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Synthesis, characterization and screening of novel glycoside derivatives of thiourea for antimicrobial activity

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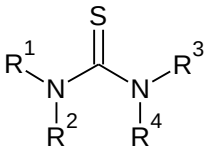
ABSTRACT

This research highlights synthesis of glycoconjugates and recent progress in the development of glycosylated derivative therapeutics. This study lies in the discussion of recent mechanistic theories and supporting experimental evidences on chemical glycosylation on compounds. Here synthesized 10 novel glycosylated derivatives of Thiourea. Glycosylation reaction is achieved by using D-glucose and D-fructose. They were characterized by elemental analysis, IR, and ^{1}HNMR spectroscopies. All the synthesized derivatives were screened for in-vitro antibacterial activity against Bacillus subtilis, Staphylococcus aureus, Escheria coli and Pseudomonas aeruginosa.

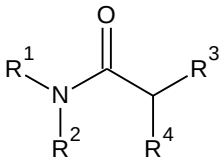
Keywords— *Glycosylation, Thiourea, Phenol, Aromatic aldehyde, Antibacterial activity*

1. INTRODUCTION

Thiourea is the class of organic compounds containing sulphur. Thiourea otherwise called as 2-thiourea, thiocarbamide, and sulfourea with the general formula for Thiourea is (R_1R_2N) (R_3R_4N) C=S. They have structural resemblance to urea i.e., the oxygen of urea's is replaced by a sulphur atom. Inspite of these resemblances, the chemical properties of urea and Thiourea are quite different from each other.



Thiourea



Urea

Thiourea's are being used in all aspects of medicine. They are versatile chemicals with outstanding biological applications. Most prominent biological applications of Thiourea is for treatment of co-infection, as antioxidant, as anti-thyroid drugs, as anti-epileptic drugs, as antimicrobials, as anti-hypertensive, as anti-cancer drug and as urease inhibitors.

Glycosylation is an enzymatic process in which sugars are added to other molecules to produce a glycosylated product. Carbohydrate can be linked to an aromatic aglycone through O-, N-, and C-glycosydic bonds to give O-,N- and C-aryl glycosides respectively. Glycosylation of natural product may affect solubility, stability or molecular recognition associated with the biological target. Glycosylation is a promising strategy for modulating the physiochemical properties of drugs and for improving their absorption through biological membranes. The introduction of carbohydrate moieties changes the physiological properties of drugs, which can improve their bioavailability. The formation of a glycosydic linkage allows for the synthesis of complex glucoside which may plays important roles in biological processes and pathogenesis and therefore allows for further studies with respect to their biological importance.

There are number of compounds which contain glycoside units. These residues can be crucial for their activity and sometimes it only improves pharmacokinetic parameters. Glycosides are generally more water soluble than the respective aglycon and hence influences membrane transport.

Glycosylation of therapeutic proteins has a profound impact on their safety and efficacy. Many factors influence glycosylation of bio therapeutics. Glycosylation is a post translational modification of proteins. Glycans imparts a range of functions on their

protein carriers. Glycosylation of biopharmaceutical drugs has a crucial role in their safety and efficacy by modulating a wide range of drug properties including immunogenicity, in vivo circulatory half-life and effector functions.

The advantages of glycosylation include:

- Targeting specific organs and enhancing bio distribution in tissues
- Improving penetration through biological membrane
- Increasing metabolic stability and lowering the clearance rate

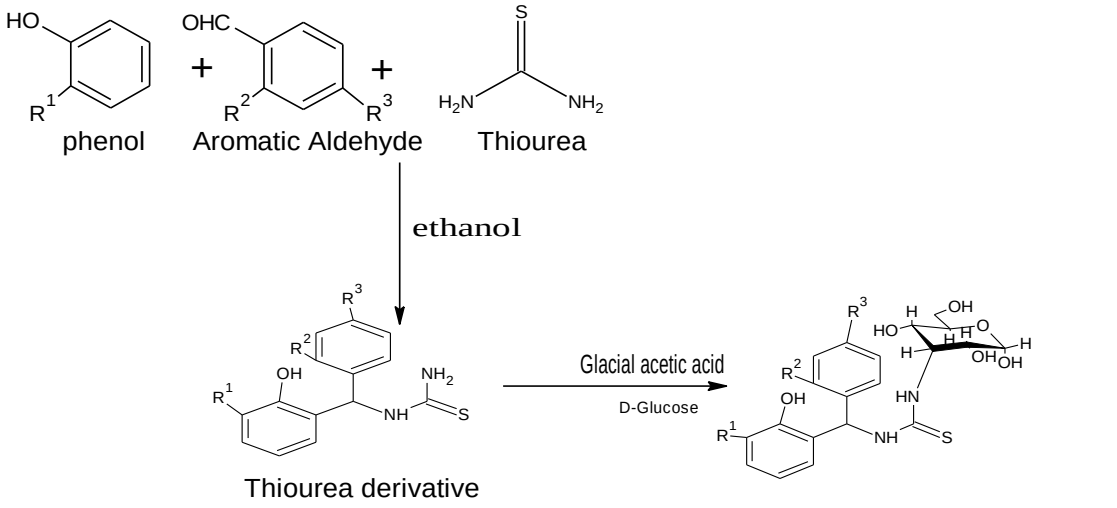
Progress in the area of chemical glycosylation has significantly improved the ability to synthesize various glycosydic linkages with impressive yields and stereo selectivity.

2. MATERIALS AND METHODS

All the chemicals are obtained from commercial suppliers and used without further purification. All the melting points were determined on 'VEEGO' apparatus and are uncorrected. Silica gel G plates of 3*8cm (Sigma-Aldrich)were used for TLC and spots were located by UV chamber. The IR spectra were recorded in the 4000-400 cm^{-1} range using KBr discs on FT-IR 8400 Shimadzu spectrometer. ^{1}HNMR spectra were recorded on varianMercury (30 MHz) spectrometer in $CDCl_3$ with TMS as an internal standard and values are expressed in ppm. Elemental analysis was performed for C, H, and N and was found within ±0.4% of theoretical values.

3. SYNTHETIC PROCEDURE

3.1General Scheme



Derivative	R1	R2	R3
R1	NH_2	-Cl	-Cl
R2	NH_2	-H	-Cl
R3	NH_2	-H	$-OCH_2C_6H_5$
R4	NH_2	$-NO_2$	-H
R5	NH_2	-H	-H

3.2 Synthesis of 1-(2-hydroxy,4-aminophenyl)2,4-dichlorophenyl) methyl-3-(1-deoxy-β-D-glucopyranosyl) thiourea

A mixture of 2,4-dichloro benzaldehyde (1mmol), Amino phenol (1mmol), Thiourea (1mmol) was allowed to heat at 100^0C for 1-3hrs with stirring. The residue recrystallized by ethanol.

A mixture of amide derivative (1mmol) and series of glucose (1mmol) was refluxed in absolute ethanol (10ml) in the presence of drops of glacial acetic acid for 1-3 hrs. After cooling the solid was filtered off and recrystallized from ethanol.

3.3 Synthesis of 1-2-(hydroxyl,4-aminophenyl)4-chlorophenyl) methyl-3-(1-deoxy-β-D-glucopyranosyl) Thiourea

A mixture of 4-chloro benzaldehyde (1mmol), Amino phenol (1mmol), Thiourea (1mmol) was allowed to heat at 100^0C for 1-3hrs with stirring. The residue recrystallized by ethanol.

A mixture of amide derivative (1mmol) and series of glucose (1mmol) was refluxed in absolute ethanol (10ml) in the presence of drops of glacial acetic acid for 1-3 hrs. After cooling the solid was filtered off and recrystallized from ethanol.

3.4 Synthesis of 1-(2-hydroxy,-aminophenyl)4-benzyloxy)methyl-3-(1-deoxy-β-D-glucopyranosyl)Thiourea

A mixture of 4-benzyloxy benzaldehyde (1mmol), Amino phenol (1mmol), Thiourea (1mmol) was allowed to heat at 100^0C for 1-3hrs with stirring. The residue recrystallized by ethanol.

A mixture of amide derivative (1mmol) and series of glucose (1mmol) was refluxed in absolute ethanol (10ml) in the presence of drops of glacial acetic acid for 1-3 hrs. After cooling the solid was filtered off and recrystallized from ethanol.

3.5 Synthesis of 1-(2-hydroxy,4-aminophenyl)2-nitro methyl-3-(1-deoxy-β-D-glucopyranosyl)Thiourea

A mixture of 2-Nitro benzaldehyde (1mmol), Amino phenol (1mmol), Thiourea (1mmol) was allowed to heat at 100^0C for 1-3hrs with stirring. The residue recrystallized by ethanol.

A mixture of amide derivative (1mmol) and series of glucose (1mmol) was refluxed in absolute ethanol (10ml) in the presence of drops of glacial acetic acid for 1-3 hrs. After cooling the solid was filtered off and recrystallized from ethanol.

3.6 Synthesis of 1-(2-hydroxy,4-aminophenyl)methyl-3-(1-deoxy-β-D-glucopyranosyl)Thiourea

A mixture of benzaldehyde (1mmol), Amino phenol (1mmol), Thiourea (1mmol) was allowed to heat at 100^0C for 1-3hrs with stirring. The residue recrystallized by ethanol.

A mixture of amide derivative (1mmol) and series of glucose (1mmol) was refluxed in absolute ethanol (10ml) in the presence of drops of glacial acetic acid for 1-3 hrs. After cooling the solid was filtered off and recrystallized from ethanol.

4. ANTIBACTERIAL STUDY

Anti-bacterial screening of newly synthesized compounds was carried out on four organisms by disc diffusion method by measuring the zone of inhibition in millimeters. Both gram positive and gram-negative bacteria were used. Gram positive bacteria include*bacillus subtilis (*NCM NO.*2063), Staphylococcus aureus (NCIM No.5021), and gram negative bacteria Pseudomonas aeruginosa (*NCIM No.5021*), Escheria coli(*NCIM.2065*),* Ciprofloxacin 30µg antibiotic is used as standard. Test drug include 200µg/ml.

The study was carried out in a laminar air flow unit. UV light was switched on for half an hour before working in the laminar hood to avoid any accidental contamination. Placed agar plates right side up in the incubator heated to 37ºC for 10min to 20min with the cover adjusted so that the plates are slightly opened.Labelled the covers of each of the plates with the name of the test organism and the synthesized glycosylated thiourea derivative (R1-R5) Petri dishes and other glass wares were sterilized in the autoclave at 121ºC and at a pressure of 151*lbs/sqinch* for 15mins.Micropipette tips, culture media, cork bore, forceps, blank disks and so forth, were also sterilized.Bacterial inoculums were prepared and inoculated into the entire surface of solid agar plate with a sterile cotton tipped swab to form an even lawn. The paper disc 6mm in diameter impregnated with diluted test drug solution (100µg/ml in ethanol) was placed on the surface of each of agar plates using sterile pair of forceps (flame sterilized). The plates were incubated for 2-3 days at 20-25ºC and observed without opening them and the **zone of inhibition** was measured.

5. RESULT AND DISCUSSION

5.1 Physicochemical properties

Table1: Physicochemical properties

Sample code	Molecular Formula	Molecular Weight	Physical state	Color	Melting point(^{0}C)	R_f value
R1	$C_{20}H_{23}Cl_2N_3O_6S$	402.48	Crystalline	Yellow	139	0.85
R2	$C_{20}H_{24}ClN_3O_6S$	469.95	Crystalline	Brownish yellow	127	0.44
R3	$C_{27}H_{31}N_3O_7S$	472.60	Crystalline	Brown	141	0.60
R4	$C_{20}H_{24}N_4O_8S$	480.50	Crystalline	Yellow	135	0.79
R5	$C_{20}H_{25}N_3O_6S$	435.50	Crystalline	Brownish red	131	0.88

5.2 Solubility profile

Table 2: Solubility Profile

Sample Code	Solvents					
	n-Hexane	Chloroform	Ethyl Acetate	Acetone	Alcohol	Water
R1	Sparingly Soluble	Soluble	Soluble	Soluble	Soluble	Sparingly Soluble
R2	Sparingly Soluble	Soluble	Soluble	Soluble	Soluble	Sparingly Soluble
R3	Sparingly Soluble	Soluble	Soluble	Soluble	Soluble	Sparingly Soluble
R4	Sparingly Soluble	Soluble	Soluble	Soluble	Soluble	Sparingly Soluble
R5	Sparingly Soluble	Soluble	Soluble	Soluble	Soluble	Sparingly Soluble

5.3 Antibacterial Activity by measuring the radius of the zone of inhibition

Table 3: Antibacterial activity by measuring the radius of the zone of inhibition

Sample code	Zone of inhibition(radius in mm)			
	Gram positive		Gram negative	
	B.subtilis	*S.aureus*	*E.coli*	*P.aeruginosa*
Ciprofloxacin	**17**	**16**	**16**	**17**
R1	**4.5**	**6**	**-**	**-**
R2	**8**	**4.5**	**1.5**	**-**
R3	**5**	**5**	**-**	**-**
R4	**10**	**6**	**4**	**-**
R5	**11**	**10**	**6**	**-**

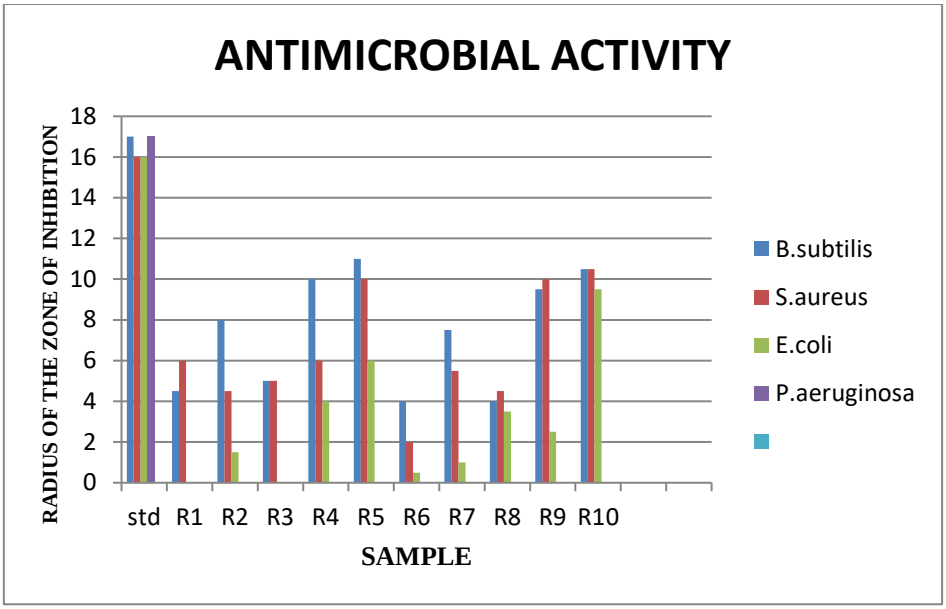


Fig 1: Graphical Representation of Antibacterial Activity

6. SPECTRAL INTERPRETATION

1-(2-hydroxy,4-aminophenyl)2,4-dichlorophenyl)methyl-3-(1-deoxy-β-D- glucopyranosyl)Thiourea(R1) : Yield 55.25, yellow crystalline solid, m.p.139^{0}C, ^{1}HNMR(DMSO):δ7.570(m,6H,Ar-H),δ8.417(dH,NH),δ9.300(s,Ar-C-OH),IR(KBr,cm^{-1}): 3389.71(Ar-OH), 1584.5 (C=S),1479(asymmetric stretching)

1-2-(hydroxyl,4-aminophenyl)4-chlorophenyl)methyl-3-(1-deoxy-β-D-glucopyranosyl)Thiourea(R2): Yield 53.60,brownish yellow, crystalline solid, m.p 127^{0}c, ^{1}HNMR(DMSO): δ7.579(m,7H,Ar-H),δ 6.824(d,2H,NH_2), δ9.069(s,Ar-C-OH),IR(KBr,cm^{-1}):3304(Ar-OH),1624(C=O stretching), 1583(C=S).

1-(2-hydroxy,-aminophenyl)4-benzyloxy)methyl-3-(1-deoxy-β-D-glucopyranosyl)Thiourea (R3): Yield 55.40,brown ,crystallineSolid,mp.141^{0}c,^{1}HNMR(DMSO): δ3.392(OCH_2),7.472-7.9(m,7HAr-H),δ9.87(d,H,NH),IR(KBr,cm^{-1}):3055(NH stretching),1454(CN asymmetric stretching).

1-(2-hydroxy,4-aminophenyl)2-nitro methyl-3-(1-deoxy-β-D-glucopyranosyl)Thiourea (R4):Yield 56.30,yellow crystalline solid,m.p.135^{0}c,^{1}HNMR(DMSO):δ9.257(s,Ar-C-OH),δ8.43(d,H,NH),δ6.8(d,2H,Ar-NH_2),IR(KBr,cm^{-1})3365(Ar-OHstretching,1504(NO2)

1-2-hydroxy,4-aminophenyl)methyl-3-(1-deoxy-β-D-glucopyranosyl)Thiourea(R5):yield 65.45,brownish red crystalline Solid, mp.131,1HNMR(DMSO):δ7.74(m,7H,Ar-H),δ3.41(d,H,NH),δ6.99(d,2H,Ar-NH_2),IR (KBr,cm^{-1})5573(NH stretching)3304(Ar-OH)

7. SUMMARY

The titled compounds R1-R5 were obtained by reacting various derivatives of aromatic aldehyde ,O-Amino phenol,Thiourea and Glucose in the presence of glacial acetic acid.They were characterized by IR (KBr) shows peak at 1583 due to presence of (C=S) and appearance of strong bands in the region of 3464cm^{-1} due to asymmetric stretching vibrations of (–NH_2) group. IR spectra show a peak at 1143cm^{-1} due to glycosydic group. Also, the absorption band at the range near to 1400cm^{-1} is due to the presence of C-N stretching of amide. The structures were confirmed were confirmed by ^{1}H NMR and elemental analysis. Antibacterial screening of all newly synthesized derivatives were carried out on four microorganisms using disc diffusion method by measuring the radius of the zone of inhibition produced by the corresponding derivative on the agar plate. Ciprofloxacin (30µg) was chosen as the standard drug. Out of these synthesized derivatives R4 and R5 showed good activity.

8. CONCLUSION

The present study was aimed at evaluating the antibacterial potential of the glycosylated Thiourea derivatives by providing various substitution at the second and fourth position of aromatic aldehyde. A series of five compounds were synthesized as outlined in the scheme.All the synthesized compounds were then biologically screened for antibacterial activity by disc diffusion method.1-(2-hydroxy,4-aminophenyl)methyl-3-(1-deoxy-β-D glucopyranosyl)thiourea**(R5)** shows highest activity against gram positive bacteria *B.subtilis.*1-(2-hydroxy,4-aminophenyl)2,4-dichlorophenyl)methyl-3-(1-deoxy-β-D-glucopyranosyl)thiourea**(R1)** and 1-(2-hydroxy,4-aminophenyl)4-benzyloxy)methyl-3-(1-deoxy-β-D-glucopyranosyl)thiourea**(R3)** shows no activity in gram negative organisms. . None of the synthesized compounds showed activity against *Pseudomonas aeruginosa.* The increase in extending of activity seemed to be inorder decrease in the bulkiness of the substitution on the synthbesized derivatives. This may be due to the absence of stearic hindrance. So the highest activity is obtained by unsubstituted derivatives(**R5)**.The compound 1-(2-hydroxy,4-aminophenyl)2-nitro methyl-3-(1-deoxy-β-D-glucopyranosyl)Thiourea (**R4)** also shows higher activity,may be because of benzene containing strong electron withdrawing group enhances the antibacterial activity.

9. ACKNOWLEDGMENT

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10. REFERENCES

[1] Shakeel A. Altaf AA. Quereshi AM and Badshah A. Thiourea derivatives in drug design and medicinal chemistry: A short Review. Journal of drug design and medicinal chemistry. 2016; 2(1): 10-20.

[2] Moradi SV. Hussein WM. Varamini P. Simerska P and Toth I. Glycosylation,an effective synthetic strategy to improve the bioavailability of therapeutic peptides. chemical science. 2016; 7: 2492-2500.

[3] Kren V and Martinkova L. Glycosides in medicine: The role of glycosydic residue in biological activity. Current medicinal chemistry. 2001 ;8: 1303-1328.

[4] Nigudkar SS and Demchenko AV. Stereo controlled 1,2-cis glycosylation as the driving force of progress in synthetic carbohydrate chemistry. Chemical science. 2015; 6: 2687-2704.

[5] Seitz O. Glycopeptide synthesis and the effects of Glycosylation on protein structure and activity. Chembiochem. 2000; 1: 214-246.

[6] Mydock LK and Demchenko A. Mechanism of chemical O-glycosylation: from early studies to recent discoveries. organic and biomolecular chemistry. 2010; 8: 497-510.

[7] Sharma BK. Instrumental method of chemical: analytical chemistry. 23rd ed. Meerut: Goel Publishing House; 2004: 241-264.

[8] Silverstein RM. Webster FX. Kiemle DJ. Spectroscopic identification of organic compounds 7thedition. Newyork; John Wiley and sons. 2005:72-108.

[9] Brunton L. Parker K. Blumenthal D. Buxton. Goodman's and Gilmans Manual of pharmacology and therapeutics. USA: McGraw-Hill Companies, 2008: 707-715.

[10] Pelczar MJ. Chan ECS. Krieg NR Microbiology. 5th ed.New Delhi: Tata McGraw-Hill; 2005: 519-536.

[11] Cappuccino GJ. Sherman N. Microbiology A Laboratory Manual. 6thed.India: Pearson Education; 2006: 279-285.

[12] KD Tripathy Essential of medical pharmacology.5th edition.627-632.369-370.405-407.

[13] Halim AA and Ngaini Z. synthesis and bacteriostatic activities of bis(thiourea)derivatives with variable chain length; journal of chemistry; 2016;27(3): 9832.

[14] Madhava G. Venkata SK. Sreenivasulu R and Raju CN. Synthesis of novel urea and thiourea derivatives of diphenylphosphoramidate and their antimicrobial activity. Der Pharmacia Lettre. 2012; 4(4): 1194-1201.

[15] Saeed S. Rashid N. Jones PG. Ali M. Hussain R. Synthesis, characterization and biological evaluation of some Thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents. European journal of medicinal chemistry; 2010; 4; 123-1331.

[16] Celen AO. Kayamakcoglu B. GumruS.Toklu H Z.Aricoglu F; synthesis and anticonvulsant activity of substituted thiourea derivatives; marmara pharmaceutical journal. 2011;5: 43-47.

[17] Ibrahim ES. Elgemeie G.E.H. Abbasi M M. Abbas YA. Elbadawi M A and Attia M E. Synthesis of N-Glycosylated pyridines as New Antiviral Agents. Nucleosides and Nucleotides; 1995; 14: 1415-1423.

[18] Sayed EI. Khalaf HS. Mohamed SF. Hussien HA. Kutkat OM. Amr AE. Synthesis and antiviralactivity of 1,2,3-Triazole glycosides based substituted pyridine via click cycloaddition. Russian journal of general chemistry.2017;10:2444-2453.

[19] Ghoneim AA and Morsy N. A facile synthesis of new heterocyclic compounds from thiourea and urea which links with some hexoses. orgChem:anind J. 2017; 13(3): 114.

[20] http://www.molinspiration.com/.

[21] http://www.pharmaexpert.ru/passonline/.

[22] http://icmr.nic.in/bioethics/final_cpcsea.pdf, CPCSEA guidelines for laboratory animal facility.

[23] Kumar P. Sharma A. Gupta S K. Synthesis, characterization and anticonvulsant activity on novel Thiourea derivatives. Int.J.Pharm.Med.Res. 2014; 2(3): 116-122.

[24] Jambi MSS.Characterization and synthesis of Novel Thiourea Derivatives. International Journal of ChemTech Research. 2019; 12(3):177-187.

[25] Zhong Z. Xing R. Liu S. Wang L. Cai S. Li P. Synthesis of acyl Thiourea derivatives of chitosan and their antimicrobial activities in vitro. carbohydrate research. 2008; 343(3): 566-570.

[26] OECD Guidelines http://www.oecd.org/chemicalsafety/testing/oecdguidelinesfortestingofchemicals.html

[27] Martis M. Campos G. Silvestre S. Falcao A and Alves G. Early preclinical evaluation of dihydropyrimidin(thi)ones as potential anticonvulsant drug candidates. European journal of pharmaceutical sciences.2017;102:264-274.

[28] Mannam R. Devineni SR. Pavuluri CM. Chamarthi NR. andKottapalli RSP. Urea and thiurea derivatives of 3-(trifluromethyl)-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-a]pyrazine:synthesis,characterization,antimicrobial activity and docking studies. Phosphorous, sulphur, and silicon and the related elements, 2019;1: 1563-5325 .

[29] Thakur AS. Deshmukh R. Jha AK. And Kumar SP. Molecular docking study and anticonvulsant activity of synthesized 4-((4,6-dimethyl-6H-1,3-thiazin-2-yl)phenylsulfonyl)urea/Thiourea derivatives. Journal of king Saudi university-science. 2018; 30(3): 330-336.

[30] Vinayaditya S. Ameeta A. Vikash M. Ramsneh R and Savita A. Synthesis, structural analysis and biological evaluation of anticonvulsant activity of pyrazole derivatives containing Thiourea. 2011; 1(3): 125-146.

[31] Struga M. Kossakowski J. Kedzierska E. Fidecka S. and Stefanska J. Synthesis and pharmacological activity of urea and Thiourea derivatives of 4- Azatricyclo[5.2.2.$0^{2,6}$]undec-8-ene-3,5-dione. Chem.Pharm.Bull. 2007;55(5):796-799.

[32] Lien. EJ. And Kumler WD. Dipole momets and pharmacological activity of cyclic ureas, cyclic thioureas and the N,N-Diethylated compounds, Chembio. 1968;11(2):214-219.

[33] Korkmaz N. Obaidi OA. Senturk M. Astley D. Ekinci D. and Supuran CT. Synthesis and biological activity of novel thiurea derivatives as carbonic anhydrous inhibitors. Informa healthcare. 2013; 10: 1-6.

[34] Beale MT. and Taylor SM. Synthesis of cardiac glycoside analogs by catalyst controlled, regioselective glycosylation of digitoxin. Organic letters. 2013; 15(36): 1358-1361.

[35] Olano C. Abdelfattah MS. Gullon S. Brana A and Salas J. Glycosylated derivatives of steffimycin: insights into the role of sugar moieties for the biological activity. Chembiochem. 2008; 9: 624-633.
[36] Kahne D. Walker S. Cheng Y. and Engen DV. Glycosylation of unreactive substrates. J.am.chem.soc. 1989;111(17): 6881-6882.
[37] Abu-Zaied MA. And Elgemeie GH. Thiazole in glycosylation reaction: Novel synthesis of thiazolethioglycosides. Heteroatom chemistry. 2017; 10: 1-6.

