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Synthesis and Antimicrobial Activity of 1-Aryl-5-Hepta-*O*-Benzoyl-B-D-Lactosyl-2-*S*-Benzyl-2, 4-Isodithiobiurets

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ABSTRACT

Several 1-aryl-5-hepta-*O*-benzoyl-β-D-lactosyl-2-*S*-benzoyl-2, 4-isodithiobiurets have been synthesized by the interaction of hepta-*O*-benzoyl-β-D-lactosyl isothiocyanate with several 1-aryl-*S*-benzyl isothiocarbamides. These compounds were screened for their antibacterial and antifungal activities against–*Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Klebsiella pneumonie*, *Psudomonasaeruginosa*, *Aspergillus niger* and *Candida albicance*. The newly synthesized compounds have been characterized by analytical and IR, ^{1}H NMR and Mass spectral studies. These compounds show appreciable activity towards these microorganisms.

Keywords: Lactosyl isothiocyanate, 1-aryl-S-benzyl isothiocarbamides, isodithiobiurets and antimicrobial activity.

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INTRODUCTION

Aryl/alkyl isothiocarbamides, due to their basic nature are found to interact with isothiocyante to form corresponding isodithiobiurests. Several non-glycosidic isodithiobiurests are known for their anticonvulsant and hypnotic activities, Glycobiology[1] has gained much attention because the oligosaccharide part and other glycoconjugates are responsible for their function in various biological processes viz. cell growth. Regulation, immunological responses, inflammation and bacterial and viral infections[2-4]. Literature survey reveals that synthesis of amino, diamino derivatives which exhibit biological and pharmaceutical activities such antimalarial effect[5, 6]. Glycosyl thiourea has been widely used as important intermediate in the synthesis of nucleoside anologs[7-9].Thiobiurets,imidazoles and thiazolines also shows anti inflammatory, antitumor, hypnotic activities[10,11]. In recent years, steadily increasing research effort has centered on the production of glycosylbiurets because these compounds have been shown to possess many different biological activities. Some carbohydrate base urea exhibit relevant biological properties such as the antibiotic SF-1993, CV-1. Nitroso urea have shown to be alpha-glycosidase inhibitors, possesses antitumor activity. In the last years the intensive use of antibiotic has lead to an increase of the emergence of resistant bacteria.[12]. There is a growing need for new class of antibacterial compounds having different mechanism of action compared to existing drugs.

MATERIALS AND METHOD

Experimental

Melting points of all synthesized compounds were determined using open capillary tube on Mac digital melting point apparatus and were uncorrected. The reagent grade chemicals were obtained from commercial sources and purified by either distillation or recrystallization before use. IR spectra were recorded in solid phase KBr disks on SHIMADZU IR affinity-1 FTIR spectrometer and $_1$H NMR spectra in $CDCl_3$ on Bruker DRX-300 of NMR spectrometer 300 MHz. The Mass spectra were recorded on Waters UPLC-TQD Mass Spectrometer. Optical rotations were measured on Equip-Tronics EQ 800 Digital Polarimeter in $CHCl_3$. Purity of synthesized compounds has been checked by thin layer chromatography. It was performed on E. Merck pre-coated silica gel plates.

General Procedure

Preparation of hepta-*O*-benzoyl-β-D-lactosylisothiocyanate

To a suspension of hepta-*O*-benzoyl-α-D-lactosyl bromide (0.01 M, 15 g) in sodium dried xylene (80 ml) was added lead thiocynate (0.01 M, 4.2 g). The reaction mixture was refluxed gently for 3 hr. With frequent shaking. This solution was then cooled and librated lead bromide was removed

by filtration. The xylene filtrate was then treated with petroleum ether (60 – 80°C) with steering a white solid mass obtained (13 g). This solid was expected hepta-*O*-benzoyl-β-D-lactosylisothiocyanate(I). It was purified by dissolving it in minimum quantity of chloroform and reprecipitating with petroleum ether. m.p.-118-120°C. (I) (Scheme-I).

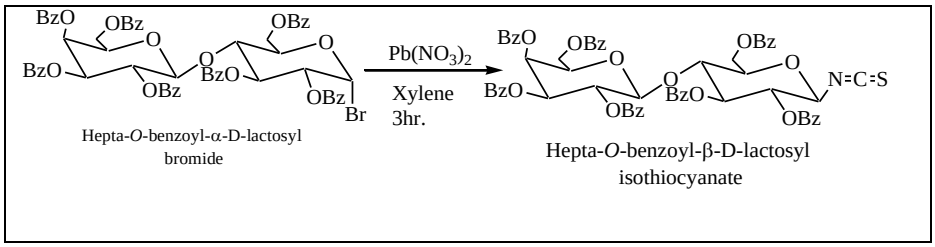

Figure 1: Synthesis of Hepta-*O*-benzoyl-β-D-lactosylisothiocyanate

Preparation of 1-aryl-*S*-benzyl isothiocarbamides

The required 1-aryl-*S*-benzyl isothiocarbamides were prepared by already well known procedure. A solution of hydroxy phenyl thiocarbamide (0.02 M, 3.36) in ethanol (25 ml) was mixed with benzyl chloride (0.02 m, 2.35 g) in ethanol (25 ml) and the reaction mixture was refluxed for 90 min. afterwards, it was cooled and rendered basic with dilute ice cold ammonia solution as a sticky residue was obtained which on standing for 2 hr., solidified then it was filtered and washed with petroleum ether. This solid was expected to be 1-aryl-*S*-benzyl isothiocarbamides II (a-f) (Scheme-II).

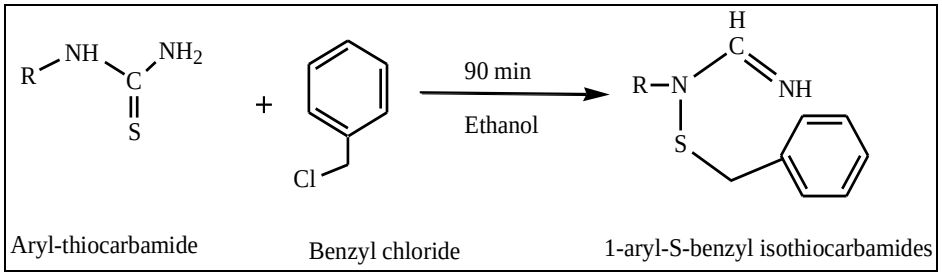

Figure 2: Synthesis of 1-aryl-*S*-benzyl-isothiocarbamide

Preparation of 1-*o*-hydroxy-phenyl-5-hepta-*O*-benzoyl-β-D-lactosyl-2-*S*-benzyl-2, 4-isodithiobiurete

A benzene solution of 1-*o*-hydroxy phenyl-*S*-benzyl isothiocarbamide (0.002 M, 0.518g in 20 mL) was added to a benzene solution of hepta-*O*-benzoyl-1-β-D-lactosyl isothiocyanate (0.002 M, 2.22 g in 20 mL). The reaction mixture was refluxed for 6 hr and monitored by TLC afterwards, solvent was removed under reduced pressure to obtain sticky residue. This was triturated with petroleum ether (60 – 80°C) to afforded a pale yellow solid (88.99%). The crude product was 1.21 g crystallized by ethanol water m.p. 77°C, Anal. Calcd. For $C_{76}H_{64}O_{18}N_3S_2$, Required: C, 66.61; H, 4.67; N, 3.06; S, 4.67: III (a-f) (Scheme-III).

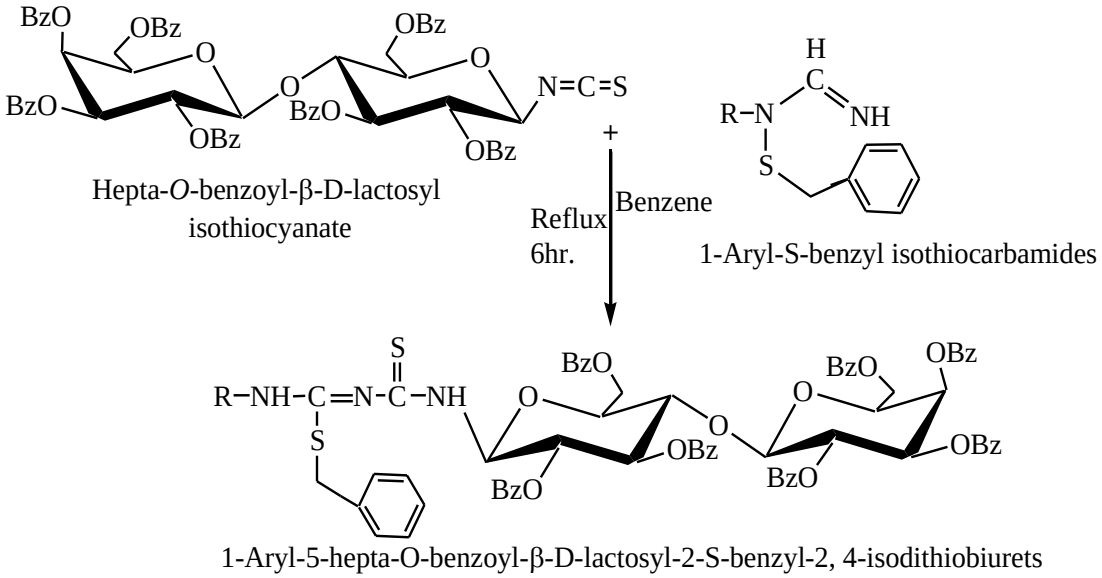

Figure 3: 1-aryl-5-hepta-*O*-benzoyl-β-D-lactosyl-2-*S*-benzyl-2, 4-isodithiobiurets

Where, OBz = Benzoyl

R = a) *o*-hydroxyphenyl b) *m*-hydroxyphenyl c) *p*-hydroxyphenyl

d) *o*-methoxy phenyl e)*m*-methoxy phenyl f) *p*-methoxy phenyl

RESULTS AND DISCUSSION

Herein, we report the synthesis of various 1-hydroxy phenyl-5-hepta-*O*-benzoyl-β-D-lactosyl-2-*S*-benzoyl-2, 4-isodithiobiurets III(a-g) have been synthesized by the interaction of hepta-*O*-benzoyl-β-D-lactosylisothiocyanate (I) with several 1-aryl-*S*-benzyl isothiocarbamidesII(a-g) in benzene medium. All products were crystallized from ethanol before recording the physical data (Table-1). The purity of compounds was checked by TLC. The spectral analysis[13-15]IR, $_1$H NMR and Mass spectra of the product were observed. Optical rotation of the product was also recorded III (a-g) (Scheme-II).

Table 1: Physial characterization of 1-aryl-5-hepta-*O*-benzoyl-β-D-lactosyl-2-*S*-benzoyl-2, 4-isodithiobiurets (IIIa-f) (Scheme III)

Sr. No	Comp.	Yield%	m. p. °C	Elemental analysis Found (Required) N	S	$[\alpha]_D^{28}$ (c, CHCl3)	R_f Petroleum ether:EtoAc,7: 3)
1	IIIa	64%	77°C	3.08(3.06)	4.65 (4.67)	+22.55°,0.115	0.87
2	IIIb	82 %	120°C	3.05(3.06)	4.62 (4.67)	+57.59° ,0.111	0.75
3	IIIc	85 %	107°C	3.10(3.06)	4.70 (4.67)	+102.9°,0.132	0.50
4	IIId	76.5%	128-130	3.08(3.03)	4.66 (4.62)	-128°, 0.112	0.65
5	IIIe	80%	132	3.05(3.03)	4.65 (4.62)	+36.8°, 0.133	0.48
6	IIIf	65%	158-160	2.98(3.03)	4.58 (4.62)	-87.23, 0.143	0.58

Reactants: i) Hepta-*O*-benzoyl-β-D-lactosylisothiocyanate, ii) 1-aryl-S-benzyl isothiocarbamids

Spectral Data

IIIa) 1-*o*-hydroxy-phenyl-5-hepta-*O*-benzoyl-β-D-lactosyl-2-*S*-benzyl-2, 4-isodithiobiuret: m.p.: 77^{o}C; Yield : 64.00%; $[\alpha]_{D}^{28}$: + 22.55^{o} (c,0.115 in $CHCl_3$);

IR (KBr, cm-1):υ, 3458 (N-H stretch), 3061 (Ar-H stretch), 2954 (Ali C-H stretch)1730 (C=O), 1492 (C=N), 1313 (C-N), 1379 (C-O), 1026 and 910 (characteristic of lactose), 746 (C-S);

1H NMR ($CDCl_3$, ppm):δ8.11- 7.18 ppm(44H, m, Ar-H), 6.70 -6.60(2H, m, NH), δ6.22 – 3.69 (14H, m, lactosyl protons);

Mass (m/z): (M^+)-1369,(M^+-OH) -1352, (HBL^+)-1053, (HBL^+-C_7H_5O)-948, (HBL^+-C_7H_5O)-948, (HBL^+-$C_5H_5O_2$) -931, (TBG)-579, (TBG-C_6H_5)-474, ($C_6H_5O_2$)-109,C_7H_7-91,. (Anal.Calcd. For $C_{76}H_{64}O_{18}N_3S_2$, Required: C, 66.61; H, 4.67; N, 3.06; S, 4.67 Found: C, 66.65; H, 4.70; N, 3.08; S, 4.65 %.).

IIIb) 1-*m*-hydroxy-phenyl-5-hepta-*O*-benzoyl-β-D-lactosyl-2-*S*-benzyl-2, 4-isodithiobiuret: m.p. :120^{o}C; Yield : 82.00%; $[\alpha]_{D}^{28}$: + 57.59^{o} (c,0.111 in $CHCl_3$);

IR (KBr, cm-1): υ, 3352 (N-H stretch), 3061 (Ar-H stretch), 2916 (Ali C-H stretch)1730 (C=O), 1492 (C=N), 1315 (C-N), 1379 (C-O), 1026 and 910 (characteristic of lactose), 709 (C-S);

IIIc) 1-*p*-hydroxy-phenyl-5-hepta-*O*-benzoyl-β-D-lactosyl-2-*S*-benzyl-2, 4-isodithiobiuret: m.p.:107^{o}C; Yield: 85.00%; $[\alpha]_{D}^{28}$: + 102.9^{o} (c, 0.132 in $CHCl_3$);

IR (KBr, cm-1): υ, 3462 (N-H stretch), 3061 (Ar-H stretch), 2976 (Ali C-H stretch) 1730 (C=O), 1492 (C=N), 1315 (C-N), 1379 (C-O), 1070 and 908 (characteristic of lactose), 707 (C-S);

IIId) 1-*o*-methoxy-phenyl-5-hepta-*O*-benzoyl-β-D-lactosyl-2-*S*-benzyl-2, 4-isodithiobiuret: m.p. :128-130^{o}C; Yield : 76.50%; $[\alpha]_{D}^{28}$: -128^{o} (c,0.112 in $CHCl_3$);

IR (KBr, cm-1): υ, 3462 (N-H stretch), 3062 (Ar-H stretch), 2976 (Ali C-H stretch)1730 (C=O), 1452 (C=N), 1315 (C-N), 1379 (C-O), 1026 and 916 (characteristic of lactose), 711 (C-S);

1H NMR ($CDCl_3$, ppm):δ8.133- 6.221 ppm (44H, m, Ar-H), δ 6.19 (2H, m, NH), δ6.221 –4.587 (14H, m, lactosyl protons), δ3.870 (3H, s CH_3);

Mass (m/z): (M^+)-1383, (M^+-OCH_3)-1352, (M^+-C_7H_8ON)-1261, (HBL^+)-1053, (HBL-C_5H_8)-984, (HBL-$C_{14}H_{15}O_7$)-757, (TBG)-579, (TBG-$C_7H_8O_2$)-455, (TBG-$C_5H_{10}O_6$)-413, ($C_{12}H_{22}O_{11}$)-342, (TBG-$C_{13}H_{22}O_8$)-273 (100%), (C8H4N3S2)-206, (C7H5N2S)-149. (Anal. Calcd. For $C_{77}H_{66}O_{18}N_3S_2$Required: C, 66.81; H, 4.77; N, 3.03; S, 4.62 Found: C, 66.85; H, 4.73; N, 3.08; S, 4.66 %.).

IIIe) 1-*m*-methoxy-phenyl-5-hepta-*O*-benzoyl-β-D-lactosyl-2-*S*-benzyl-2, 4-isodithiobiuret: m.p.:132^{o}C; Yield: 80.00%; $[\alpha]_{D}^{28}$: + 36.8^{o} (c,0.133 in $CHCl_3$);

IIIf) 1-*p*-methoxy-phenyl-5-hepta-*O*-benzoyl-β-D-lactosyl-2-*S*-benzyl-2, 4-isodithiobiuret: m.p.:158-160^{o}C; Yield: 65.00%; $[\alpha]_{D}^{28}$: -87.23^{o} (c, 0.143 in $CHCl_3$);

Antimicrobial Studies

Antibacterial Activity

All the compounds have been screened for antimicrobial activity by using disc diffusion assay. The disc bearing plates were incubated at 37^{0}C for 24 hrs. Inhibition zones read after incubation at 37^{0}C for 24 hr. for bacterial strains. The compounds were taken at a concentration or 1mg/ml using dimethyl sulphoxide as a solvent. Amikacin (100 ug/ml) was used as standard for antibacterial. These compounds were screened for antibacterial activity against *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiellapneumonie*, *Psudomonasaeruginosa* in nutrient agar medium.

Antifungal Activity

All the compounds were also screened for their antifungal activities by using disc diffusion assay. These compounds were taken at a concentration or 1mg/ml using dimethyl sulphoxide as a solvent. Fluconazole (100ug/ml) was used as standard for antifungal activity. The results are presented in Table 2.

Table: 2 Antimicrobial activities of of newly synthesized1-aryl-5-hepta-*O*-benzoyl-β-D-lactosyl-2-*S*-benzoyl-2, 4-isodithiobiurets (IIIa-f).

Compounds	*E. coli*	*S. aureus*	*P. vulgaris*	*Ps. aeruginosa*	*S. typhi*	*K.pneumonie*	*A. niger*	*C.albicance*
IIIa	11	12	10	12	15	11	20	18
IIIb	10	16	11	14	17	-	18	20
IIIc	10	12	13	13	19	-	-	-
IIId	15	18	14	13	14	13	12	-
IIIe	13	19	17	10	15	10	22	15
IIIf	12	-	13	-	16	-	17	22
DMSO	-	-	-	-	-	-	-	-
Amikacin	18	21	23	19	20	20	-	-
Fluconazole	-	-	-	-	-	-	24	24

Zone size was interpreted by

Sample	Disc content	Resistant	Intermediate	Sensitive
Amikacin	100ug/ml	≤ 15 mm	16-20 mm	≥ 21 mm
Fluconazole	100ug/ml	≤ 15 mm	16-20 mm	≥ 21 mm

CONCLUSION

In this research work, the characterizations of newly synthesized products were established on the basis of UV, IR, ^{1}H NMR, & Mass spectral studies. Various 1-aryl-5-hepta-*O*-benzoyl-β-D-lactosyl-2-S-benzoyl-2, 4-isodithiobiurets were synthesized and yield of product ranged from 64-85%. Some of the compounds synthesized showed promising antimicrobial activities.

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