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Synthesis and Antimicrobial Activity of Lactosyl Triazino Substituted Benzothiazole (Hydrochlorides)

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ABSTRACT: Certain lactosyl fused triazino substituted benzothiazoles have been prepared by the interaction of 1-hepta-*O*-acetyl-β-D-lactosyl-3-substituted benzothiazolyl thiocarbamides and *N*-phenyl isocyanodichloride. The structures of newly synthesized compounds were characterized by spectral and analytical data. The compounds were screened for their antibacterial and antifungal activity against various pathogenic bacteria and fungi and most of the compound showed good inhibition against the pathogens.

Keywords: Benzothiazolyl thiocarbamides, *N*-phenyl isocyanodichloride, lactosyl triazino substituted benzothiazole and antimicrobial activities.

INTRODUCTION

Heterocycles containing thiazole ring system are found to exhibit broad-spectrum of biological activities especially benzothiazoles/substituted benzothiazoles have attracted great deal of attention in medicinal chemistry due to their diverse range of biological properties such as antimicrobial, antitumor, antitubercular, anticancer, antilieshmanial, insecticidal and anti-inflammatory, anti-HIV activities[1-5]. Similarly, carbohydrates represent one of the most diverse and versatile class of functional natural products and their primary significance is attributed to their major importance in large number of crucial biological functions [6]. As a result sugar heterocycle [7-10] are of prime importance specially in the field of drug discovery. Among these sugar based benzothiazoles display important biological activities. In the view of above advantages we decided to explore further these important class of compounds by selecting lactose as a sugar template. So, herein we report the synthesis of novel lactosyl triazino substituted benzothiazoles and their study as antibacterial and antifungal agents.

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EXPERIMENTAL

Melting points were taken in open capillary tubes on Mac digital melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer spectrum RXI FTIR spectrometer 4000-450 cm^{-1}. The ^1H NMR spectra were recorded in $CDCl_3$ at 300 MHz on a Bruker DRX-300 NMR spectrometer. The FAB Mass spectra were recorded on a Jeol SX-102/Da-600 mass spectrometer / data system using argon/xenon (6 KV, 10mA) as the FAB gas. The accelerating voltage was 10 KV, and the spectra were recorded at room temperature, Optical rotations $[\alpha]_D^{31}$ were measured on Equip-Tronics EQ-800 Digital Polarimeter at 31° C in Chloroform. Thin layer chromatography (TLC) was performed on E. Merck pre-coated silica gel plates.

The required 1-hepta-*O*-acetyl-β-D-lactosyl-3-substituted benzothiazolyl thiocarbamides [11,12] **(Ia-g)** were prepared by the interaction of hepta-*O*-acetyl-β-D-lactosyl isothiocyanate and substituted 2-amino benzothiazoles. The requisite substituted 2-amino benzothiazoles were prepared in excellent yield by oxidative cyclization of substituted aryl thiocarbamides with molecular bromine in chloroform. *N*-phenyl isocyanodichloride[13] (**II**) was prepared by the interaction of phenyl isothiocyanate and excess of chlorine.

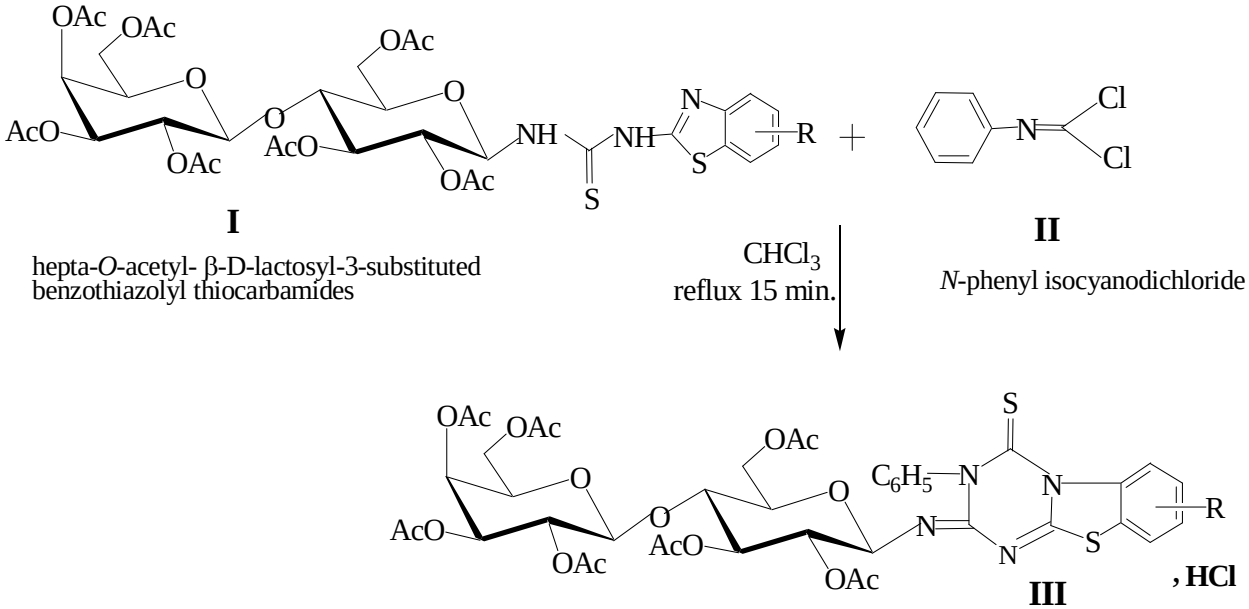
***General procedure for synthesis of compounds* (IIIa-g)**

4H,4-thio-2-hepta-*O*-acetyl-β-D-lactosylimino-3-phenyl-2,3-dihydro-(1,3,5)-triazino(2,1b) -6, 7 or 8-substituted benzothiazole (hydrochlorides)

Interaction of 1-hepta-*O*-acetyl–β-D–lactosyl-3-substituted benzothiazolyl thiocarbamide (**I**) (0.0025 M) and *N*-phenyl iso-cyanodichloride (**II**) (0.0025) was carried out in boiling $CHCl_3$ for 20 mins. The evolution of hydrogen chloride was noticed. After condensation, the solvent was distilled off to obtain a sticky residue. The residue was triturated with petroleum ether (60–80°C) to afford a pale yellow solid (**III**). It was crystallized by ethanol- petroleum ether. The physical characterization of compounds **IIIa-g** is exhibited in **Table 1.** The reaction sequence leading to the formation of the desired heterocyclic compounds are outlined in **Figure I**.

ANTIMICROBIAL ACTIVITY

The antibacterial and antifungal activities of synthesized compounds **(IIIa-g)** were tested *in vitro* against bacteria *Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium* and fungi *Aspergillus niger* and *Rhizoctonia* by cup plate agar diffusion method[14,15]. Amikacin (100µg/ml) was used as a standard drug for antibacterial activity and Fluconazole (100µg/ml) as a standard drug for antifungal activity. The antibacterial activity was carried out in nutrient agar medium and antifungal 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. All the synthesized compounds (100 µg/ml) were placed serially in the cavities with the help of micropipette and allowed to diffuse for 1.0 hr. DMSO was used as a solvent for all the compounds and as a control. These plated were incubated at 37 ºC for 24 hr and 28 ºC for 48 hr, for antibacterial and antifungal activities respectively. The zone of inhibition observed around the cups after respective incubation was measured in mm. The results are presented in **Table 2**.



4H, 4- thio-2-hepta -*O* -acetyl- β-D-lactosylimino-3-phenyl-2,3-dihydro -(1,3,5)- triazino-(2,1b)-6, 7 or 8-aryl benzothiazole (hydrochlorides)

Where, Ac-= $COCH_3$

R = a) H, b) 6-Cl,c) 7-Cl,d) 8-Cl,e) 6-Me, f) 7-Me, g) 8-Me.

Figure 1

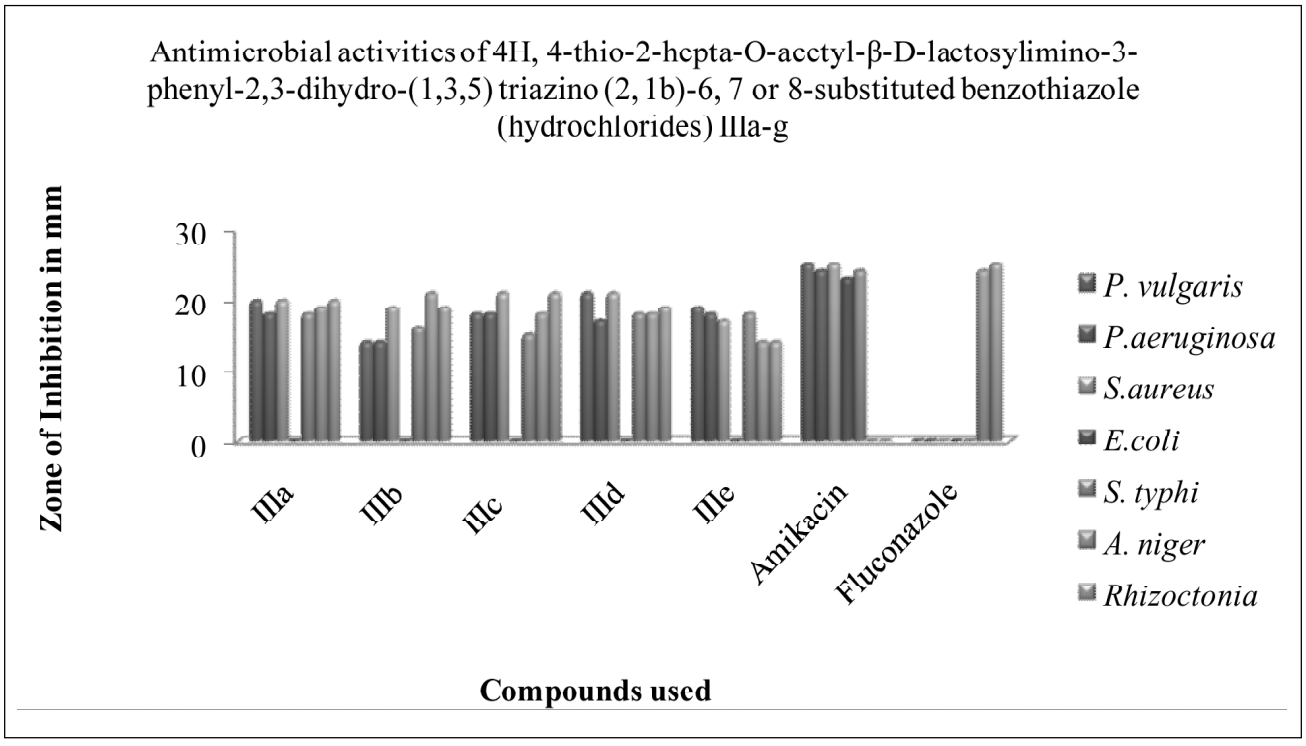
Table 1
Physical characterization and analytical data of compounds (IIIa-g)

Product	*Yield (%)*	*m.p. (°C)*	R_f	$[\alpha]_D^{32}$ *(c,1.013 in $CHCl_3$)*	*% Found (Calculated)* *N*	*S*
(IIIa)	82.98	98- 100	0.85	+42.10	5.76 (5.80)	6.65(6.63)
(IIIb)	84.27	122-124	0.88	+20.09	5.57(5.61)	6.44(6.41)
(IIIc)	76.61	115-117	0.52	+32.08	5.55(5.61)	6.37(6.41)
(IIId)	92.33	103-105	0.59	+57.14	5.54(5.61)	6.38(6.41)
(IIIe)	93.44	128-130	0.70	+61.05	5.67(5.72)	6.56(6.54)
(IIIf)	76.63	108-110	0.65	-23.07	5.65(5.72)	6.48(6.54)
(IIIg)	69.67	100-102	0.79	-13.07	5.69(5.72)	6.59(6.54)

Table 2
Antimicrobial activities of 4H,4-thio-2-hepta-*O*-acetyl-β-D-lactosylimino-3-phenyl-2,3-dihydro-(1,3,5)-triazino(2,1b)-6,7 or 8-substituted benzothiazole (hydrochlorides). IIIa-g

Product	*Antimicrobial activity*						
	Antibacterial activity					*Antifungal activity*	
	P. vulgaris	*P.aeruginosa*	*S.aureus*	*E.coli*	*S. typhi*	*A. niger*	*Rhizoctonia*
IIIa	20	18	20	——	18	19	20
IIIb	14	14	19	——	16	21	19
IIIc	18	18	21	——	15	18	21
IIId	21	17	21	——-	18	18	19
IIIe	19	18	17	——	18	14	14
Amikacin	25	24	25	23	24	——	——
Fluconazole	——	——	——	——	——	24	25

Zone of inhibition in mm, (12-14) weak activity, (15-18) moderate, and above 18 mm, strong activity.



RESULTS AND DISCUSSION

We have synthesized novel fused lactosyl triazino substituted benzothiazole (hydrochlorides) **(IIIa-g) Fig. 1** from precursors 1-hepta-*O*-acetyl-β-D-lactosyl-3-substituted benzothiazolyl thiocarbamide (**Ia-g**) and *N*-phenyl isocyanodichloride (**II**). The synthesized compounds were soluble in common organic solvents and insoluble in water. The structural elucidation was confirmed by elemental and spectral analysis. The IR spectra of the compounds showed strong characteristic absorption of lactose unit in the range of 900-910, 1000-1100 cm^{-1} for stretching vibration of C-H bond. The stretching band for acetyl C=O has appeared in the region 1749-1750 cm^{-1}. The absorption band for C=N has appeared in the region 1542-1547 cm^{-1}. ^1H NMR spectrum of the products showed characteristic of lactosyl protons at δ 6.25-3.57 ppm and resonance signals for aromatic protons at δ 7.92- 6.25 ppm. Acetyl protons appeared at δ 3.12-0.83 ppm. Mass spectra [16,18] exhibited molecular ion peak along with characteristic fragments of lactose unit at m/z 619, 560, 331, 169 and 109.

SPECTRAL ANALYSIS

4H,4-thio-2-hepta-*O*-acetyl-β-D-lactosylimino-3-phenyl-2,3-dihydro-(1,3,5)triazino(2,1b) -6, 7 or 8 (H) benzothiazole (hydrochloride) (IIIa)

IR (ν/cm^{-1}): 2945 C-H (CH_2), 1750 (C=O), 1543 (C=N), 1230 (C-O), 1051 & 906 (lactose), 601 (C-S); ^1H NMR ($CDCl_3$) (δ ppm): 7.92-6.16 (m, 9H, Ar-H), 6.16 –3.58 (m, 14H, lactose unit), 2.18 -1.73 (m, 21H, $7COCH_3$); Mass (m/z): (M^+ +1) 965, 830, 619, 560, 331, 211, 109.

4H,4-thio-2-hepta-*O*-acetyl-β-D-lactosylimino-3-phenyl-2,3-dihydro-(1,3,5)-triazino(2,1 b) -7-chloro benzothiazole (hydrochloride) (IIIc)

IR (ν/cm^{-1}): 2960 C-H (CH_2), 1750 (C=O), 1544 (C=N), 1233 (C-O), 1050 & 906 (lactose), 602 (C-S); ^1H NMR ($CDCl_3$) (δ ppm): 7.70–6.15 (m, 8H, Ar-H), 6.25 – 3.77 (m, 14H, lactose unit), 3.21-0.83 (m, 21H, $7COCH_3$); Mass (m/z): (M^+) 998, 888, 619, 560, 331, 211, 169, 109.

4H,4-thio-2-hepta-*O*-acetyl-β-D-lactosylimino–3–phenyl–2,3-dihydro-(1,3,5)-triazino(2, 1b)-6-methyl benzothiazole (hydrochloride) (IIIe)

IR (ν/cm^{-1}): 2944 C-H (CH_2), 1749 (C=O), 1546 (C=N), 1232 (C-O), 1051 & 906 (lactose), 601 (C-S); ^1H NMR ($CDCl_3$) (δ ppm): 7.73–6.25 (m, 8H, Ar-H), 5.74–3.74 (m, 14H, lactose unit), 2.66–1.84 (m, 21H, $7COCH_3$), 1.266 (s, 3H, CH_3); Mass (m/z): (M^+) 978, 619, 560, 331, 169, 109.

ANTIMICROBIAL STUDIES

Antibacterial activity

The results of the title compounds for preliminary antimicrobial testing are shown in **Table 2.** The compounds **(IIIa-g)** exhibit strong inhibition against *S. aureus* and *P. vulgaris*, moderate activity against *Ps. aeruginosa, S. typhi,* while no activity against *E. coli.*

Antifungal activity

The compound IIIe exhibited low activity against *A. niger* and *Rhizoctonia* whereas the rest compounds exhibited moderate to strong activity against *A. niger* and *Rhizoctonia*.

CONCLUSION

The present study reports the synthesis of novel fused 4H, 4-thio-2-hepta-*O*-acetyl-â-D-lactosylimino-3-phenyl-2,3-dihydro-(1,3,5)-triazino (2,1b)-6,7 or 8-substituted benzothiazole. The method adapted for synthesis is efficient and inexpensive and is useful in synthesizing pharmacologically important molecules.

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