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# Synthesis, Characterization And Microbial Studies of N-Lactosylated Isothiobiurets

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## ABSTRACT

*Several 1-Hepta-O-acetyl- β-D-lactosyl-5-aryl-isothiobiurets were synthesized by the interaction of Hepta-O-acetyl-β-D-lactosyl thiocarbamides with various aryl isocyanates respectively and were screened for their antibacterial and antifungal activities against some selected pathogenic organisms like E. coli, S. aureus, Ps. aeruginosa, P. vulgaris, B. cereus and A. niger, C. albicans to get potent bioactive molecule. The identities of these new N-lactosides have been established on the basis of elemental analysis, IR, $^{1}$HNMR, $^{13}$CNMR and MS spectral studies.*

**Keywords:** Lactosyl thiocarbamides, lactosyl isothiobiurets, synthesis, antimicrobial Activity.

## INTRODUCTION

Lactosylated Thiocarbamides, and Isothiobiurets have a long history of applications in pharmaceutical and agrochemical industries. Thiobiurets (mono and di) are important derivatives of thiourea which may increase the biological activity of (thio)ureas. The mono and dithiobiuret derivative are effective fungicides, bactericides, herbicides and also have demonstrated effective growth regulating activity[1] .

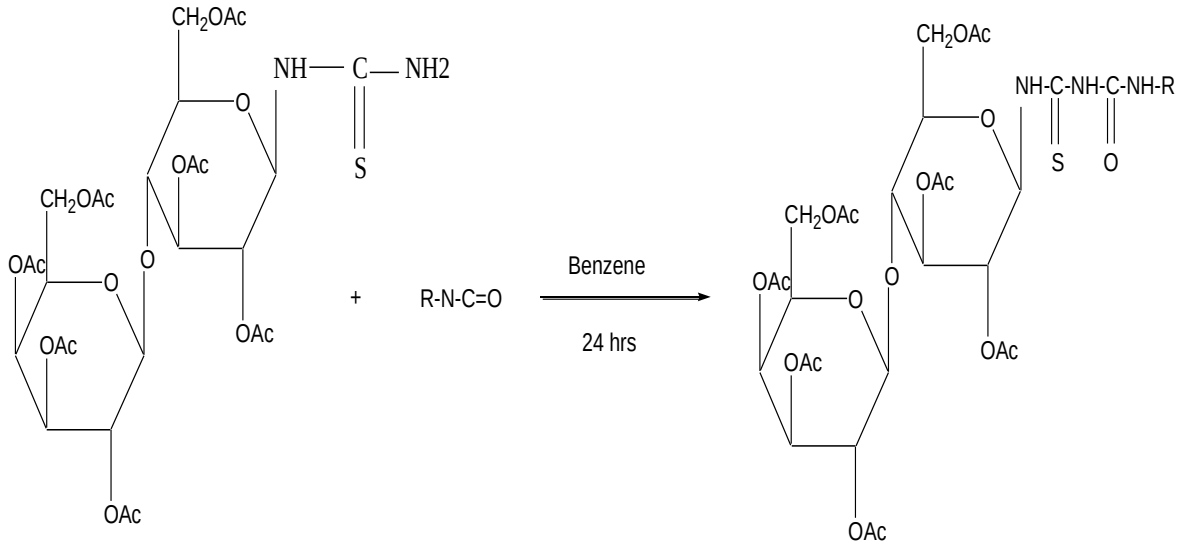
Some thiobiuret derivatives also showed analgesic[2], anticonvulsant and hypnotic activity[3,4]. Glycosyl urea and their biuret derivatives are reported as potential glycoenzyme inhibitors[5]. Thioureas and derivatives are biologically important compounds and are useful fungicides, herbicides[6] and antibacterial agents[7].They have also found use in organocatalysis [8, 9].

Many of the substituted thioureas are active as anti-HIV[10], antiviral[11], antimicrobial [12],antituberculor[13], antitumor [14], antihypertensive[15] and anticarcinogenic agents[16].

The high acidity of the -NH-CS-NH- protons, in correlation with strong hydrogen bonding property has been exploited in the design of self-assembling macromolecules and stabilization of secondary structures[17]. Thioureamoieties embedded into macromolecules like pseudo-oligosaccharides provide anchoring points for hydrogen bonding recognition of complementary functional groups with specific orientation[18]. They are also used as catalysts for asymmetric organic synthesis [19-22].

## MATERIALS AND METHODS

Melting points the synthesized compounds were recorded on electro thermal melting point apparatus are uncorrected. Specific rotations of the newly synthesized compounds were measured on Equip-Tronic digital polarimeter model no. EQ 800 at $30^0$C in $CHCl_3$. IR spectra were recorded on a Shamazdu FTIR spectrometer. $^1$HNMR were obtained on a Bruker DRX-300 (300 MHz FT NMR) NMR spectrometer in $CDCl_3$ solution with TMS as an internal reference. The MS spectra were recorded on a Jeol SX -102 FAB mass spectrometer and $^{13}$CNMR were recorded in $CDCl_3$. Purity of the compounds was checked by thin layer chromatography using Merck silica gel coated aluminum plates and petroleum ether: ethyl acetate as eluent. The synthetic strategy involved the interaction of Hepta-*O*-acetyl-β-D-lactosyl thiocarbamides with aryl isocyanates. As a first step the required Hepta-*O*-acetyl-β-D-lactosyl thiocarbamides was prepared by earlier known method[23] by interaction of Hepta-*O*-acetyl-β-D-lactosyl isothiocyanate with ammonia in benzene medium. The various aryl isocyanates were purchased from Sigma-Aldrich. All solvents were freshly distilled before use.




1 2 3

Where, Ac - $COCH_3$
R = a) o-Tolyl, b) m-Tolyl, c) p-Tolyl, d) m-Cl-Phenyl, e) p-Cl-Phenyl, f) o-Anisyl, g) m-Anisyl, h) p-Anisyl, i) 1-Naphthyl.

**Synthesis of 1-Hepta-*O*-acetyl-β-D-lactosyl-5-aryl-isothiobiurets (5a-i) :** 1-Hepta-O-acetyl-β-D-lactosyl-5-aryl-isothiobiurets **(3a-i)** were synthesized by mixing 1-Hepta-O-acetyl-β-D-lactosyl-thiocarbamide **(1)** (0.005M, 3.475 g) with aryl isocyanate (0.66mL) **(2a-i)** in dry benzene medium and stirring at room temperature for 24 h while monitoring the reaction by TLC. Benzene was distilled off and the resultant syrupy mass was triturated several times with petroleum ether ($60\text{-}80^0$), to afford white granular solid **(3a-i) (Table-1)**. Crystallized from ethanol. The characterization of products **(3a-g)** was established by IR, $^1$HNMR, $^{13}$CNMR and Mass spectral studies.

**Table 1**. Synthesis of 1-Hepta-*O* -acetyl- β -D -lactosyl-5-aryl-isothibiurets (3a-i)

| Sr.No. | Product (3a-i) | Reactants (2a-i) | Yield (%) | M.P. ($^oC$) | $[\alpha]_D^{32}$ (0.1, in $CHCl_3$) | Found (Required) N | Found (Required) S | R*f* (pet ether:EtOAC) (1:1) |
|---|---|---|---|---|---|---|---|---|
| 1 | 3a | -5-*o-tolyl* | 78 | 132 | +164 | 4.98 (5.0) | 3.90 (3.86) | 0.58 |
| 2 | 3b | -5-*m-tolyl* | 89 | 169 | +179 | 4.95 (5.0) | 3.92 (3.86) | 0.72 |
| 3 | 3c | -5-*p-tolyl* | 82 | 248 | -138 | 4.97 (5.0) | 3.94 (3.86) | 0.84 |
| 4 | 3d | -5- *m*-Cl-*phenyl*- | 85 | 196 | +187 | 4.92 (4.95) | 3.81 (3.77) | 0.82 |
| 5 | 3e | -5-*p*-Cl-*phenyl*- | 76 | 166 | -224 | 4.91 (4.95) | 3.83 (3.77) | 0.34 |
| 6 | 3f | -5-*o-anisyl*- | 91 | 154 | +107 | 4.96 (4.98) | 3.85 (3.79) | 0.70 |
| 7 | 3g | -5-*m-anisyl*- | 93 | 147 | +173 | 4.93 (4.98) | 3.80 (3.79) | 0.59 |
| 8 | 3h | -5-*p-anisyl*- | 82 | 171 | -230 | 4.95 (4.98) | 3.84 (3.79) | 0.78 |
| 9 | 5i | -5-α- *naphthyl*- | 94 | 191 | +112 | 4.82 (4.87) | 3.78 (3.71) | 0.86 |

Satisfactory C and H analysis were found in all cases.

## RESULTS AND DISCUSSION

Thus the synthesized novel 1-Hepta-*O*-acetyl-β-D-lactosyl-5-aryl-isothiobiurets exhibits comparable antibacterial and antifungal activities against the organism tested. The method adopted in the synthesis and investigation is simple, efficient and inexpensive in synthesizing pharmacologically important molecules.

**3c .1-Hepta-*O* -acetyl- β -D –lactosyl-5-*p* -tolyl-isothiobiurets : IR (KBr) cm-1:** 3306 (NH stretching), 3021 (Aromatic -H), 1750 (C=O), 1518 (C=N), 1374 (C-N), 669 (C=S), 926 (char. of lactose unit), 760 (para substituted). **$^1$HNMR ($CDCl_3$) δ ppm :** δ 7.276-7.0 (t, 3H, Ar-H), δ 5.466-3.652 (m, 15H, lactose unit), δ 2.338-1.84 (m, 26H , OAc), δ 1.253(s, 1H, $CH_3$ **MS** 826 [M+],717, 733, 675, 503, 478. **$^{13}$CNMR** ($CDCl_3$, 90 MHz) **δ ppm**; δ 171.69-169.29 (C=O), 29.87(OAc), δ 60.95-77.65 (C of lactose ring), δ 89.13 (C=S), δ 130.16-122.58 (Ph-C). (Anal. Calcd. For $C_{35}H_{45}O_{18}N_3S$: C 50.7, H 5.44, O 34.82, N 5.0, S 3.86 Found C 50.3, H 5.40, O 34.77, N 4.97, S 3.94 %).

**3d. 1-Hepta-*O* -acetyl- β -D -lactosyl-5-*m*-chloro-isothiobiurets : IR (KBr) cm-1:** 3356 (NH stretching ), 3019 (Aromatic-H), 1752 (C=O), 1527 (C=N), 1478 (C=N), 669(C=S), 927(char. of lactose unit), 757 (meta substituted). **$^1$HNMR (CDCl3) δ ppm :** δ 7.242-7.0 (s, 1H, Ar-H)), δ 2.140-1.97 (m, 24H, –OAc), δ 6.642 (s, 1H, NH), δ 1.255 (s, NH). **MS** 828 M+, 733, 675, 659. **$^{13}$CNMR** (CDCl3, 90 MH z) ppm δ21.08-20.67 (OAc), δ 77.65-60.80 (carbons of lactosyl ring), δ 185.32 (C=S), δ 169.88-169.30 ( -C=O, 1C), δ 171.47-170.34 (OAc). **(**Anal. Calcd. For $C_{34}H_{42}O_{10}N_3SCl$: C, 48.17, H 4.95, O 34, N 4.95, S 3.77,Cl 4.13 Found C 48.12, H 4.89, O 33.92, N 4.92, S 3.81 Cl 4.10%).

**3i. 1-Hepta-*O*-acetyl- β -D-lactosyl-5- α -naphthyl-isothiobiurets: IR (KBr) cm-1:** 3452 (NH stretchin g), 3022 (Aromatic H), 1750 (C=O), 1553 (C=N),671 (C=S), 758 (para substituted), 925 (char. of lactose unit). **$^1$HNMR (CDCl3) δ ppm :** δ 7.8-7.2 (t, 3H, Ar-H)), δ 2.154-1.972 (m, 23H –OAc), δ 5.357 (s, 1H, NH), δ 1.256 (s, 1H, NH), δ 5.226-3.663 (m, 13H, lactose unit). **$^{13}$CNMR (CDCl3, 90 MHz)** δ 170.60-170.36 (-COCH3), δ 169.59 (-C=O), δ δ 77.65-60.94 (carbons of lactose ring), δ 21.05-20.73 (-COCH3).

(Anal. Calcd. For $C_{38}H_{44}O_{10}N_3S$: C 52.9, H 5.10, O 33.41, N 4.87, S 3.71, Found C 48.12, H 5.5, O 33.78, N 4.82, S 3.78%).

**3f. 1-Hepta-*O*-acetyl- β -D-lactosyl-5-*o*-anisyl-isothiobiurets : IR (KBr) cm-1:** 3414 (NH stretching), 3021 (Aromatic -H), 1750 (C=O), 1527 (C=N),670 (C=S), 760 (ortho substituted), 924 (char. of lactose unit). **$^1$HNMR (CDCl3) δ ppm :** δ 8.123-7.310 (t, 3H, Ar-H)), δ 2.184-1.968 (m, 23H –OAc), δ 6.989-6.866 (t, 3H, NH), δ 5.498-3.652 (m, 17H, lactose unit). **$^{13}$CNMR (CDCl3, 90 MHz)** δ 171.11- 170.07 (-COCH3), δ 169.49 (-C=O), δ 88.97-55.75 (carbons of lactose ring), δ 21.02-20.58 (-COCH3), 152.82-143.96 (R-C=O-NH). (Anal. Calcd. For $C_{35}H_{45}O_{19}N_3S$: C 49.82, H 5.33, O 36.06, N 4.98, S 3.79, Found C 49.79, H 5.28, O 36.0, N 4.96, S 3.85%).

**Antimicrobial Activity:** Newly synthesized isothiobiurets were tested against following pathogenic microbes for their antibacterial and antifungal activities using cup plate agar diffusion method[24-26]. *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. The compounds were taken at a concentration of 1mg/ml using dimethyl sulphoxide as solvent. Gentamycin (100µg $ml^{-1}$) was used as a standard for antibacterial and Nystatin (100µg $ml^{-1}$) as a standard for antifungal activity. Most of the synthesized compounds exhibited mild to moderate anti-microbial activity against the tested microorganisms. Compounds were found to possess significant antibacterial and antifungal activity when compared to standard drug (*Gentamycin and Nystatin* for antibacterial and antifungal respectively).The entire synthesized compounds exhibited mild to moderate activity are shown in **Table 2.**

**Table 2.** Antibacterial and Antifungal Activities of Compounds (3a – i)

| Compounds | Inhibition zone diameter in mm* | | | | | | |
|---|---|---|---|---|---|---|---|
| | Bacteria | | | | | Fungi | |
| | *E. coli* | *P. vulgaris* | *S. aureus* | *P.aeruginosa* | *B. cereus* | *A. niger* | *C. albicans* |
| 3a | 12 | 15 | 13 | 18 | 16 | 14 | 18 |
| 3b | 19 | 12 | 15 | 18 | 17 | 11 | 12 |
| 3c | 13 | 19 | 17 | 13 | 16 | 19 | 14 |
| 3d | 20 | 16 | 12 | 17 | 19 | 18 | 15 |
| 3e | 14 | 19 | 17 | 15 | 19 | 14 | 17 |
| 3f | 18 | 19 | 16 | 14 | 12 | 13 | 19 |
| 3g | 15 | 17 | 19 | 15 | 16 | 20 | 14 |
| 3h | 19 | 12 | 19 | 18 | 14 | 16 | 15 |
| 3i | 12 | 17 | 15 | 16 | 13 | 17 | 20 |
| *Gentamycin* | *19* | *19* | *19* | *19* | *19* | --- | --- |
| *Nystatin* | --- | --- | --- | --- | --- | *20* | *20* |

## APPLICATIONS

The synthesized isothiobiurets showed significant antimicrobial activities and lead for the development of new drugs due to the nature presence of sulphur and nitrogen present in it. The applicability of synthesized compounds is also supported by the various references quoted in the script.

## CONCLUSIONS

In the present research, several novel isothiobiurets were synthesized and screened for their antimicrobial activities. Compounds **IIIb, IIId, IIIe, IIIg and IIIh** were found to possess significant antibacterial and antifungal activity.

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