SYNTHESIS, CHARACTERIZATION AND MICROBIAL ASSAY OF GLYCOSYL SUPHANILAMIDES

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Abstract:
1-glycosyl-3-sulphanilamido thiocarbamides have been synthesized by the interaction of various 1- glycosyl isothiocyanantes with sulphanilamide which on further interaction with aryl isothiocyanates gives 1- glycosyl -3-(1-sulphanilamido-3-aryl thiocarbamide) thiocarbamides. The identities of these new compounds have been established on the basis of chemical transformations and spectral studies. In the present investigation the *In-vitro* bacterial assay of compounds has been evaluated by using several bacteria such as *Escherichia coli, Staphylococcus aureus, S. Typhi* and *Pseudomonas aeruginosa.* All compounds studied shows satisfactory bacterial assay.
Key words: Glycosyl, Sulphanilamides, Aryl isothiocyanate, Thiocarbamide Microbial assay.

Introduction:-
Sulfanilamide is a sulfonamide antibacterial. Chemically, it is an organic compound consisting of an aniline derivatized with a sulfonamide group[1]. Modern antibiotics have supplanted sulfanilamide on the battlefield; however, sulfanilamide remains in use for treatment of vaginal yeast infections.

As a sulfonamide antibiotic, sulfanilamide functions by competitively inhibiting (i.e., by acting as a substrate analogue) enzymatic reactions involving para-aminobenzoic acid (PABA)[2]. PABA is needed in enzymatic reactions that produce folic acid, which acts as a coenzyme in the synthesis of purines and pyrimidines. Mammals do not synthesize their own folic acid so are unaffected by PABA inhibitors, which selectively kill bacteria.

Carbohydrate derivatives have been extensively investigated, including synthesis, characterization and biological activity, partly due to facts that many naturally occurring saccharides and synthesized analogues exhibit various and potent biological activities like anti-inflammatory, analgesic, fungicidal, herbicidal and pesticide agents[6-8] and have been widely employed as agrochemicals and pharmaceuticals[9-11].

To investigate the chemistry of new related compounds with reference to their synthetic applications towards glycosyl compounds in medical chemistry and in many other ways. It appeared quite interesting to carry out the synthesis of following *N*-glycosylated sulphanilamide containing compounds. 1-glycosyl-3-sulphanilamido thiocarbamide (3a-b) have been synthesized by the interaction of 1-glycosyl isothiocyanante (1) with sulphanilamide (2) which on further interaction with aryl isothiocyanates (4) gives 1- glycosyl -3-(1-sulphanilamido-3-aryl thiocarbamide) thiocarbamides (5a-d).

Results and Discussion:-
Synthesis of 1-Hepta-O-acetyl- β -D-lactosyl-3-(1-sulphanilamido-3-aryl thiocarbamide) thiocarbamides (5a-b)

Condensation of 1-Hepta-*O*-acetyl- β -D-lactosyl-3-isothiocyanate (1) with Sulphanilamide (2) gives 1-Hepta-*O*-acetyl- β -D-lactosyl-3-sulphanilamido thiocarbamide (3a) which on further reaction with aryl isothiocyanates (4) gives1-Hepta-*O*-acetyl- β -D-lactosyl-3-(1-sulphanilamido-3-aryl thiocarbamide) thiocarbamides (5a-b). The solvent was distilled off. The sticky masses obtained were triturated several times with

petroleum ether (60-80 ^{o}C) afforded a as granular solid. It was purified by ethanol-water.

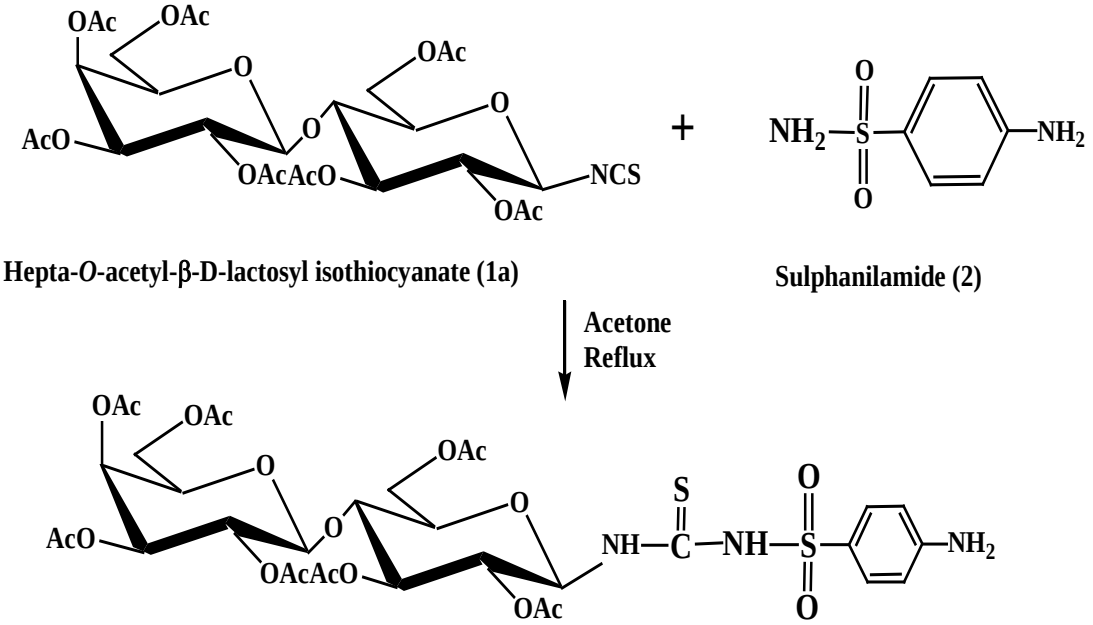
Synthesis of 1-Hepta-O-benzoyl- β -D-maltosyl -3-(1-sulphanilamido-3-aryl thiocarbamide) thiocarbamides (5c-d)

Condensation of 1- Hepta-*O*-benzoyl- β -D-maltosyl -3- isothiocyanate (1) with Sulphanilamide (2) gives 1- Hepta-*O*-benzoyl- β -D-maltosyl -3-sulphanilamido thiocarbamide (3b) which on further reaction with aryl isothiocyanates (4) gives1- Hepta-*O*-benzoyl- β -D-maltosyl -3-(1-sulphanilamido-3-aryl thiocarbamide) thiocarbamides (5c-d). The solvent was distilled off. The sticky masses obtained were triturated several times with petroleum ether (60-80 ^{o}C) furnishes as a granular solid. It was purified by ethanol-water.

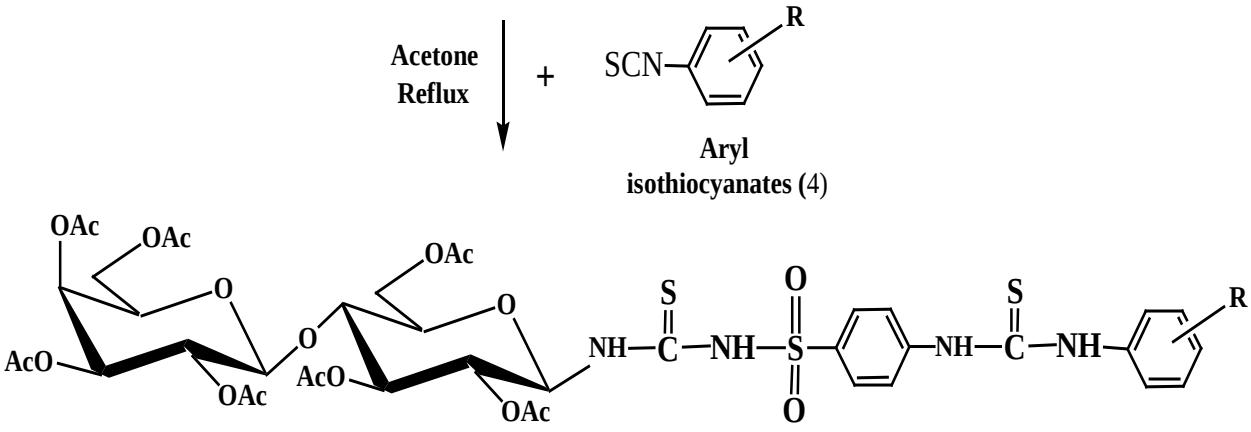
The IR, ^{1}H NMR and Mass[12-15] spectral analysis and elemental analysis indicate the products and design the structures of the compounds.

Schemes:

Scheme 1:



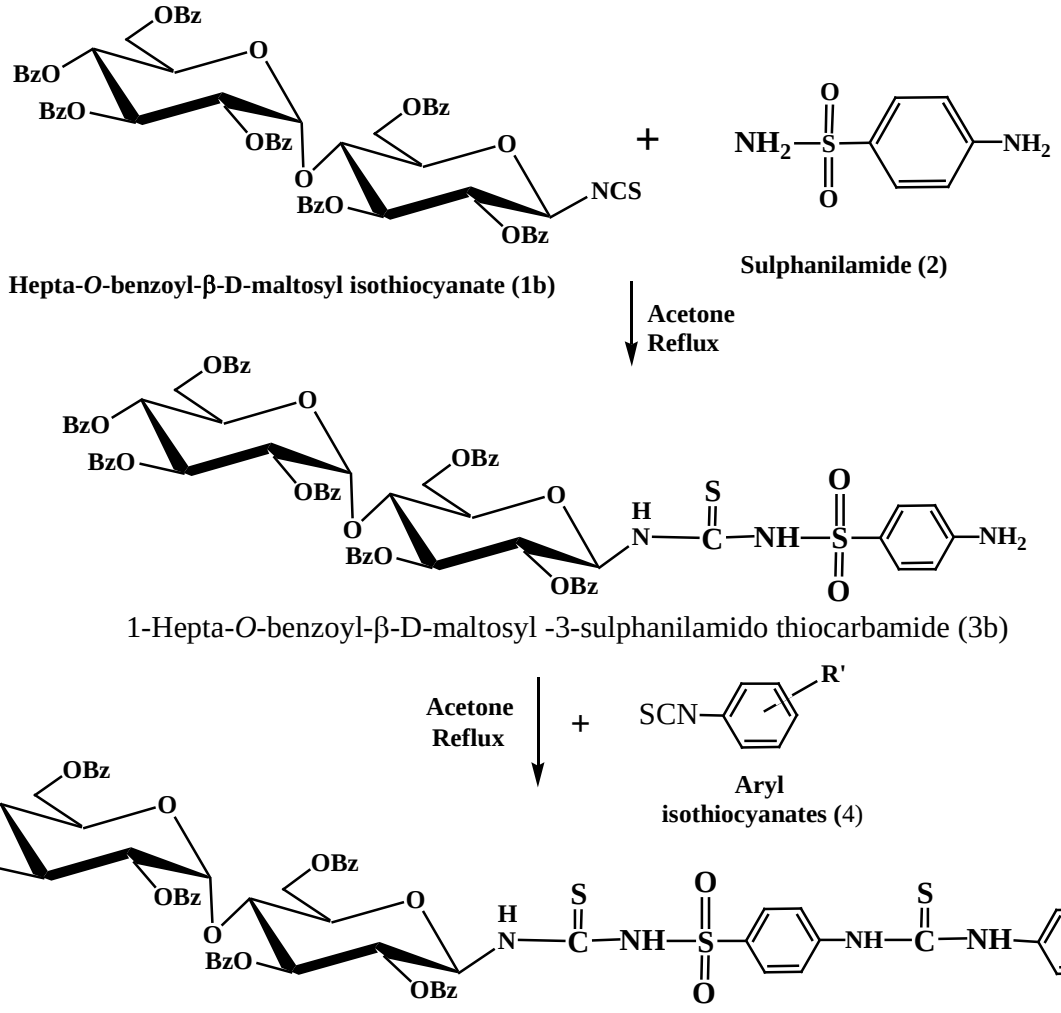
1-Hepta-*O*-acetyl-β-D-lactosyl-3-sulphanilamido thiocarbamide (3a)



1-Hepta-*O*-acetyl-β-D-lactosyl-3-(1-sulphanilamido-3-aryl thiocarbamide) thiocarbamides (5a-b)

Where, Ac - -$COCH_3$, R= a) *p*-methoxy, b) *o*-chloro.

Scheme 2:



1-Hepta-*O*-benzoyl-β-D-maltosyl -3-(1-sulphanilamido-3-aryl thiocarbamide) thiocarbamides (5c-d)
Where, Bz- -COC_6H_5, R'= a) H, b) *p*-chloro.

Material and Method

The reagents required for the given synthesis are obtained as follows-

Synthesis of Glycosyl isothiocaynates (1):-

Glycosyl isothiocaynates were synthesized by the interaction of Glycosylbromides with lead thiocyanate in anhydrous xylene medium. The product was isolated from petroleum ether and crystallized from chloroform-petroleum ether mixture.

Sulphanilamide (2):-

Sulphanilamide used for synthesis was of commercial grade having melting point 160^0C with formula $C_6H_8N_2O_2S$.

Synthesis of aryl isothiocyanates (4):-

The aryl isothiocyanates were prepared by known method i.e. by oxidative decomposition of ammonium aryl dithiocarbamates with Lead nitrate.

Experimental Section:-

The melting point of compounds were determined with the help of Thermonic melting point apparatus and were found uncorrected. The structures of newly synthesized compounds were confirmed on the basis of elemental and spectral analysis. IR Spectra were recorded on KBr disks on SHIMADZU IR affinity-1 FTIR spectrometer. ^{1}H NMR was obtained on Bruker DRX-300 NMR Spectrometer. Samples were prepared in $CDCl_3$ with TMS as an internal reference. The mass spectra were obtained on JEOL-AccuTof JMS-T100LC and Thermo Fennigan LCQ Advantage max ion trap mass spectrometer. Thin layer chromatography (TLC) was performed on silica gel G for TLC (Merck) and spot were visualized by iodine vapour.

Spectral and Elemental analysis[12-16]:-

1-Hepta-*O*-acetyl- β -D-lactosyl-3-sulphanilamido thiocarbamide (3a)

IR(KBr cm^{-1}): 3356 (N-H), 3105 (Aromatic C-H), 2945 (Aliphatic C-H), 1749 (C=O), 1531 (C=C), 1230 (C-O), 1155, 1051 and 906 (Characteristics of Lactose), 736 (C-S). (Found: C, 37.42; H, 4.95; O, 36.89; N, 4.80; S, 7.35 %, $C_{27}H_{43}O_{20}N_3S_2$ Required : C, 37.45; H, 4.97; O, 36.99; N, 4.85; S, 7.39 %).

1-Hepta-*O*-acetyl-β -D-lactosyl-3-(1-sulphanilamido-3-*p*-methoxy phenyl thiocarbamide) thiocarbamide (5a)

IR(KBr cm^{-1}): 3336 (N-H str.), 3103 (Aromatic C-H str.), 2943 (Aliphatic C-H str.), 1749 (C=O str..), 1508 (C=C str.), 1159 (C-N str.), 1230 (C-O str.), 1053 (Characteristics of lactose), 688 (C-S str.); (Found : C, 40.72; H, 4.80; O, 32.52; N, 4.80; S, 9.25 %, $C_{35}H_{50}O_{21}N_4S_3$ Required : C,40.77; H, 4.85; O, 32.62; N, 4.43; S, 9.32 %).

1-Hepta-*O*-acetyl- β -D-lactosyl-3-(1-sulphanilamido-3-*o*-chloro phenyl thiocarbamide) thiocarbamide (5b)

IR(KBr cm^{-1}): 3331 (N-H str.), 3097 (Aromatic C-H str.), 2978 (Aliphatic C-H str.), 1747 (C=O str..), 1527 (C=C str.), 1159 (C-N str.), 1228 (C-O str.), 1056 (Characteristics of lactose), 690 (C-S str.); **^{1}H NMR ($CDCl_3$, ppm):** δ 7.28-7.12, 6.26 (4H, m, NH protons), 8.17-7.138 (8H, m, Aromatic protons), 5.65-3.77 (14H, m, lactosyl protons), 2.19-1.97 (21H, m, acetyl protons) **Mass (m/z):** 1034 (M^+),. (Found : C, 39.38; H, 4.44; O, 30.85; N, 5.35; S, 9.25 %, $C_{34}H_{47}O_{20}N_4S_3Cl$ Required : C,39.45; H, 4.54; O, 30.94; N, 5.41; S, 9.28 %).

1-Hepta-*O*-benzoyl- β -D-maltosyl -3-sulphanilamido thiocarbamide (3b)

IR(KBr cm^{-1}): 3475 (N-H str.), 3064 (Aromatic C-H str.), 2958 (Aliphatic C-H str.), 1730 (C=O str..), 1531 (C=C str.), 1095 (C-N str.), 1267 (C-O str.), 1240, 1098 and 1030 (Characteristics of maltose), 709 (C-S str.). (Found : C, 63.59; H, 4.42; N, 3.25; S,4 7.96 %, $C_{68}H_{57}O_{19}N_3S_2$ Required : C, 63.60; H, 4.44; N, 3.27; S, 4.98 %).

1-Hepta-*O*-benzoyl- β -D-maltosyl -3-(1-sulphanilamido-3-phenyl thiocarbamide) thiocarbamide (5c)

IR(KBr cm^{-1}): 3448 (N-H str.), 3062 (Aromatic C-H str.), 2958 (Aliphatic C-H str.), 1726 (C=O str..), 1531 (C=C str.), 1093 (C-N str.), 1267 (C-O str.), 1260, 1098 and 1030 (Characteristics of maltose), 707 (C-S str.). (Found : C, 63.44; H, 4.35; N, 3.92; S, 6.75%, $C_{75}H_{62}O_{19}N_4S_3$ Required : C,63.46; H, 4.37; N, 3.94; S, 6.77 %).

1-Hepta-*O*-benzoyl-β-D-maltosyl -3-(1-sulphanilamido-3-*p*-chloro phenyl thiocarbamide) thiocarbamide (5d)

IR(KBr cm^{-1}): 3246 (N-H str.), 3064 (Aromatic C-H str.), 2958 (Aliphatic C-H str.), 1730 (C=O str..), 1546 (C=C str.), 1095 (C-N str.), 1269 (C-O str.), 1155, 1098 and 1030 (Characteristics of maltose), 709 (C-S str.); **^{1}H NMR ($CDCl_3$, ppm):** δ 3.990-3.928 (4H, m, NH), 8.17-7.138 (43H, m, Aromatic protons), 6.244-3.916 (14H, m, Maltosyl protons); **Mass (m/z):** 11452 (M^+), 1159, 1117, 1053, 1026, 931, 579. (Found : C, 61.96; H, 4.19; N, 3.83; S, 6.60 %, $C_{75}H_{61}O_{19}N_4S_3Cl$Required : C,61.98; H, 4.20; N, 3.85; S, 6.61%).

Table 1:-

Characterization of Several Glycosyl Suphanilamides-

Sr. No.	Compounds	m.p. (^oC)	Yield (%)	R_f value Ethyl acetate : petroleum ether 7 : 3
1	1-Hepta-*O*-acetyl- β -D-lactosyl-3-sulphanilamido thiocarbamide (IIIa)	98	71	0.804
2	1-Hepta-*O*-acetyl- β -D-lactosyl-3-(1-sulphanilamido-3-*p*-methoxy phenyl thiocarbamide) thiocarbamide (Va)	103	59	0.77
3	1-Hepta-*O*-acetyl- β -D-lactosyl-3-(1-sulphanilamido-3-*o*-chloro phenyl thiocarbamide) thiocarbamide (Vb)	115	62	0.75
4	1-Hepta-*O*-benzoyl- β -D-maltosyl -3-sulphanilamido thiocarbamide (IIIb)	175	61	0.85

5	1-Hepta-*O*-benzoyl- β -D-maltosyl -3-(1-sulphanilamido-3-phenyl thiocarbamide) thiocarbamide (Vc)	140	61	0.78
6	1-Hepta-*O*-benzoyl-β-D-maltosyl -3-(1-sulphanilamido-3-*p*-chloro phenyl thiocarbamide) thiocarbamide (Vd)	210	71	0.79

Microbial assay:

The microbial assay of synthesized compounds have been studied using cup plate agar diffusion method[17-18] by measuring the inhibition zone in mm. the compounds were taken at a concentration of 1 mg/ml using dimethyl sulphoxide (DMSO) as solvent.

Bacterial assay:

The bacterial assay of compounds was studied against *Escherichia coli*, *Staphylococcus aureus and Pseudomonas aeruginosa* in nutrient agar medium. Amikacin (100 µg/ml) was used as standard for antibacterial activity. The results are presented in Table2.

It has been observed that some of these compound exhibited interesting microbial activities. 3a, 5c and 5d exhibited most significant activity against *Escherichia coli*, 3a and 5b exhibited most significant activity against *Staphylococcus aureus*, 3b and 5b exhibited most significant activity against *S. Typhi* and 3a and 5d exhibited most significant activity against *Pseudomonas aeruginosa* respectively. All the other compounds exhibited low to moderate activity. (Table-2).

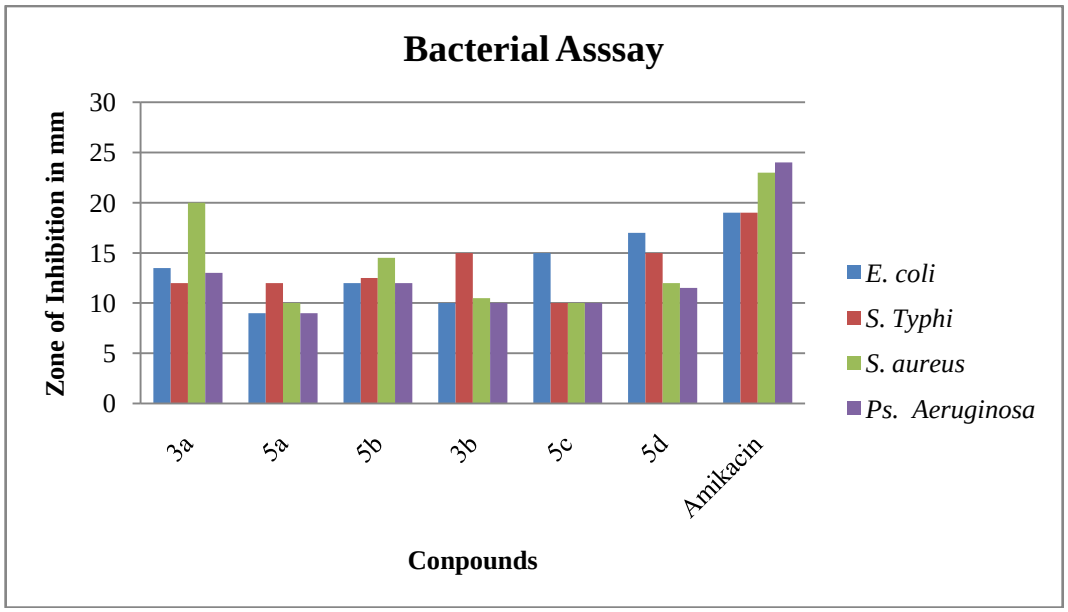
Table2:

Microbial assay of Several Glycosyl Suphanilamides**.**

Compounds	**Antibacterial****			
	E. coli	*S. Typhi*	*S. aureus*	*Ps. Aeruginosa*
3a	13.5	12	20	13
5a	9	12	10	9
5b	12	12.5	14.5	12
3b	10	15	10.5	10
5c	15	10	10	10
5d	17	15	12	11.5
Amikacin	19	19	23	24

**zone of inhibition in mm (15 or less) resistance, (16-20mm) moderate and (more than 20mm) sensitive. *Escherichia coli* (*E*. coli), *Staphalococcus aureus* (*S*. aureus) and *Psudomonas auriginosa* (*Ps. auriginosa*), *S. Typhi.*

Graphical Representation:



Conclusion:

A series of new Glycosyl Suphanilamides were synthesized and characterized by IR, ^{1}H NMR and Mass Spectral and Elemental analysis. The synthesized compounds were evaluated for their antibacterial activities. Thus, the newly synthesized Suphanilamides derivatives, exhibits comparable antibacterial 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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