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Synthesis and Characterization of novel Formamidines, benzothiazolyl formamidines and formimidic acid alkyl ester as potential antimicrobial agent

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ABSTRACT

Formamidines, Thiazoles, dithiazolidines, thiadiazines and other ring system derivatives have a long history of applications in pharmaceutical and agrochemical industries. Our interest in the chemistry of the formamidine nucleus and its derivatives prompted us to explore the synthesis of 1-hepta-*O*-acetyl- β-D-lactopyranosyl-3-H/Aryl formamidines, *N*-hepta-*O*-acetyl- β-D-lactopyranosyl-*N'*-benzothiozolyl formamidines and *N*-hepta-*O*-acetyl-β-D–lactopyranosyl formimidic acid alkyl esters by reductive desulphurization of 1-hepta-*O*-acetyl- β-D–lactopyranosyl–H / aryl thiocarbamides, 1-hepta-*O*-acetyl-β-D-lactopyranosyl-3-[2-*N*-substituted benzothiazolyl] thiocarbamides and 1-hepta-*O*-acetyl-β-D–lactopyranosyl-O-alkyl thiocarbamates respectively using Raney Nickel. The identities of these newly synthesized derivatives have been established on the basis of chemical transformations and IR, ^{1}H NMR and Mass spectral studies. The title compounds have been assayed for their biological activity against gram-positive as well as gram negative microorganisms. These compounds show most promising activity towards these micro-organisms.

Keywords: Synthesis, Lactosylated formamidines and its derivatives, Antimicrobial activity.

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INTRODUCTION

Resistance of pathogenic bacteria towards available antibiotics is rapidly becoming a major worldwide problem, the design of new compounds to deal with resistant bacteria has become one of the most important areas of antibacterial research today. In addition, it is known that antifungal drugs do not have selective activity because of the biochemical similarity between human cell and fungi forms. Therefore there are many studies focused on antibacterial and antifungal compounds[1–3]. Formamidines and its derivatives have been used as a precursor to synthesize some important biologically active heterocycles. Glycosylation of such heterocycles that are rich in biological activity is a field of increasing interest because various glycosylated derivatives display improved biological property with respect to their nonglycosylated counterparts. Beside these formamidines have been found use as antimalarial[4], anti cancer & antitumor activity[5], antifungal agents, anti-inflammatory[6], anti-edema, anti-hypersensitive, Ultra Violet light absorbers[7] and in many other ways.

In view of applications of these compounds some efforts are done to synthesize formamidines and its derivatives. In this communication we report the synthesis of 1-hepta-*O*-acetyl- β-D-lactopyranosyl-3-H/Aryl formamidines **(IIa-l)**, *N*-hepta-*O*-acetyl- β-D-lactopyranosyl-*N'*-benzothiozolyl formamidines **(IVa-f)** and *N*-hepta-*O*-acetyl-β-D–lactopyranosyl formimidic acid alkyl esters **(VIa-g)** by reductive desulphurization of 1-hepta-*O*-acetyl- β-D–lactopyranosyl–H / aryl thiocarbamides **(Ia-l)**, 1-hepta-*O*-acetyl-β-D-lactopyranosyl-3-[2-*N*-substituted benzothiazolyl] thiocarbamides **(IIIa-f)** and 1-hepta-*O*-acetyl-β-D–lactopyranosyl-O-alkyl thiocarbamates **(Va-g)** respectively using Raney Nickel and study their antimicrobial activities against gram-positive as well as gram negative microorganisms.

MATERIALS AND METHOD

Experimental:

Melting points of 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 Model - Agilent Cary 630 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 Agilent 6520 Q-TOF (ESI-HRMS) mass spectrometer. Purity of the compounds was checked by thin layer chromatography using Merck silica gel coated aluminum plates and petroleum ether: ethyl acetate as eluent.

Materials

All solvents and reagents were commercial and used without further purification.

Preparation of 1-hepta-*O*-acetyl- β-D–lactopyranosyl-3-H / aryl thiocarbamides (Ia-l)

1-hepta-*O*-acetyl- β-D–lactopyranosyl-3-H / aryl thiocarbamides were prepared by earlier known method[8] by interaction of 1-hepta-*O*-acetyl- β-D–lactopyranosyl isothiocyanate and aryl amines.

Preparation of Raney Nickel (W-2)

The required raney Nickel (W-2) was prepared by earlier reported method[9] by action of Sodium Hydroxide solution on powdered Ni-Al alloy.

Preparation of 1-hepta-*O*-acetyl- β-D–lactopyranosyl-3-H / aryl formamidines (IIa-l)

To a toluene solution of Raney nickel (W-2; 15.0g in 100ml), the toluene solution of 1-hepta-*O*-acetyl- β-D–lactopyranosyl-3-aryl thiocarbamides (7.70g; 0.01 mol in 50 ml) was added with constant stirring. The reaction mixture was heated gently over heating mantle for 150 min with TLC monitoring. After the completion of reaction the reaction mixture was filtered hot to remove wasted Raney nickel (Nickel sulphide). The solvent was distilled off to afford a semisolid which on triturating with petroleum ether 60-80^0 C for several times afforded faint yellow solid. The latter were then recrystallized from ethyl alcohol to afford pure solid. **(Scheme 1)**

Preparation of 1-hepta-*O*-acetyl-β-D-lactopyranosyl-3-[2-*N*-substituted benzothiazolyl thiocarbamides (IIIa-f)

1-hepta-*O*-acetyl-β-D-lactopyranosyl-3-substituted benzothiazolyl thiocarbamides were prepared by earlier known method[10] by the interaction of 1-hepta-*O*-acetyl-β-D–lactopyranosyl isothiocyanate with 2-amino benzothiazoles / Substituted benzothiazoles.

Preparation of *N*-hepta-*O*-acetyl- β-D-lactopyranosyl-*N'*-benzothiozolyl formamidines (IVa-f)

To a toluene solution of Raney nickel (W-2; 15.0g in 100ml), the solution of 1-hepta-*O*-acetyl-β-D-lactopyranosyl-3-[2-*N*-substituted benzothiazolyl thiocarbamide (8.27g; 0.01 mol in 50 ml) was added with constant stirring. The reaction mixture was heated gently over heating mantle for 4 hours. It was filtered to remove wasted Raney nickel. The solvent was distilled off to afford a semisolid which on triturating with petroleum ether 60-80^o C for several times afforded faint yellow solid. It was recrystallized from ethyl alcohol to afford pure solid. **(Scheme 2)**

Preparation of 1-hepta-*O*-acetyl-β-D–lactopyranosyl-O-alkyl thiocarbamates (Va-g)

1-hepta-*O*-acetyl-β-D–lactopyranosyl-O-alkyl thiocarbamates were prepared by interaction of 1-hepta-*O*-acetyl- β-D–lactopyranosyl isothiocyanate and alkyl alcohols.

Preparation of *N*-hepta-*O*-acetyl-β-D–lactopyranosyl formimidic acid alkyl esters (VIa-g)

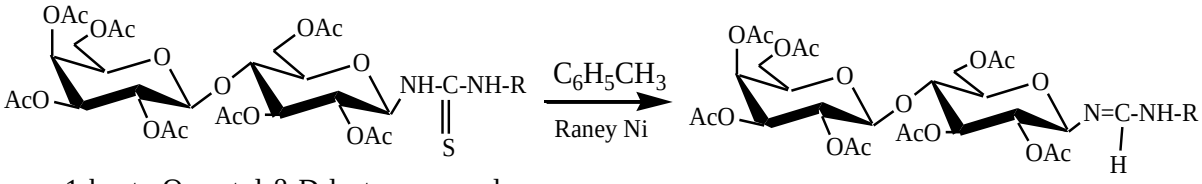
To a toluene solution of 1-hepta-*O*-acetyl- β-D–lactopyranosyl-O-alkyl thiocarbamate (6.77 g; 0.01 mol in 50 ml), solution of Raney nickel (W-2; 15.0 g in 100 ml) was added with constant stirring. The reaction mixture was heated gently over heating mantle for 3 hours with TLC monitoring. The reaction mixture was filtered to remove Raney nickel. The solvent was distilled off to afford a semisolid which on triturating with petroleum ether 60-80^0 C for several times afforded faint yellow solid. The latter were then recrystallized from ethyl alcohol to afford pure solid. **(Scheme 3)**

RESULTS AND DISCUSSION

Herein, we report the synthesis of various lactosyl formamidines **(IIa-l)**, lactosyl benzothiazolyl formamidines **(IVa-f)** and lactosyl formimidic acid alkyl esters **(VIa-g)** by the reductive desulphurization of lactosyl thiocarbamides **(Ia-l)**, lactosyl benzothiazolyl thiocarbamides **(IIIa-f)** and lactosyl thiocarbamates **(Va-g)** using Raney nickel in toluene medium.

All products were crystallized from ethanol before recording the physical data (Table 1). The purity of compounds was checked by TLC. Optical rotation of the product was also recorded**.** The IR, ^{1}H NMR, Mass spectral analysis[10-14] supported the title compounds. The title compounds have been assayed for their biological activity against different microorganisms. These compounds show most promising activity towards these micro-organisms.

Scheme for synthesis shown as follows:

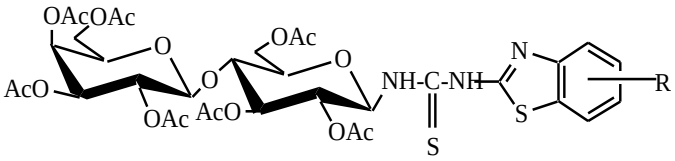


1-hepta-O-acetyl-β-D-lactopyranosyl-3-H / aryl thiocarbamides **(Ia-l)**

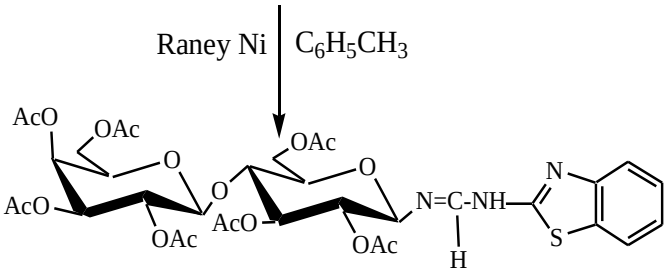
1-hepta-O-acetyl-β-D-lactopyranosyl-3-H / aryl formamidines **(IIa-l)**

Scheme 1

Where, R= a) H; b) Phenyl; c) *o*-tolyl; d) *m*-tolyl; e) *p*-tolyl; f) *o*-Cl-phenyl; g) *m*-Cl-phenyl; h) *o*-methoxy phenyl; i) *m*-methoxy phenyl; j) *p*-methoxy phenyl; k) *m*-OH phenyl; l) *p*-OH phenyl.



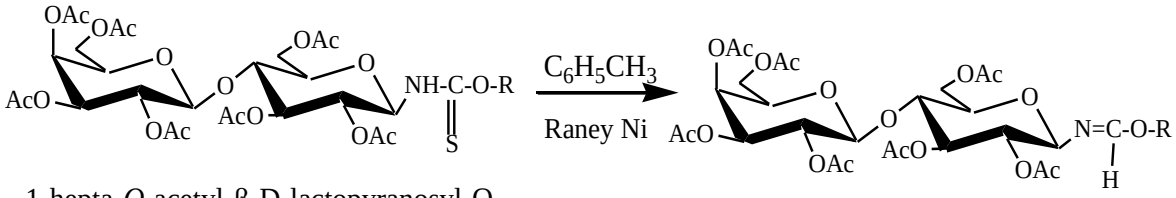
1-hepta-*O*-acetyl-lactopyranosyl 3-[2-*N*-substituted benzothiazolyl thiocarbamides,**(IIIa-f)**



1-hepta-*O*-acetyl-lactopyranosyl-3-substituted benzothiazolyl formamidines, **(IVa-f)**

Scheme-2

Where, R = a) Phenyl, b) o-tolyl, c) p-tolyl, d) o-Cl-phenyl, e) m-Cl-phenyl f) p-Cl-phenyl



1-hepta-*O*-acetyl-β-D-lactopyranosyl-O-alkyl thiocarbamates **(Va-g)**

N-hepta-O-acetyl-β-D-lactopyranosyl formimidic acid alkyl esters **(VIa-g)**

Scheme 3

Where, R= a) methyl, b) ethyl, c) 1-propyl, d) 2-propyl, e) 1-butyl, f) 2-butyl, g) 2-amyl
Ac = $-COCH_3$

Table 1: Physical Characterization of 1-hepta-*O*-acetyl- β-D-lactopyranosyl-3-H/Aryl formamidines (IIa-l), *N*-hepta-*O*-acetyl- β-D-lactopyranosyl-*N'*-benzothiozolyl formamidines (IVa-f) and *N*-hepta-*O*-acetyl-β-D–lactopyranosyl formimidic acid alkyl esters (VIa-g)

Sr.No.	Lactosyl formamidines **(IIa-l)**, lactosyl benzothiazolyl formamidines **(IVa-f)** and lactosyl formimidic acid alkyl esters **(VIa-g)**	Yield (%)	M.P. (^oC)	R_f Value 6:4 EtOAc : Pet. Ether	$[\alpha]_D^{31}$ (c, in $CHCl_3$)	Elemental analysis (%) Found (Required) N
1.	2a	75.24	84	0.67	+26.88	4.21(4.22)
2.	2b	84.08	95	0.91	+45.26	3.38(3.79)
3.	2c	79.25	130	0.56	+86.25	3.61(3.72)
4.	2d	82.36	125	0.93	+58.68	3.65(3.72)
5.	2e	78.02	132	0.48	+63.14	3.68(3.72)
6.	2f	82.01	110	0.86	+53.21	3.60(3.62)
7.	2g	77.69	113	0.66	+72.36	3.58(3.62)
8.	2h	83.63	103	0.89	+66.68	3.62(3.64)
9.	2i	87.26	105	0.84	+68.76	3.60(3.64)
10.	2j	85.39	100	0.68	+86.37	3.57(3.64)
11.	2k	83.04	135	0.74	+56.17	3.68(3.70)
12.	2l	79.08	122	0.63	+44.01	3.65(3.70)
13.	4a	79	155	0.54	+130.43	5.28(5.22)
14.	4b	80	150	0.48	+142.85	5.15(5.19)
15.	4c	82	135	0.85	+113.40	5.12(5.19)
16.	4d	83	140	0.74	-145.83	5.01(5.06)
17.	4e	79	156	0.65	+204.08	5.00(5.06)
18.	4f	81	130	0.73	-161.29	5.03(5.06)
19.	6a	85.24	102	0.84	+53.21	2.01(2.06)
20.	6b	75.08	112	0.68	+72.36	1.97(2.02)
21.	6c	76.25	130	0.74	+86.37	1.92(1.98)
22.	6d	80.36	125	0.67	+56.17	1.95(1.98)
23.	6e	78.02	136	0.91	+44.01	1.88(1.94)
24.	6f	85.01	110	0.56	+66.68	1.90(1.94)
25.	6g	74.69	135	0.93	+68.76	1.86(1.90)

Table-2: Antimicrobial activity of 1-hepta-*O*-acetyl-□□D-lactopyranosyl-3-H/Aryl formamidines (IIa-l), *N*-hepta-*O*-acetyl- β-D-lactopyranosyl-*N'*-benzothiozolyl formamidines (IVa-f) and *N*-hepta-*O*-acetyl-β-D–lactopyranosyl formimidic acid alkyl esters (VIa-g)

Compounds	Antibacterial**			
	E. coli	*S. aureus*	*S.typhi*	*Ps. aeruginosa*
IIa	15	18	17	23
IIb	22	19	16	22
IIc	23	20	17	20
IId	17	12	19	19
IIe	19	16	18	18
IIf	20	20	20	17
IIg	12	14	19	20
IIh	15	17	18	19
IIi	21	15	18	18
IIj	20	16	20	20
IIk	20	22	19	22
III	15	21	20	21
IVa	15	16	16	22
IVb	13	20	16	20
IVc	18	20	18	22
IVd	13	12	17	25
IVe	19	18	20	25
IVf	12	16	17	20
VIa	18	12	16	19
VIb	19	21	17	18
VIc	17	18	16	20
VId	16	19	17	21
VIe	15	17	18	22
VIf	22	16	19	23
VIg	20	19	20	24
Amikacin	25	24	22	27

**Zone of inhibition measured in mm, (15 or less) resistance, (16-20 mm) moderate and (more than 20 mm) sensitive. *Escherichia coli* (*E.* coli), *Staphylococcus aureus* (*S.* aureus), *Salmonella typhi* (*S. typhi*), *Psudomonas aeruginosa* (*Ps. aeruginosa*).

Spectral Analysis:

IIb) 1-hepta-*O*-acetyl- β-D-lactopyranosyl-3-phenyl formamidines:

IR(KBr cm^{-1}): 3407 (N-H), 3023 (Aromatic C-H), 1749 (C=O), 1656 (C=N), 1373 (C-N), 1223 (C-O), 1056 (Characteristics of lactose); **^{1}H NMR ($CDCl_3$):** δ 7.3-7.0 (5H, m, aromatic protons), 6.9 (1H, s, NH), 5.2-3.7 (14H, m, lactosyl protons), 2.1-2.0 (21H,m, acetyl protons), 1.9 (1H, s, C-H proton); **Mass (*m/z*)** 739 ($M^{+\cdot}$+1), 619, 559, 331 (base peak), 168.

IVa) *N*-hepta-*O*-acetyl- β-D-lactopyranosyl-*N'*-benzothiozolyl formamidines:

IR(KBr cm^{-1}): ν 3415 (N-H), 3011 (Aromatic C-H), 2960 (Aliphatic C-H), 1745 (C=O), 1512 (C=N), 1370 (C-N), 1225 (C-O), 1056 (Characteristics of lactose), 601 (C-S); **^{1}H NMR (CDCl$_3$):** δ 7.2-7.0 (4H, m, Ar-H), 5.3-3.7 (14H, m, lactosyl protons), 2.1-2.0 (21H, m, acetyl protons), 1.9 (1H, s, C-H proton), 0.8 (1H, s, N-H proton); **Mass (*m/z*):** 796 ($M^{+\cdot}$+1), 619, 559, 331, 229, 169, 127, 109.

VIa) *N*-hepta-*O*-acetyl-β-D–lactopyranosyl formimidic acid methyl esters:

IR(KBr cm^{-1}): ν 2960 (Aliphatic C-H), 1745 (C=O), 1512 (C=N), 1370 (C-N), 1225 (C-O), 1056 (Characteristics of lactose); **^{1}H NMR (CDCl$_3$):** δ 5.2-3.8 (14H, m, lactosyl protons), 2.1-2.0 (21H, m, acetyl protons), 1.9 (1H, s, C-H proton), 1.2 (3H, s, methyl protons) ; **Mass (*m/z*):** 678 ($M^{+\cdot}$+1), 619, 559, 331, 229, 169, 127, 109.

Antimicrobial activity:

All the compounds have been screen for both antbacteriall activity using cup plate agar diffusion method[15-16] 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. The compounds were screen for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi species* by using Nutrient 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 hours for antibacterial activities. 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 all compounds exhibited most significant activity against *Ps. aeruginosa* and *S. typhi*. IIb, IIc, IIe, IIf, Iii, IIj, IIk, IVc, IVe, VIa, VIb, VIf and VIg shows appreciable activity towards *E. coli*. IIa, IIb, IIc, IIf, IIk, IIl, IVb, IVc, IVe, VIb, VIc, VId and VIg are effective towards *S. aureus*. All the other compounds exhibited low to moderate activity.

CONCLUSION

N-Substituted lactopyranosyl formamidines, lactosyl benzothiazolyl formamidines and lactosyl formimidic acid alkyl esters derivatives were synthesized and characterized for their structure

elucidation. Various chemical and spectral data supported the structures. The method adopted in this investigation is simple, efficient and inexpensive. Thus, the newly synthesized title compounds, exhibits comparable antibacterial activities against the organisms tested. So these compounds due to their appreciable activity towards these micro-organisms are biologically active and useful in the future for the preparation of pharmacologically important derivatives.

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REFERENCES

1. Gold H S, Moellering R C, Engl N J Med 1996; 335: 1445.
2. Bossche V H, Marichal P, Odds F C, Trends Microbial 1994; 2: 393.
3. Cohen M L, Science 1992; 257: 1050.
4. Sandrine D, Sophie G, Fouad DA, Louis M, Philippe G, Christian S, Bull 2001; 49(8): 933-37.
5. Jolanta P, Jolanta T, Edward B, Magorzata W, Irena O, C. RW, Acta Biochimica polonica 2000; 47: 141-47.
6. Jack RD, Sunnyvale, Ferenc MP, Walnut C, Eugene GT, El Cerrito; U.S. Pat. No.3; 959: 368.
7. Joseph AV, Wayne, Emanuel H, Fairfield, Isaac DC, U.S. Pat. No.5; 243: 055.
8. Tale PV, Deshmukh SP, Heteroatom Chemistry 2006; 17(4): 306-09.
9. Ralph M, Org Synth 1941; 21: 15.
10. Silverstein RM, Webster FX. Spectrometric identification of organic compounds, 6th ed. New York (NY): John Wiley and Sons Inc; 2011.
11. Williams DH, Fleming I. Spectroscopic methods in organic chemistry. 5th ed. Tata McGraw-Hill; 2004.
12. Dyer JR. Applications of absorption spectroscopy of organic compounds. New Delhi: PHI Learning Private Limited; 2010.
13. Verma R, Kulkarni SY, Jose CI, Pansave VS. Carbohydr Res 1984;25:133.
14. Colthup NB, Daly LH, Weberley SE. Introduction of infrared and raman spectroscopy. New York (NY): Academic Press; 1964.

15. Kawangh F. Analytical microbiology. New York (NY): Academic press; 1963.
16. British pharmacopoeia-II. Biological assay and tests. London: The Stationary Office Ltd.; 1998;A-205.