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Synthesis and biological studies of unsymmetrical mannopyranosyl thiocarbamides

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ABSTRACT

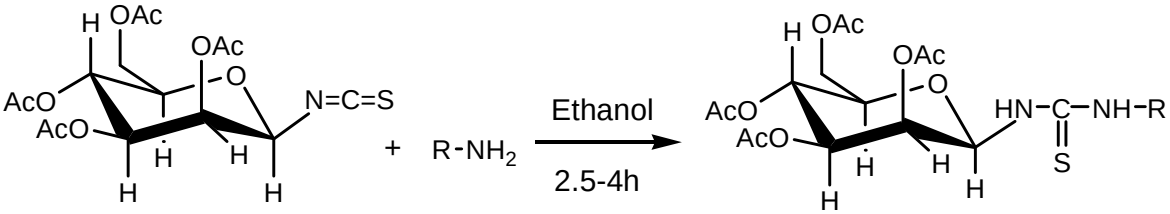
Synthesis of unsymmetrical N,N'-disubstituted Mannopyranosyl thiocarbamides by the interaction of N-tetra-O-acetyl-β-D-Mannopyranosyl isothiocyanate and free amines in ethanol. The polar solvent which increases the rate of reaction which was monitored by TLC while, the yield measured after completion of reaction with column chromatography. These compounds were screened for their antibacterial and antifungal activity against E. coli, S. aureus, P. vulgaris, Pseudomonas, Bacillus, S. typhi, A. niger and Fusarium.The identities of these newly synthesized compounds were established on the basis of elemental analysis UV, IR, ^{1}H NMR, ^{13}C NMR & Mass spectral studies.

Keywords. Synthesis, Characterization, Mannopyranosyl isothiocyanate & thiocarbamides.

INTRODUCTION

Symmetric and unsymmetric thiocarbamides have been reported and tested for their biological activity. The conventional methods reported for thiocarbamides synthesis are essentially based on the reaction of amines with isothiocyanate[1-3]. In our own effort to develop and synthesized the some new N,N'-disubstituted unsymmetric thiocarbamides having Mannosyl moiety. Unsymmetric N,N'-disubstituted mixed thiocarbamides are found as main product in the reaction involving mannopyranosyl isothiocyanate **1** with free aliphatic & aromatic amines (**2a-h**). Here nucleophilic addition of free amines to the heterocumulated carbon atom is a main decomposition route of isothiocyanates[4] (scheme -1). In further investigation, we discovered that the yield of unsymmetric thiocarbamides significantly increased during the attempted coupling between mannopyranosyl isothiocyanate & free amines as reactive

nucleophiles[5-6]. In this investigation no products arising from O→N acyl migration were detected but different mechanistic pathway in the reaction mixture containing mannopyranosyl isothiocyanate & amines. In view of this we recently synthesized newer types of several N-tetra-O-acetyl-β-D-Mannopyranosyl-3-substituted thiocarbamides by the interaction of N-tetra-O-acetyl-β-D-Mannopyranosyl isothiocyanate & free amines. These compounds were screened for their antibacterial and antifungal activity against *E. coli, S. aureus, P. vulgaris, Pseudomonas, Bacillus, S. typhi, A. niger and Fusarium*[**7**].



1 **2a-h** **3a-h**

Where,

R= a) H b) c) d) e) f) g) h)

Ac = CH_3-CO- (Acetyl Group)

Scheme -1

EXPERIMENTAL SECTION

4.1. General Methods.

Optical rotations $[\alpha]_D$ were measured on a EQUIP-TRONICS Digital Polarimeter model no. EQ 800 at 29 ^{0}C in $CHCl_3$. UV spectra were recorded on a UV-VIS Spectrophotometer 117 (λmax 400-240 nm in $CHCl_3$). IR spectra were recorded on a Perkin Elmer spectrum RXI (4000-450 cm^{-1}) FTIR Spectrophotometer. ^{1}H NMR was obtained on a Bruker DRX-300 (300 MHz FT NMR) NMR Spectrometer in $CDCl_3$ with TMS as an internal reference. The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer / date system using argon / xenon (6Kv, 10 mA) as the FAB gas. The accelerating voltage was 10 Kv, and the spectra were recorded at room temperature. m-Nitrobenzyl alcohol (NBA) was used as matrix unless specified otherwise. TLC was performed with E. Merck precoated TLC plates, aluminium silica Gel_{60} $F_{254,}$ with visualization by Iodine vapour and by charring with UV light or 10% H_2SO_4 & data was shown in Table - III. The compounds were screened for their antibacterial and antifungal activities by cup-plate method.

4.1.1 N-Tetra-O-acetyl-β-D-Mannopyranosyl isothiocyanate (1).

N-tetra-O-acetyl-β-D-Mannopyranosyl isothiocyanate (**1**) was prepared by the condensation of N-tetra-O-acetyl-β-D-Mannopyranosyl Bromide (0.01M, 4.11g) and lead thiocyanate (0.005M, 1.615g) in boiling sodium dried xylene (20 mL) for 3h while reaction monitoring by TLC. Lead Bromide that formed was filtered and solvent was distilled off and the resultant sticky residue triturated several times with petroleum ether to afford the syrupy semisolid mass. The products were separated as semisolid by column chromatography using 1:1 EtOAc/CCl_4. The mannopyranosyl isothiocyanate was desulphurised by alkaline plumbite test & it also gives positive test of isothiocyanate.

Yield, 2.25g (57.84%), mp 86-88^0(d), $[\alpha]_D^{29}$ -72^0 (c, 0.921 in $CHCl_3$), R_f, 0.66 (1:1; CCl_4: EtOAc). UV ($CHCl_3$), λmax, 264.5 nm. IR υmax cm^{-1} ($CHCl_3$), 2965, 2137, 2015, 1750, 1378, 1214, 1085. ^{1}H NMR ($CDCl_3$), δ= 5.69-4.14(m, 5H, Man. ring H_1-H_5), 4.07(s, 2H, O-CH_2-), 2.17(s, 12H, 4 –$COCH_3$). ^{13}C NMR ($CDCl_3$), δ= 171.3, 170.3, 169.0, 168.5(C=O), 146.2(S=C=N), 82.0(C-1), 75.1(C-5), 70.6(C-3), 69.3(C-2), 65.5(C-4), 62.5(C-4), 20.5($COCH_3$). FABMS (m/z), 389(M^+), 331(M – N=C=S). "Anal. Calcd. for $C_{15}H_{19}NO_9S$: C, 46.27; H, 4.88; N, 3.59; S, 8.22. Found: C, 46.03; H, 4.79; N, 3.42; S, 7.98".

4.1.2. N-Tetra-O-acetyl-β-D-Mannopyranosyl-3-(H) thiocarbamide (3a).

N-Tetra-O-acetyl-β-D-Mannopyranosyl-3-(H) thiocarbamide (3a) was prepared by the condensation of N-tetra-O-acetyl-β-D-Mannopyranosyl isothiocyanate (**1**, 0.01M, 3.89 g) and ammonia (**2a**, 0.01M, 0.51 g) in boiling ethanol (25 mL) for 2.5h while monitoring reaction by TLC. The solvent was distilled off and the resultant sticky residue triturated several times with petroleum ether to afford the syrupy semisolid mass (3a). The products were separated as semisolid by column chromatography using 1:1 EtOAc / Hexane as eluent & the product was desulphurised by alkaline plumbite test. UV ($CHCl_3$), λmax, 262.0 nm. IR υmax cm^{-1} ($CHCl_3$), 3450, 3380, 2980, 2885, 1753, 1220, 1185, 1037. ^{1}H NMR ($CDCl_3$), δ= 7.50(s, 2H, NH_2), 7.42(br,s, 1H, NH), 5.70-4.15(m, 5H, Man. ring H_1-H_5), 4.20(s, 2H, O-CH_2-), 2.15(s, 12H, 4 –$COCH_3$). ^{13}C NMR ($CDCl_3$), δ= 182.7(C=S), 170.2, 169.0, 168.5, 167.9(C=O), 80.2(C-1), 72.7(C-5), 72.0(C-6), 71.9(C-2), 70.7(C-4), 68.1(C-3), 20.2($COCH_3$). FABMS (m/z), 406(M^+), 331(M – N=C=S). "Anal. Calcd. for $C_{15}H_{22}N_2O_9S$: C, 44.33; H, 5.41; N, 6.89; S, 7.88. Found: C, 44.03; H, 4.27; N, 6.55; S, 7.61".

The several other extending products (3b-h) were unequivocally identified by spectroscopic comparison UV, IR, ^{1}H NMR, ^{13}C NMR & Mass spectral studies.

4.1.3. N-Tetra-O-acetyl-β-D-Mannopyranosyl-3-Phenyl thiocarbamide (3b).

UV ($CHCl_3$), λmax, 265 nm. IR υmax cm^{-1} ($CHCl_3$), 3450, 3010, 2980, 2887, 1751, 1225, 1182, 1032, 710. ^{1}H NMR ($CDCl_3$), δ= 7.48(m, 5H, Ar-H), 7.12(br,s, 2H, 2NH), 5.55-4.13(m, 5H, Man. ring H_1-H_5), 4.02(s, 2H, O-CH_2), 2.07(s, 12H, 4–$COCH_3$). ^{13}C NMR ($CDCl_3$), δ= 179.3(C=S), 171.0, 170.8, 168.7, 168.0(C=O), 137.2(Ph, C-1), 130.0(Ph, C-2,6), 129.2(Ph, C-3,5), 127.1 (Ph, C-4), 79.7(C-1), 73.4(C-5), 72.5(C-6), 70.9(C-2), 69.2(C-4), 68.8(C-3), 20.9($COCH_3$). FABMS (m/z), 482(M^+), 405(M - C_6H_5), 390(M - C_6H_5-NH), 331(M – $C_7H_7N_2S$). "Anal. Calcd. for $C_{21}H_{26}O_9N_2S$: C, 52.28; H, 5.39; N, 5.80; S, 6.63. Found: C, 52.02; H, 5.02; N, 5.51; S, 6.29".

4.1.4. N-Tetra-O-acetyl-β-D-Mannopyranosyl-3-o-tolyl thiocarbamide (3c).

UV ($CHCl_3$), λmax, 259 nm. IR υmax cm^{-1} ($CHCl_3$), 3451, 3011, 2980, 2885, 1750, 1225, 1180, 1031, 770. ^{1}H NMR ($CDCl_3$), δ= 7.42(m, 4H, Ar-H), 7.25(br,s, 2H, 2NH), 5.60-4.32(m, 5H, Man. ring H_1-H_5), 4.26(s, 2H, O-CH_2), 2.29(s, 3H, Ar-CH_3), 2.09(s, 12H, 4–$COCH_3$). ^{13}C NMR ($CDCl_3$), δ= 180.1(C=S), 169.7, 169.0, 168.0, 167.2(C=O), 138.2(Ph, C-1), 139.3(Ph, C-2,6), 130.1(Ph, C-3,5), 129.1 (Ph, C-4), 80.1(C-1), 73.3(C-5), 71.1(C-6), 70.7(C-2), 68.3(C-4), 67.6(C-3), 21.0($COCH_3$). FABMS (m/z), 496(M^+), 481(M – CH_3), 405(M - C_6H_4-CH_3), 331(M – $C_8H_9N_2S$). "Anal. Calcd. for $C_{22}H_{28}N_2O_9S$: C, 53.22; H, 5.64; N, 5.64; S, 6.45. Found: C, 52.99; H, 5.36; N, 5.32; S, 6.08".

4.1.5. N-Tetra-O-acetyl-β-D-Mannopyranosyl-3-m-tolyl thiocarbamide (3d).

UV ($CHCl_3$), λmax, 245 nm. IR υmax cm^{-1} ($CHCl_3$), 3445, 3015, 2985, 2885, 1755, 1226, 1182, 1030, 801. ^{1}H NMR ($CDCl_3$), δ= 7.39(br,s, 2H, 2NH), 7.25-7.12(m, 4H, Ar-H), 5.66-4.39(m, 5H, Man. ring H_1-H_5), 4.21(s, 2H, O-CH_2), 2.25(s, 3H, Ar-CH_3), 2.14(s, 12H, 4–$COCH_3$). ^{13}C

NMR ($CDCl_3$), δ= 181.5(C=S), 173.2, 171.8, 170.5, 168.0(C=O), 137.7(Ph, C-1), 136.7(Ph, C-2,6), 131.2(Ph, C-3,5), 129.8 (Ph, C-4), 78.7(C-1), 75.1(C-5), 72.1(C-6), 71.6(C-2), 69.7(C-4), 68.9(C-3), 20.3($COCH_3$). FABMS (m/z), 496(M^+), 405(M - C_6H_4-CH_3), 331(M – $C_8H_9N_2S$). "Anal. Calcd. for $C_{22}H_{28}N_2O_9S$: C, 53.22; H, 5.64; N, 5.64; S, 6.45. Found: C, 52.82; H, 5.29; N, 5.33; S, 6.07".

4.1.6. N-Tetra-O-acetyl-β-D-Mannopyranosyl-3-p-tolyl thiocarbamide (3e).

UV ($CHCl_3$), λmax, 261 nm. IR υmax cm^{-1} ($CHCl_3$), 3451, 3012, 2980, 2880, 1751, 1225, 1183, 1039, 796. ^1H NMR ($CDCl_3$), δ= 7.41-7.30(m, 4H, Ar-H), 7.16(br,s, 2H, 2NH), 5.47-4.35(m, 5H, Man. ring H_1-H_5), 4.22(s, 2H, O-CH_2), 2.27(s, 3H, Ar-CH_3), 2.10(s, 12H, 4–$COCH_3$). ^{13}C NMR ($CDCl_3$), δ= 181.7(C=S), 170.5, 169.2, 168.7, 167.3(C=O), 139.7(Ph, C-1), 138.2(Ph, C-2,6), 131.7(Ph, C-3,5), 130.0 (Ph, C-4), 81.7(C-1), 76.7(C-5), 72.9(C-4), 71.2(C-2), 68.5(C-3), 67.7(C-6), 20.8($COCH_3$). FABMS (m/z), 496(M^+), 405(M - C_6H_4-CH_3), 331(M – $C_8H_9N_2S$). "Anal. Calcd. for $C_{22}H_{28}N_2O_9S$: C, 53.22; H, 5.64; N, 5.64; S, 6.45. Found: C, 52.89; H, 5.29; N, 5.39; S, 6.15".

4.1.7. N-Tetra-O-acetyl-β-D-Mannopyranosyl-3-o-Chlorophenyl thiocarbamide (3f).

UV ($CHCl_3$), λmax, 275 nm. IR υmax cm^{-1} ($CHCl_3$), 3430, 3060, 2980, 2886, 1735, 1224, 1185, 1031, 772. ^1H NMR ($CDCl_3$), δ= 7.52(br,s, 2H, 2NH), 7.45-7.32(m, 4H, Ar-H), 5.50-4.32(m, 5H, Man. ring H_1-H_5), 4.25(s, 2H, O-CH_2), 2.15(s, 12H, 4–$COCH_3$). ^{13}C NMR ($CDCl_3$), δ= 181.4(C=S), 170.9, 170.0, 169.1, 168.0(C=O), 139.7(Ph, C-1), 137.7(Ph, C-2), 135.0(Ph, C-6), 134.2 (Ph, C-4),131.2(Ph, C-3,5), 80.2(C-1), 74.1(C-5), 72.8(C-6), 70.2(C-2), 68.7(C-4), 67.6(C-3), 21.6($COCH_3$). FABMS (m/z), 516(M^+), 405(M - C_6H_4-Cl), 331(M – $C_7H_6N_2SCl$). "Anal. Calcd. for $C_{21}H_{25}ClN_2O_9S$: C, 48.78; H, 4.84; Cl, 6.87; N, 5.42; S, 6.19. Found: C, 48.42; H, 4.44; Cl, 6.55; N, 5.07; S, 5.85".

4.1.8. N-Tetra-O-acetyl-β-D-Mannopyranosyl-3-m-Chlorophenyl thiocarbamide (3g).

UV ($CHCl_3$), λmax, 268 nm. IR υmax cm^{-1} ($CHCl_3$), 3427, 3045, 2975, 2889, 1730, 1212, 1175, 1029, 804. ^1H NMR ($CDCl_3$), δ= 7.49(br,s, 2H, 2NH), 7.41-7.17(m, 4H, Ar-H), 5.52-4.34(m, 5H, Man. ring H_1-H_5), 4.15(s, 2H, O-CH_2), 2.11(s, 12H, 4–$COCH_3$). ^{13}C NMR ($CDCl_3$), δ= 180.9(C=S), 172.1, 171.3, 170.2, 169.1(C=O), 139.0(Ph, C-1), 138.1(Ph, C-3), 137.0(Ph, C-5), 135.5(Ph, C-2,6), 131.0 (Ph, C-4), 82.1(C-1), 74.0(C-5), 70.9(C-6), 69.3(C-2), 68.0(C-4), 67.2(C-3), 21.2($COCH_3$). FABMS (m/z), 516(M^+), 405(M - C_6H_4-Cl), 331(M – $C_7H_6N_2SCl$). "Anal. Calcd. for $C_{21}H_{25}ClN_2O_9S$: C, 48.78; H, 4.84; Cl, 6.87; N, 5.42; S, 6.19. Found: C, 48.62; H, 4.59; Cl, 6.49; N, 5.13; S, 5. 98".

4.1.9. N-Tetra-O-acetyl-β-D-Mannopyranosyl-3-p-Chlorophenyl thiocarbamide (3h).

UV ($CHCl_3$), λmax, 259 nm. IR υmax cm^{-1} ($CHCl_3$), 3399, 3051, 2981, 2884, 1735, 1217, 1181, 1033, 805. ^1H NMR ($CDCl_3$), δ= 7.50(br,s, 2H, 2NH), 7.39-7.24(m, 4H, Ar-H), 5.45-4.44(m, 5H,Man. ring H_1-H_5), 4.19(s, 2H, O-CH_2), 2.07(s, 12H, 4–$COCH_3$). ^{13}C NMR ($CDCl_3$), δ= 179.7(C=S), 171.6, 170.0, 169.1, 167.1(C=O), 138.1(Ph, C-1), 137.1(Ph, C-4), 135.4(Ph, C-2,6), 131.7(Ph, C-3,5), 80.7(C-1), 76.8(C-5), 72.0(C-6), 70.2(C-2), 69.2(C-4), 67.2(C-3), 21.0($COCH_3$). FABMS (m/z), 516(M^+), 405(M - C_6H_4-Cl), 331(M – $C_7H_6N_2SCl$). "Anal. Calcd. for $C_{21}H_{25}ClN_2O_9S$: C, 48.78; H, 4.84; Cl, 6.87; N, 5.42; S, 6.19. Found: C, 48.51; H, 4.46; Cl, 6.61; N, 5.09; S, 5.97".

Table - I

Compds	*E. coli (mm)*	*S. aureus (mm)*	*P. Vulgaris (mm)*	*Pseudomonas (mm)*	*Bacillus (mm)*	*S. typhi (mm)*
3a	9.0	9.3	9.5	8.8	8.5	9.7
3b	8.5	9.0	8.7	8.5	8.2	9.5
3c	7.2	6.9	6.6	6.2	6.4	7.5
3d	6.5	6.4	6.1	6.1	6.7	7.1
3e	9.1	8.2	8.8	8.3	8.0	8.5
3f	7.5	6.7	7.7	7.1	6.0	6.5
3g	5.2	5.9	5.3	5.5	4.9	4.4
3h	9.7	8.7	9.2	9.0	8.8	8.7
Co-trimazine	11.2	11.8	10.7	10.3	10.5	10.9

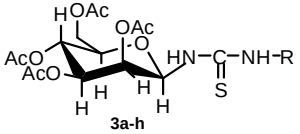
**8.0 mm & above – Highly active, 6.0 to 7.9 mm- Moderately active & Below 6.0 mm- Less active*

Table - II

Compounds	*A. Niger (mm)*	*Fusarium (mm)*
3a	8.7	8.0
3b	9.0	8.2
3c	7.0	6.5
3d	9.2	8.5
3e	8.5	9.0
3f	7.2	6.9
3g	6.5	7.1
3h	8.3	8.7
Griseofulvin	10.2	10.5

**8.0 mm & above – Highly active, 6.0 to 7.9 mm- Moderately active & Below 6.0 mm- Less active*

Table – III: Synthesis of N-tetra-O-acetyl-β-D-Mannopyranosyl-3-substituted thiocarbamides

S.No	R-NH_2 2a-h (g)	Reaction Time (h)	 3a-h	M.P. ^{O}C	Yield g, (%)	$[\alpha]_D^{29}$ (c)	Rf (EtOAc / Hexane)
1.	2a, R = H (0.51)	2.5	3a, R= NH_2	112-116^0(d)	3.30 (81.28)	+37.2^0 (0.879)	0.54 (1:1)
2.	2b, R =  (0.93)	2.5	3b, R= 	127-131^0(d)	3.65 (75.72)	+27.7^0 (0.821)	0.47 (1:1.5)
3.	2c, R =  (1.07)	3.15	3c, R= 	117-121^0(d)	3.15 (63.50)	+38.3^0 (0.789)	0.77 (1:2)
4.	2d, R =  (1.07)	3.45	3d, R= 	124-128^0(d)	2.75 (55.44)	-12.4^0 (0.812)	0.68 (1:1.5)
5.	2e, R =  (1.07)	2.75	3e, R= 	132-137^0(d)	3.80 (76.61)	+37.0^0 (0.931)	0.44 (1:1)
6.	2f, R=  (1.27)	3.25	3f, R= 	142-147^0(d)	3.15 (61.04)	+9.1^0 (0.686)	0.37 (1:1.5)
7.	2g, R =  (1.27)	4.0	3g, R= 	150-154^0(d)	2.65 (51.35)	-7.7^0 (0.876)	0.49 (1:2)
8.	2h, R =  (1.27)	3.20	3h, R= 	146-151^0(d)	3.25 (62.98)	-12.4^0 (0.819)	0.21 (1:2.5)

Note:- Reactant- N-Tetra-O-acetyl-β-D-Mannopyranosyl isothiocyanate (1, 0.01M, 3.89g)

RESULT AND DISCUSSION

N-Tetra-O-acetyl-β-D-Mannopyranosyl-3-substituted thiocarbamides (**3a-h**) were synthesized by the condensation of N-tetra-O-acetyl-β-D-Mannopyranosyl isothiocyanate (**1**) and free amines (**2a-h**) in ethanol, while monitoring the reaction by TLC. Formation of the N,N'-disubstituted Mannopyranosyl thiocarbamides requires heating for 2.5-4 h. The solvent was distilled off and the resultant sticky residue was triturated several times with petroleum ether to afford the syrupy semisolid mass (**3a-h**). The products were separated as semisolid by column chromatography using 1:1 EtOAc / Hexane. The characterization of products (3a-h) were established by UV, IR, ^{1}H NMR, ^{13}C NMR & Mass spectral studies[3,8].

3. Microbial Activity

3.1 Antibacterial activity

All the compounds were screened for their antibacterial activities against various pathogenic bacteria such as *E. coli, S. aureus, P. vulgaris, Pseudomonas, and Bacillus, S. typhi,* by cup-plate method at concentration 100 μgmL^{-1} in DMF by using standard Co-trimazine (25 μgmL^{-1}) for bacteria. Amongst the compounds tested for antibacterial activity, compounds **3a, 3b 3e & 3h** were highly active and compounds **3c, 3d & 3f** were moderately active while compound **3g** was less active (Table-I).

3.2 Antifungal activity.

All the compounds were also screened for their antifungal activities by cup-plate method at a concentration at 100 μgmL^{-1} in DMF by using standard Griseofulvin (10 μgmL^{-1}) against *A. niger* and *Fusarium.* The compounds **3a, 3b, 3d, 3e** & **3h** were highly active while compounds **3c, 3f & 3g** were moderately active (Table – II).

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