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Synthesis And Antimicrobial Activity Of Hepta-*O*-Benzoyl-β-D-Lactosyl-3-(2)-Hydrazino-1, 3-Benzothiazolyl Thiocarbamides

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ABSTRACT

Benz-fused compounds have been employed in the synthesis of various compounds which show very potential pharmacological activities. Carbohydrate is the key element in variety of biological phenomena and its *N*-linked sugar derivatives also exhibit wide range of medicinal activities. Keeping in this view, when one biological active molecule is linked to another, the resultant molecule generally has increased potency.Hence for the first time, in present work, we have interacted two pharmocophores, hepta-*O*-benzoyl-β-D-lactosyl isothiocyanate and substituted 2-hydrazino-1,3-benzothiazoles in acetone medium to yield hepta-*O*-benzoyl-β-D-lactosyl-3-(2)-hydrazino-1,3-benzothiazolyl thiocarbamides. The identities of these newly synthesised hepta-*O*-benzoyl-β-D-lactosyl-3-(2)-hydrazino-1,3-benzothiazolyl thiocarbamides have been established on the basis of usual chemical transformations and IR , ^{1}H NMR and Mass spectral studies. The antibacterial and antifungal activities of also reported. Some of these derivatives exhibit significant antimicrobial activity.

Key words: Lactosyl isothiocyanate, hydrazino benzothiazoles, lactosyl hydrazino benzothiazolyl thiocarbamides, antimicrobial activity.

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INTRODUCTION

Benzothiazole, a multifaceted nucleus, has been under research for the last two decades. Being a heterocyclic compound, benzothiazole finds use in research as a starting material for the synthesis of larger, usually bioactive structures. Its aromaticity makes it relatively stable, although as a heterocycle, it has reactive sites which allow for functionalization[1]. From the literature survey, it has been found that extensive work has been reported on 2-substituted benzothiazole derivatives in past and evaluated for different activities like antibacterial[1], anticancer[2], antiviral[3], antitumor[4], antifungal[5], antiinflammatory[6], antioxidative and radioprotective[7], antidiabetic[8,9], anthelmintic[10], anti-leishmanial[11], anticonvulsant[12], neuroprotective[13], a topical carbonic anhydrase inhibitor and an antihypoxic. Taking this into view, and in continuation of our search for biologically potential benzothiazole derivatives, a certain new derivatives were synthesized taking benzothiazole as the basic moiety.

The *N*-lactosylated compounds also have been known for their great biological importance. They have been found use as diuretic agents, analgesics, antidiabetic compounds, bacteriostatic agents and some remarkable significant activities[14].

Hence, in present work, different benzothiazoles react with hydrazine and this hydrazino benzothiazoles then focused to fuse with *N*-lactosylated compound.

MATERIALS AND METHODS

Experimental

The reagent grade chemicals were obtained from commercial sources and purified by either distillation or recrystallization before use. Melting points of all synthesized compounds were determined using open capillary tube on Mac digital melting point apparatus and were uncorrected. 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-lactosyl isothiocyanate:

To the suspension of hepta-*O*-benzoyl-α-D-lactosyl bromide (0.039 M, 15 g) in sodium dried xylene (60 mL) was added lead thiocyanate (0.039 M, 4.5 g). The mixture was refluxed gently

for 3 h. with frequent shaking. The xylene filtrate was then treated with petroleum ether (60-80^{o}C) with stirring, a solid was obtained. This solid was expected hepta-*O*-benzoyl-β-D-lactosyl isothiocyanate **(I)**. It was purified by dissolving it in minimum quantity of chloroform and reprecipitating with petroleum ether. m.p.-118-120^{o}C. **(I) (Scheme-I).**

Preparation of 2- hydrazino-1, 3-benzothiazoles:

Concentrated HCl (1mL) was added drop wise to hydrazine hydrate (0.2 M, 1mL 80%) at 5-10^{o}C followed by ethylene glycol (20mL). To the above solution 2-aminobenzothiazole (0.01 M, 1.85g) was added in portions. It was then refluxed for 3 h, cooled and poured onto crushed ice. The separated solid was filtered, dried and recrystalized from ethanol. **II(a-g). (Scheme-II)**

Preparation of hepta-*O*-benzoyl-β-D-lactosyl-3-(2)-hydrazino-1,3-benzothiazolyl thiocarbamide:

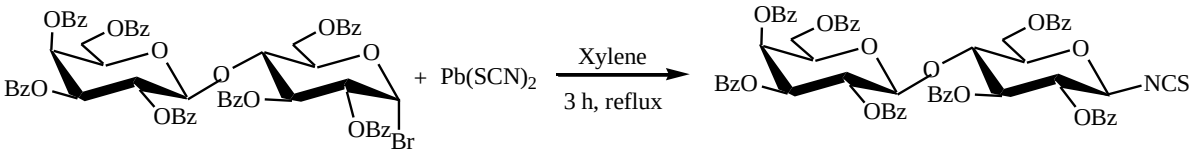
A acetone solution of hepta-*O*-benzoyl-β-D-lactosyl isothiocyanate (0.025M, 2.5g in 20mL) was mixed with acetone solution of 2-hydrazino-1,3-benzothiazole (0.025M, 0.37g in 10mL), and mixture after shaking for sometime was kept at room temperature for 24 h. Acetone was distilled off to obtained sticky residue. This residue was triturated several times with petroleum ether to afford a light coloured solid. **III(a-g). (Scheme-III).**

RESULTS AND DISCUSSION

Herein, we report the synthesis of various hepta-*O*-benzoyl-β-D-lactosyl-3-(2)-hydrazino-1,3-benzothiazolyl thiocarbamides **III(a-g)** by interaction of hepta-*O*-benzoyl-β-D-lactosyl isothiocyanate **(I)** and substituted 2- hydrazino-1,3-benzothiazole **II(a-g)** in acetone medium.

All products were crystallized from ethanol before recording the physical data (Table-1). The purity of compounds were checked by TLC. The spectral analysis[15-17] 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).**

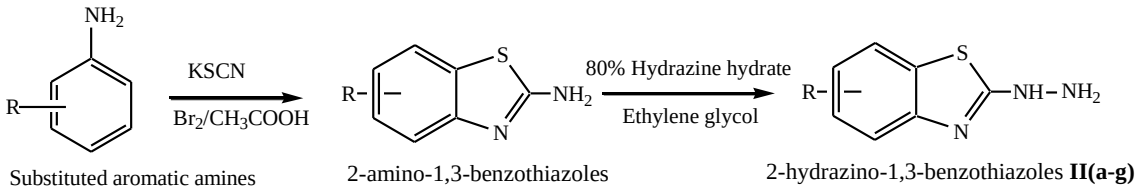
Scheme for synthesis shown as follows:



hepta-O-benzoyl-α-D-lactosyl bromide

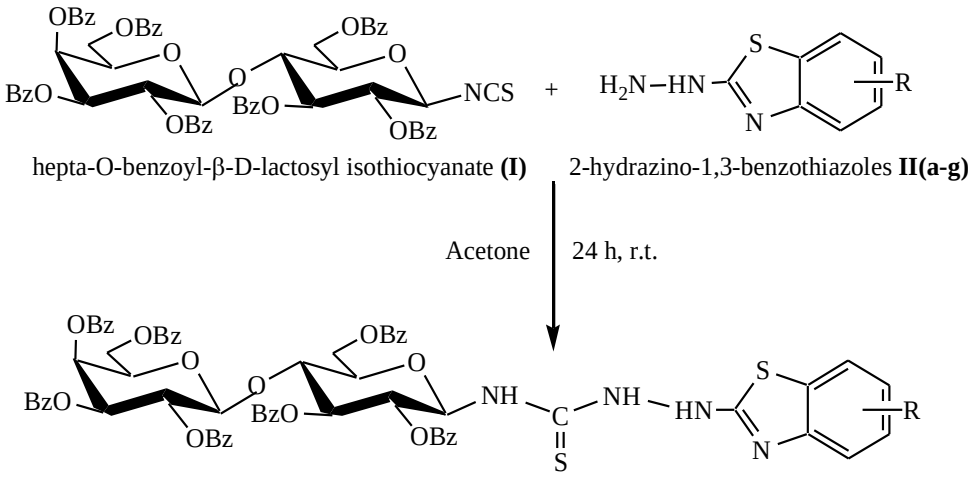
hepta-O-benzoyl-β-D-lactosyl isothiocyanate **(I)**

Scheme-I



Substituted aromatic amines — 2-amino-1,3-benzothiazoles — 2-hydrazino-1,3-benzothiazoles **II(a-g)**

Scheme-II



hepta-O-benzoyl-β-D-lactosyl isothiocyanate **(I)** 2-hydrazino-1,3-benzothiazoles **II(a-g)**

hepta-O-benzoyl-β-D-lactosyl-3-(2)-hydrazino-1,3-benzothiazolyl thiocarbamide **III(a-g)**

Scheme-III

Where,

Bz = COC_6H_5, R = a) Hydrogen, b) 4-Chloro, c) 5-Chloro, d) 6-Chloro, e) 4-methyl, f) 5-methyl, g) 6-methyl.

Spectral Data

IIIa) Hepta-*O*-benzoyl-β-D-lactosyl-3-(2)-hydrazino-1,3-benzothiazolyl thiocarbamide: m.p. : 137-139^oC; Yield : 64.63%; $[\alpha]_D^{28}$: + 119^o (c,0.1 in $CHCl_3$);

IR (KBr, cm^{-1}) : υ, 3483.44 (N-H stretch), 3062.96 (Ar-H stretch), 1730.15 (C=O), 1600.92 (C=N), 1556.55 (N-H bend), 1450.47 (Ar C=C), 1315.45 (C-N), 1269.16 (C-O), 1095.57 (characteristic of lactose), 1070.49 (C=S), 709.80 (C-S);

^{1}H NMR ($CDCl_3$, ppm): δ 8.065-7.214 (39H, m, Ar-H), 6.749-5.720 (3H, m, NH), 5.620-3.467 (14H, m, lactosyl protons);

Mass (m/z): (M^+-$C_7H_8O_6$)-1088, (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-$C_{12}H_{21}O_8$)-286 (100%), ($C_8H_4N_3S_2$)-206, ($C_7H_5N_2S$)-149. (Found : C, 65.41; H, 4.27; N, 4.22; S, 4.96%; Calcd. for $C_{69}H_{56}O_{17}N_4S_2$: C, 65.48; H, 4.38; N, 4.38; S, 5.14 %%).

IIId) Hepta-*O*-benzoyl-β-D-lactosyl-3-(2)-hydrazino-6-chloro-1,3-benzothiazolyl thiocarbamide:

m.p. : 151-153^{o}C; Yield : 86.47%; $[\alpha]_{D}^{28}$: + 128^{o} (c,0.1 in $CHCl_3$);

IR (KBr, cm^{-1}) : υ, 3475.73 (N-H stretch), 3061.03 (Ar-H stretch), 1730.15 (C=O), 1600.92 (C=N), 1514.12 (N-H bend), 1450.47 (Ar C=C), 1315.45 (C-N), 1269.16 (C-O), 1093.64 (characteristic of lactose), 1070.49 (C=S), 709.80 (C-S), 686.66 (C-Cl);

^{1}H NMR ($CDCl_3$, ppm): δ 8.223-7.044 (38H, m, Ar-H), 6.921-6.415 (3H, m, NH), 6.258-3.236 (14H, m, lactosyl protons);

Mass (m/z): (M^{+}+3)-1313, (M^{+}+2)-1312, (M^{+}-CH_3COOH)-1248, (M^{+}-$C_8H_{14}O_7$)-1088, (HBL-C_5H_8)-984, (HBL-$C_6H_8O_7$)-860, (TBG)-579, (TBG-$C_7H_8O_2$)-455, (TBG-$C_6H_{14}O_5$)-413, (TBG-$C_8H_{18}O_7$)-356, ($C_8H_5N_3S_2Cl$)-240 (100%), ($C_7H_4N_2SCl$)-183. (Found : C, 62.98; H, 4.02; N, 4.16; S, 4.53%; Calcd. for $C_{69}H_{55}O_{17}N_4S_2Cl$: C, 63.17; H, 4.19, N, 4.27; S, 4.88%).

IIIg) Hepta-*O*-benzoyl-β-D-lactosyl-3-(2)-hydrazino-6-methyl-1,3-benzothiazolyl thiocarbamide:

m.p. : 145-147^{o}C; Yield : 91.22%; $[\alpha]_{D}^{28}$: + 118^{o} (c,0.1 in $CHCl_3$);

IR (KBr, cm^{-1}) : υ, 3488.44 (N-H stretch), 3061.03 (Ar-H stretch), 2980.92 (C-H CH_3), 1730.15 (C=O), 1600.92 (C=N), 1514.12 (N-H bend), 1452.40 (Ar C=C), 1379.10 (C-H_3 bend), 1315.45 (C-N), 1269.16 (C-O), 1093.64 (characteristic of lactose), 1070.49 (C=S), 709.80 (C-S);

^{1}H NMR ($CDCl_3$, ppm): δ 8.064-7.018 (38H, m, Ar-H), 6.229-5.971 (3H, m, NH), 5.931-3.772 (14H, m, lactosyl protons), 2.447 (3H, s, CH_3 protons);

Mass (m/z): (M^{+}+1)-1290, (M^{+}-CH_3COOH)-1228, (M^{+}-$C_7H_6O_7$)-1088, (HBL-$C_4H_7O_3$)-949, (TBG)-579, (TBG-$C_5H_{10}O_6$)-413, (TBG-$C_8H_{18}O_7$)-353, ($C_9H_8N_3S_2$)-220 (100%), ($C_8H_7N_2S$)-163. (Found : C, 64.88; H, 4.37; N, 4.15; S, 4.81 % Calcd. for $C_{70}H_{58}O_{17}N_4S_2$: C, 65.10; H, 4.49; N, 4.34; S, 4.96%)

Table : 1 Physial characterisation of hepta-*O*-benzoyl-β-D-lactosyl-3-(2)-hydrazino-1,3-benzothiazolyl thiocarbamides (IIIa-g) (Scheme-II).

Reactants: i) hepta-*O*-benzoyl-β-D-lactosyl isothiocyanate, ii) 2-hydrazino-1, 3-benzothiazoles.

Sr. No.	Compd.	Yield g (%)	m. p. (^{o}C)	Elemental Analysis Found (Required)		$[\alpha]_{D}^{28}$ (c, $CHCl_3$)	R_f (4:6, EtOAc : Pet. Ether)
				N	S		
1.	IIIa	2.1 (64.63)	137-139	4.22(4.38)	4.96(5.14)	+119^{o}(0.93)	0.63
2.	IIIb	2.8 (87.24)	145-147	4.18(4.27)	4.64(4.88)	+120^{o}(0.96)	0.59
3.	IIIc	2.8 (88.56)	142-144	4.13(4.27)	4.70(4.88)	+110^{o}(0.95)	0.47
4.	IIId	2.6 (86.47)	151-153	4.16(4.27)	4.53(4.88)	+128^{o}(0.92)	0.36
5.	IIIe	2.0 (62.39)	149-150	4.22(4.34)	4.85(4.96)	+140^{o}(0.98)	0.35
6.	IIIf	2.5 (78.13)	139-141	4.29(4.34)	4.94(4.96)	+112^{o}(0.94)	0.54
7.	IIIg	2.4 (91.22)	145-147	4.15(4.34)	4.81(4.96)	+118^{o}(0.95)	0.37

ANTIMICROBIAL STUDIES

All the compounds have been screen for both antimicrobial and antifungal activity using cup plate agar diffusion method[18-20] by measuring the inhibition zone in mm. the compounds were taken at a concentration of 1 mg/mL using Dimethyl Sulphoxide (DMSO) as solvent. Amikacin (100 µg/mL) was used as standard for antibacterial activity and Fluconazole (100 µg/mL) as standard for antifungal activity. The compounds were screen for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Klebsiyella species* by using Nutrient Agar medium and antifungal activity against *Thrichoderma harzianum* and *Verticillium species* was determined by using 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 cotton swab. After inoculation the well was punched by using sterile stainless steel cork borer of 6mm diameter. In to these wells were added 0.1 mL portion of the test compounds in solvent. The drug solution was allowed to diffuse for an hour into the medium. The plate was incubated at 37^{o}C for 24 h and 30^{o}C for 48 h for antibacterial and for antifungal activities respectively. The zone of inhibition observed around the cups after respective incubation was measured. The results are presented in Table 2.

Antibacterial studies of these compounds indicated that compounds IIIa and IIIf were found to be active against *E.coli* and rest of were found to be moderately active. IIIa exhibited most significant activity against *S.aureus* and IIIa, IIIb and IIId towards *Pseudomonas.* All the other compounds exihibited low to moderate activity. (Table2).

Table : 2 Antimicrobial activities of newly synthesized hepta-*O*-benzoyl-β-D-lactosyl-3-(2)-hydrazino-1,3-benzothiazolyl thiocarbamides (IIIa-g).

Compound	Antibacterial**					Antifungal**	
	E. coli	***S. aureus***	***P. vulgaris***	***Ps. aeruginosa***	***Klebsiyella species***	***T. harzianum***	***Verticillium species***
IIIa	20	24	19	23	18	23	21
IIIb	17	16	15	22	17	19	22
IIIc	15	16	-	12	12	14	24
IIId	19	12	16	21	20	22	20
IIIe	18	14	14	16	-	26	22
IIIf	21	16	16	17	15	20	22
IIIg	18	19	15	18	18	22	24
Amikacin	25	27	25	26	28	28	28
Fluconazole	-	-	-	-	-	28	26

**Including the well diameter of 6 mm. Zone of inhibition in mm (15 or less) resistant, (16-20 mm) moderate and (more than 20 mm) sensitive.

The results of antifungal activities are also tabulated in Table2. IIIa, IIId, IIIe, IIIf and IIIg are most effectively active against *Thrichoderma harzianum*, IIIa, IIIb, IIIc, IIIe, IIIf and IIIg actively inhibited *Verticillium species*. While other compounds inhibited moderate activity.

CONCLUSION

Derivatives were synthesized and characterized for their structure elucidation. Various chemical and spectral data supported the structures. Some of the compounds synthesized showed promising antimicrobial activities. The newly synthesized thiocarbamides exhibits comparable antibacterial and antifungal activities against the organisms tested. The method adopted in this investigation is simple, efficient and inexpensive and is useful in synthesizing pharmacologically important molecules.

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