (δ in ppm, $CDCl_3$): δ 8.25-7.13 (97H, m ,Ar-H), 6.17-3.77 (14H, m, lactose ring protons), 7.09, (1H, s, Ar-NH), 3.2, (1H, s,Ali-NH) Mass(*m/z*): 2299 (M^+), 1204 (M^+-$C_{62}H_{49}O_{18}N$), 1095 (M^+-$C_{68}H_{56}O_{17}N_2S$), 1053(HBL^+),932 (HBL^+-$C_7H_5O_2$), 579 (TBG^+), (found: C, 64.32; H, 4.51; N, 1.78; S, 1.28, calculated for $C_{124}H_{113}O_{39}N_3S$ required C, 64.72; H, 4.91; N, 1.82; S, 1.39; %).

IIId) IR (KBr, $\nu_{max,}$ cm^{-1}) : 3449 (N-H), 1728 (C=O), 1602 (C=N), 1271 (C-N), 1069 (lactosyl ring deformation), 709 (C-S) cm-1; ^{1}HNMR (δ in ppm, $CDCl_3$): δ 8025-7.13 (96H, m ,Ar-H), 6.17-3.77 (14H, m, lactose ring protons7.11, (1H, s, Ar-NH), 3.0, (1H, s , Ali-NH); Mass(*m/z*): 2333 (M^+), 1206 (M^+-C_6H_5NHCl), 1095 (M^+-$C_{68}H_{57}O_{18}N_3Cl$), 1053 (HBL^+), 932 (HBL^+-$C_7H_5O_2$), 579 (TBG^+), (found: C, 63.38; H, 4.21; N, 1.48; S, 1.24, calculated for $C_{124}H_{112}O_{39}N_3SCl$ required C, 63.78; H, 4.80; N, 1.80; S, 1.37; %).

IIIg) IR (KBr, $\nu_{max,}$ cm^{-1}) : 3465 (N-H), 1728 (C=O), 1602 (C=N), 1271 (C-N), 1026 (lactosyl ring deformation), 709 (C-S) cm-1; ^{1}HNMR (δ in ppm, $CDCl_3$): δ 8025-7.13 (96H, m ,Ar-H), 6.17-3.77 (14H, m, lactose ring protons) 6.73, (1H, s, Ar-NH), 2.8, (1H, s,Ali-NH), 2.36 (3H s, Ar-CH_3); Mass(*m/z*): 2313 (M^+), 1928 (M^+-$5C_6H_5$), 1095 (M^+-$C_{69}H_{58}O_{17}N_2S$), 1053(HBL^+), 932

(HBL^+- $C_7H_5O_2$), 579 (TBG^+), (found: C, 64.63; H, 4.62; N, 1.62; S, 1.29, calculated for $C_{125}H_{115}O_{39}N_3S$ required C, 64.85; H, 4.97; N, 1.81; S, 1.81; %).

Results And Discussion

Several 2-*S*-hepta-*O*-benzoyl lactosyl-1-aryl-5-hepta-*O*-benzoyl-β-lactosyl-2-isothiobiurets (**IIIa-f**) have been prepared by the interaction of *S*-hepta-*O*-benzoyl lactosyl-1-arylisothiocarbamides (**Ia-f**) and hepta-*O*-benzoyl-β-D-lactosyl isocyanate (**II**). After completion of the reaction, benzene was distilled off. The sticky mass thus obtained was triturated with petroleum ether (60-80°C) to afford a crystalline solid (**IIIa-f**). The product was found to be non-desulphurisable when boiled with alkaline lead acetate solution. The specific rotations were measured in chloroform. The result is summarized in Table-1. In spectral analysis of products shows bands due to Ar-H, N-H, C=O, C=N, C-N, C-S stretching and ^{1}HNMR spectra of products distinctly displayed signals due to aromatic protons and lactose ring protons. The Mass spectrum of products was also observed. The identities of these new *S*-lactosides have been established on the basis of usual chemical transformations and also IR, ^{1}H NMR and mass spectral studies[17-19].

Antimicrobial Activities

All the compounds have been screened for both antibacterial and antifungal activities 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. Amikacin (100µg/ml) was used as a standard for antibacterial and antifungal activity and fluconazole (100µg/ml) as a standard for antifungal activity. The 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 *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°c for 24h and 30°c for 48h for antibacterial and antifungal activities respectively. The zone of inhibition observed around the cups after respective incubation was measured. It has been observed that the compounds 3a-g exhibit strong inhibition against *S. typhi* and *A. niger*, weak to no activity against *P. vulgaris* and *Ps. aeruginosa*, weak activity against *E. coli* whereas, no activity against *S. aureus* (Table 2).

Table 2. Antimicrobial activity of 2-*S*-hepta-*O*-benzoyl lactosyl-1-aryl-5-hepta-*O*-benzoyl-β-lactosyl-2-isothiobiurets

Compounds	*E. coli*	*S. aureus*	*P. vulgaris*	*S. typhi*	*Ps. Aeruginosa*	*A. niger*
IIIa	10	-	9	16	10	19
IIIb	13	-	11	18	12	17
IIIc	8	-	12	22	11	20
IIId	7	-	14	17	10	21
IIIe	11	-	10	19	13	17
IIIf	14	-	9	21	8	16
Amikacin	15	-	13	21	14	-
fluconazole	-	-	-	-	-	24

Zone of inhibition in mm (15 or less), (16-20 mm) moderate and more than (20 mm) sensitive

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