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Synthesis, Characterization and Screening of Novel Glycoside Derivatives of Urea for Antibacterial Activity

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ABSTRACT

The present encompasses the synthesis of 5 new novel glycoside derivatives of urea by the reaction of urea with aromatic phenol and different aromatic aldehyde and condensed with monosaccharide like D-Glucose. The synthesized compound's structure were characterized by ^{1}H-NMR, IR and Elemental analysis. The purity of compound was confirmed by the TLC. All these synthesized compounds were screened for *In-Vitro* Anti-bacterial against both gram negative (*Pseudomonas aeruginosa* & *Escherichia coli*) and gram positive bacteria (*Bacillus subtillis* & *Staphylococcus aureus*) by Disc diffusion method. Among them some of the derivatives were found active. The glycosylation can enhance drug targeting and pharmacokinetics.

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1. INTRODUCTION

Medicinal chemistry is the science that deals with the identification, creation and modification of various molecules for therapeutic applications[1]. The structure activity relationship is the relationship between chemical structure and pharmacological activity. It is the traditional practice to modify the potency of a bioactive molecule. The Aryl urea derivative has been investigated for their various pharmacological activities. Their ability to show variety of chemical reactions made them important for molecular planning and their privileged structure became a major source of interest for many medicinal chemists to explore its enormous biological potential[20].

Glycosylation is an important biological process which is highly controlled and efficient in nature. It is the coupling reaction that takes place at the anomeric position of a saccharide (donor) and the acceptor molecule and the product formed is glycoside. The major types of glycosylation are the N -, O - and C- linked glycosylation[26].

In Glycosyl amides, the therapeutic activity is enhanced by the condensation of amides with sugar moiety. The Glycosylation of new chemical entities have significant role in enhancing and maintenance of their physiologically relevant therapeutic activity[23].

2. MATERIALS AND METHODS

All the chemicals were obtained from the chemical suppliers. The melting points were determined on 'Veego' VMP-D apparatus. For TLC Silica gel G plates of 3×8 cm (Sigma-Aldrich) Mobile phase - Ethyl acetate: chloroform (1:1) were used and spots located by UV chamber. The IR spectra recorded in 4000-400cm^{-1} range and ^{1}H-NMR spectra recorded in DMSO with TMS as internal standard. Elemental analysis was performed for C, H & N.

2.1. METHODOLOGY FOR SYNTHESIS [24]

STEP 1: Synthesis of Urea derivative: To a mixture of aromatic phenol (0.05 mol), aromatic benzaldehyde (0.05 mol), and urea in ethanol (0.05 mol) were added in drops and reaction mixture was stirred in hot water bath maintained at 80^{0}C with constant stirring for specific period according to the derivative. The solid separated on cooling was recrystallized from ethanol.

STEP 2: Glycosylation of synthesized Urea derivative: A mixture of amide derivative (1mmol) and D-Glucose (1mmol) was heated with absolute ethanol (10ml) in the presence of drops of glacial acetic acid for specific period according to the derivative. After cooling the solid was filtered off and recrystallized from ethanol. (Scheme –figure:1)

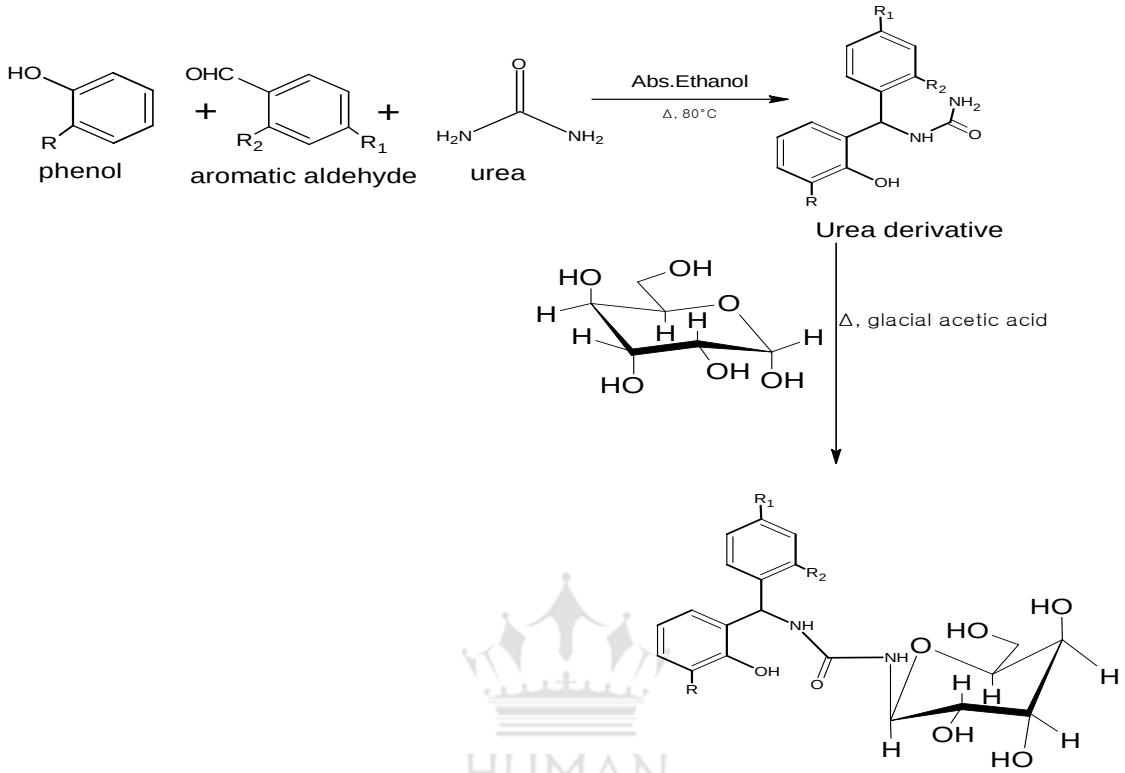

Figure No. 1: Synthesis of Glycosylated Urea derivatives

Compound	R	R1	R2
S1	NH_2	Cl	Cl
S2	NH_2	Cl	H
S3	NH_2	$OCH_2C_6H_5$	H
S4	NH_2	NO_2	H
S5	NH_2	H	H

2.2. SCREENING FOR ANTIBACTERIAL ACTIVITY

The synthesized Glycosyl urea derivatives (S1–S5) screened for antibacterial activity against different microorganisms by Disc diffusion method using Ciprofloxacin as standard. Nutrient Agar Media was used for the study. All the microorganisms were obtained from NCIM, Pune. *Bacillus subtills* (NCIM No: 2063), *Staphylococcus aureus* (NCIM No: 5021) for Gram

Positive Bacterial strain and *Pseudomonas aeruginosa* (NCIM No: 5029), *Escherichia coli* (NCIM No: 2065) for gram-negative bacterial strain.

The antibacterial screening was carried out in a laminar airflow unit and all precautions were strictly maintained to avoid contamination. UV light was switched on for half an hour before working. Placed agar plates right side up in the incubator heated to 37°C for 10 - 20 min with the cover adjusted so that the plates are slightly opened. Label the covers of each of the plates with the name of the test organism to be inoculated, and with name of the synthesized Glycosyl urea derivatives (S1-S5). Petri dishes, Micropipette tips, culture media, cork bore, forceps, blank disks and other glasswares were sterilized in the autoclave at 121°C and at a pressure of 151 lbs/sq inch for 15 min. In disc diffusion method, bacterial inoculums were prepared and inoculated into the entire surface of solid agar plate with a sterile cotton tipped swab. The paper disc 6mm in diameter impregnated with diluted test drug solution (200µg/ml in ethanol) was placed on the surface of each of agar plates using sterile pair of forceps. The plates were incubated for 2-3 days at 20-25°C and observed the zone of inhibition was measured [22].

3. RESULTS AND DISCUSSION

3.1. PHYSICOCHEMICAL PROPERTIES

Table No. 1: Physicochemical properties

Sample code	Molecular Formula	Molecular Weight	Color	Melting point (^{0}C)	R_f value
S1	$C_{20}H_{23}Cl_2N_3O_7$	488.32	Light yellow	143	0.88
S2	$C_{20}H_{24}ClN_3O_7$	453.88	Brown	177	0.46
S3	$C_{27}H_{31}N_3O_8$	463.72	Light brown	147	0.63
S4	$C_{20}H_{24}N_4O_9$	464.43	Yellow	141	0.67
S5	$C_{20}H_{25}N_3O_7$	419.43	Black	127	0.80

1.2.SOLUBILITY PROFILE

Table No. 2: Solubility Profile

Sample code	Solvents					
	Chloroform	Acetone	Ethanol	Ethyl acetate	n-Hexane	Water
S1	Soluble	Soluble	Soluble	Soluble	Sparingly soluble	Sparingly Soluble
S2	Soluble	Soluble	Soluble	Soluble	Sparingly Soluble	Sparingly Soluble
S3	Insoluble	Soluble	Soluble	Soluble	Insoluble	Sparingly Soluble
S4	Soluble	Soluble	Soluble	Soluble	Soluble	Sparingly Soluble
S5	Soluble	Soluble	Soluble	Soluble	Insoluble	Sparingly Soluble

3.3. SPECTRAL INTERPRETATION

- 1-(2-hydroxy,4-aminophenyl)2,4-dichlorophenyl)methyl-3-(1-deoxy-β-D-glucopyranosyl)urea (S1): Yield 46%, IR(KBr) : 3389(Ar-OH), 1617(C=O),1238(CN), 740(C-Cl).^{1}H-NMR (DMSO): δ7.7-7.5 (m,6H,Ar-H), δ8.3(d,H,NH), δ9.3(s,AR-C-OH).Elemental analysis calculated - C: 47.13,H:4.21,N: 7.68.

- (2-hydroxy, 4-aminophenyl) (4-chlorophenyl) methyl-3- (1-deoxy-β-D-glucopyranosyl) urea (S2): Yield 73%, IR(KBr): 3300(Ar-OH), 3045(CH-streching), 1624(C=O), 750(C-Cl). ^{1}H-NMR (DMSO): δ2.9(s,H,CH), δ6.8(d,2H,NH_2), δ7.5-6.8 (m,7H,Ar-H), δ11.8(s,H,OH). Elemental analysis calculated- C: 51.5, H:5.1, N: 8.9.

- 1-(2-hydroxy,4-aminophenyl)(4-benyloxyphenyl) methyl-3-(1-deoxy-β-D-glucopyranosyl) urea (S3): Yield 68%, IR(KBr): 3377(Ar-OH),3037(CH Streching), 1597(NH Bending), 1240(CN). ^{1}H-NMR (DMSO): δ7.9-7.5(m,7H,Ar-H), δ11.4(s,H,OH), δ9.8(d,H,NH), δ2.5(s,H,CH). Elemental analysis calculated- C: 60.5, H:4.7, N: 7.6.

- (2-hydroxy, 4-aminophenyl) (3-nitrophenyl) methyl-3- (1-deoxy-β-D-glucopyranosyl) urea (S4): Yield 80%, IR(KBr):3369(Ar-OH),1616(C=O),1514(NO_2), 1226(CN). ^{1}H-NMR (DMSO): δ9.2(s,Ar-C-OH), δ8.4-8.2(d,2H,CH-NH), δ6.8(d,2H,NH_2). Elemental analysis calculated- C: 50.23, H:4.31, N: 11.84.

- (2-hydroxy, 4-aminophenyl) (phenyl) methyl-3- (1-deoxy-β-D- glucopyranosyl) urea (S5) : Yield 88%, IR(KBr): 3371(Ar-OH),1622(C=O), 1585(NH Bending), 1235(CN). ^{1}H-NMR

(DMSO): δ7.2-7.5(m,7H,Ar-H), δ6.3(d,2H,CH-NH), δ6.9(d,2H,Ar-NH_2). Elemental analysis calculated- C: 64.7, H:5.33, N: 9.18.

3.4 ANTIBACTERIAL ACTIVITY BY MEASURING THE ZONE OF INHIBITION

Antibacterial screening of all newly synthesized Glycosyl urea derivatives were carried out on four microorganism using Disc diffusion method by measuring the radius of zone of inhibition produced by the corresponding derivatives on the Agar plate.Both gram negative (*Pseudomonas aeruginosa* & *Esherichia coli*) and gram positive bacteria (*Bacillus subtillis* & *Staphylococcus aureus*) were used. Ciprofloxacin (30mcg) antibiotic was used as standard. Test drug is used at the dose of 200 mcg. Among the 5 derivatives synthesized, all the derivatives showed activity.

Table No. 3: Antibacterial activity

Sample Code	Zone of inhibition (radius in mm)			
	Gram position organism		Gram negative organism	
	Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonas aeruginosa***
Ciprofloxacin	17	14	16	17
S1	3	2	-	-
S2	10	7	2	-
S3	4	2	-	-
S4	8	5	4	2
S5	11	9	8	-

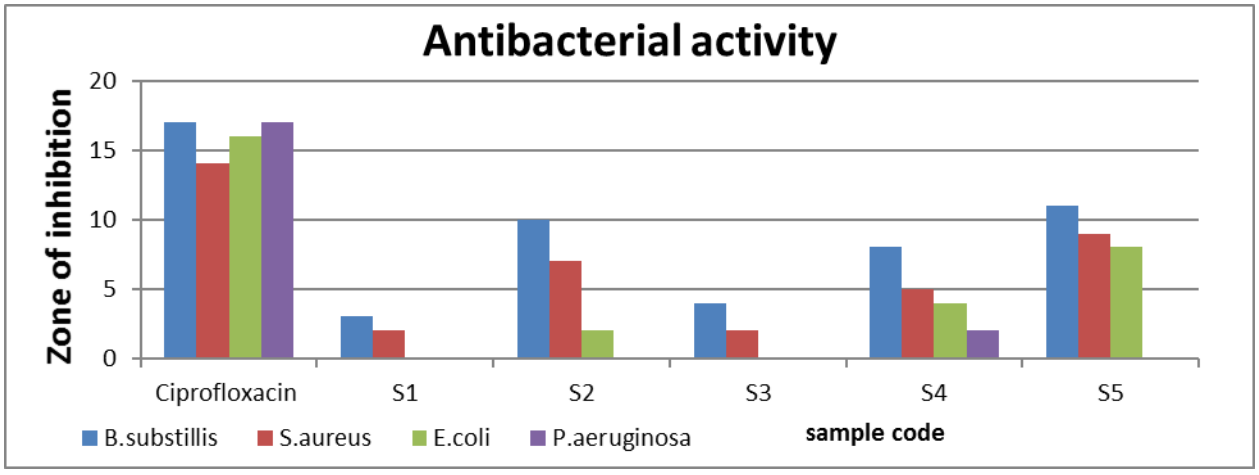

Figure No. 2: graphical representation of antibacterial activity of S1-S5.

The yields of the synthesized compounds were found between 45-90% w/w. The purity of the compound was confirmed by the TLC and the R_f value was in the range of 0.4-0.8.

All of the derivatives showed significant activity against the bacterial strains. Among them S5 (1- (2-hydroxy, 4-aminophenyl) (phenyl) methyl-3- (1-deoxy-β-D- glucopyranosyl)urea) showed good activity for both bacterial strains except for the *Pseudomonas aeruginosa*. The S4 (1-(2-hydroxy,4-aminophenyl) (3-nitrophenyl) methyl-3-(1-deoxy-β-D-glucopyranosyl) urea) showed good activity against the gram positive strains and moderate activity against the gram negative strains (*Pseudomonas aeruginosa* and *Escherichia coli)*. The most probable reason for their activity will be the unsubstituted aryl ring and presence of electron withdrawing groups. The presence of electron withdrawing substituent (NO_2) in the aryl ring at ortho position increase the antibacterial action.

4. CONCLUSION

From the present study, it can be concluded that the amide derivatives linked with Glucose can enhance the activity. All synthesized compounds showed moderate to good potency against different bacterial strains. Among them, S2, S4 & S5 showed significant activity, which can be further developed into new class of antibacterial agents.

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