Synthesis, Structural and Biological Studies of *N*-(3-Tetra-O-Acetyl-β-D-Glucopyranosyl) -1H (1,2,4-Thiadiazolo (3,4-C) (1,2,4) Thiadiazol-5(3H) Ylidene) Arylamine

M.R.Ugale, B.N.Berad, S.M.Bhiwgade

Abstract- *N*-(3-tetra-O-acetyl β-D-glucopyranosyl)-1H (1,2,4- thiadiazolo (3,4-C) (1,2,4) thiadiazol-5(3H) ylidene) arylamine have been prepared by the interaction of 3-amino- 5- arylimino-1,2,4 thiadiazoline and N- tetra-O- acetyl β-D-glucopyranosylimino chloromethane sulphenyl chloride . The synthesized compounds have been characterized by analytical and IR, NMR and mass spectral studies. The synthesized compounds were screened for their antimicrobial activities. These compounds showed moderate to good antibacterial and antifungal activities.

Keywords- Amidino thiocarbamides,3-amino- 5- arylimino-1,2,4 thiadiazoline ,N-(3-tetra-O- acetyl β-D-glucopyranosyl)-1H (1,2,4-thiadiazolo (3,4-C) (1,2,4) thiadiazol-5(3H) ylidene) arylamine, N- tetra-O- acetyl β-D-glucopyranosylimino chloromethane sulphenyl chloride,

1. INTRODUCTION

Carbohydrates play a key role in numerous biological processes including the modification of proteins, molecular recognition and immune response.[1]Thiazoline and thiazole moieties may prove perspective scaffolds for design of anticancer drugs.[2-3] The 1,3,4-thiadiazole ring is associated with diverse biological activities probably by virtue of incorporating a toxophoric –NCS-linkage.[4] Various 2-aminosubstituted amino-1,3,4-thiadiazoles and their Schiff bases have recently received significant importance because of their diverse biological properties.[5-7] Compounds with 1,3,4-and 1,2,5-thiadiazole and thiadiazolidine rings show antibacterial, fungicidal, anti-inflammatory ,and anti-tubercular activity and hence ,the investigation of novel derivatives of these compounds is of significant interest.[8-14]

Looking at the importance of glucosylated and hetrocyclic compounds, we are reporting the synthesis of N-glucosylated thiadiazole through the C-N and N-S bond formation by the interaction of 3 amino-5 arylimino-1,2,4 thiadiazoline with N--tetra-O-acetyl-β-D-glucopyranosyl imino chloromethane sulphenyl chloride.[15-18]

M.R.Ugale is Assistant Professor at G.H.Raisoni Institue of Engineering and Technology,Nagpur and pursuing Ph.D from Post Graduate Teaching Department of Chemistry, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur, Maharashtra, India-440033.
Email: manjusha.ugale@raisoni.net
B.N.Berad is Professor, Post Graduate Teaching Department of Chemistry, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur, Maharashtra, India-440033.
Email: bnberad@gmail.com

2. MATERIALS AND METHODS

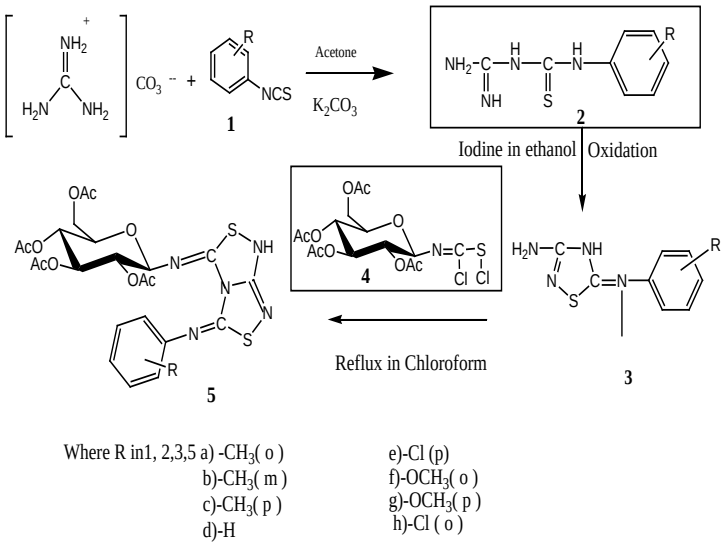
The chemicals and reagents used in present work were purchased from SD fine chem. Ltd. The reaction progress was monitored by TLC technique by using suitable mobile phase of solvent. Purification of compounds were done by recrystalization method by using suitable solvent. Determination of melting point was done by using melting point apparatus and are uncorrected. IR spectra recorded on HAPP-GENZEL. ^{1}H NMR spectra on Bruker avance-II 400 NMR spectrometer at 400 MHz in $CDCl_3$ as solvent were recorded. The mass spectra were recorded on TOF MS ES+ 2.77e^3 mass spectrometer. The compounds were screened for their antibacterial and antifungal activities by the agar diffusion method.

3. RESULTS AND DISCUSSION

The *N*-(3- tetra-*O*-acetyl-β-D-glucopyranosyl) -1H (1,2,4-thiadiazolo (3,4-C) (1,2,4) thiadiazol-5(3H) ylidene) arylamines (5) (Reaction Scheme) were synthesized by following the interaction of 3 amino-5 arylimino-1,2,4 thiadiazolines (3) 0.30 g to 0.50 g (0.0014 to 0.0022 mole) and *N*- tetra-*O*- acetyl β-D-glucopyranosylimino chloromethane sulfenyl chloride (4)0.6699 g to 1.0764g (0.0014 to 0.00234 mole) in chloroform medium by refluxing the reaction mixtures for 4 hours. The solvent was vacuum evaporated and resulting solids were recrystalized from ethanol.

The structures of the target compounds have been established by IR, ^{1}H-NMR, ^{13}C-NMR and mass structural data.

3.1 Reaction Scheme:



The N-(3- tetra-*O*-acetyl-β-D-glucopyranosyl) -1H (1,2,4-thiadiazolo (3,4-C) (1,2,4) thiadiazol-5(3H) ylidene) arylamines.(5a-h) were prepared by the above reaction and the products were isolated in good yields (table1).

3.2 Table 1 Synthesis of *N*-(3- tetra-*O*-acetyl-β-D-glucopyranosyl) -1H (1,2,4-thiadiazolo (3,4-C) (1,2,4) thiadiazol-5(3H) ylidene) arylamine (5).

Reactants:- 3-Amino-5 arylimino-1,2,4 thiadiazolines (3) And *N*-tetra- *O*- acetyl β-D-glucopyranosylimino chloromethane sulfenyl chloride (4).

3-Amino-5 arylimino-1,2,4 thiadiaz--oline (3)	***N*-(3-TAG) -1H (1,2,4-thiadiazolo (3,4-C) (1,2,4)thiadiazol-5(3H) ylidene) arylamine (5).**	**Yield**	**mp °C**	**Elemental analysis Found (cal)(%) N S**
-----5 o-tolyl--(3a)	--- o-tolyl----(5a).	96.65 %	115	11.70 10.68 (11.72) (10.79)
-----5 m-tolyl---(3b)	---- m-tolyl--(5b).	92.79 %	148	11.767 10.67 (11.72) (10.79)
----5 p-tolyl---(3c)	---- p-tolyl--(5c).	95.50 %	130	11.67 10.69 (11.72) (10.79)
---5 phenyl--(3d)	----- phenyl-(5d).	97.50 %	125	11.88 11.00 (12.08) (11.06)
---5 p-chlorophenyl--(3e)	-- p-chlorophenyl (5e).	90.90 %	135	11.25 10.31 (11.33) (10.38)
--5 o-anisyl--(3f)	-- o-anisyl--(5f).	85.5%	132	11.35 10.41 (11.41) (10.45)
--5 p-anisyl--(3g)	-- p-anisyl--(5g).	94.94 %	110	11.38 10.41 (11.41) (10.45)
--5-o-chlorophenyl (3h)	--- o-chlorophenyl---(5h).	75.05 %	142	11.29 10.29 (11.33) (10.38)

3.3 Spectral data, Colour and Molecular Formulae of 5: The *N*-(3- tetra-*O*-acetyl-β-D-glucopyranosyl) -1H (1,2,4-thiadiazolo (3,4-C) (1,2,4) thiadiazol-5(3H) ylidene) o-tolylamine (5a)

IR (KBr) ν max cm^{-1}: 3337 (N-H),3100 (C-H aromatic),1750 (C=O),1544 (C=N),1434(C=C Aromatic ring stretch),1227 (C-O),896(Glycosidic C-H deformation β-anomer),757(C-S) ; ^{1}H NMR ($CDCl_3$) δ ppm: 7.41 (s,1H,NH), 7.33-7.09 (m,4H,Ar-H), 5.72 - 4.87 and 4.24 - 3.69 (m,7H.,Glucosyl ring protons),2.29 (s,3H, Ar-CH_3),2.02 (m,12H,Acetyl protons); C^{13}-NMR($CDCl_3$) δ ppm :185.55 & 181.25 (2C, thiadiazolo ring carbon), 170.74-169.56(4C,C=O of CH_3CO),131.73-125.93(6C,Aromatic ring carbons),99.99(1C, thiadiazolo ring carbon), 82.61-61.72(6C,Glucosyl ring carbon),20.77-18.05(4C,CH_3 of CH_3CO group),17.39(1C,Ar-CH_3 carbon) ;MS(m/z): 592 (M-H Deprotonated)+, 593 (M^+).The molecular formula of 5a was established as C_{24} H $_{27}$ $O_9N_5S_2$ .Colour: cream

***N*-(3-(tetra-O-acetyl-β-D-glucopyranosyl)-1H(1,2,4 thiadiazolo (3,4-C)(1,2,4)thiadiazol-5(3H) ylidene) m-tolylamine (5b)**

IR (KBr) ν max cm^{-1}: 3243 (N-H),3100 (C-H aromatic),1750 (C=O),1542 (C=N),1431(C=C Aromatic ring stretch),1039(C-O),895(Glycosidic C-H deformation β-anomer), 779(C-S) ; ^{1}H NMR ($CDCl_3$) δ ppm: 11.34 (s,1H,NH), 7.25 - 6.94(m,4H,Ar-H),5.79-4.89 and 4.28-3.82 (m,7H.,Glucosyl ring protons),2.31 (s,3H, Ar-CH_3),2.02 (m,12H,Acetyl protons); C^{13}-NMR($CDCl_3$) δ ppm :181.25 & 180.20 (2C, thiadiazolo ring carbon), third thiadiazol ring carbon not located. 170.76-158.70(4C,C=O of CH_3CO),140.19-116.16(6C,Aromatic ring carbons),82.72-61.76(6C,Glucosyl ring carbon),21.57-20.62(4C,CH_3 of CH_3CO group),17.39(1C,Ar-CH_3 carbon) ;MS(m/z): 592 (M-H

Deprotonated)+, 593 (M^+).The molecular formula of 5b was established as C_{24} H $_{27}$ $O_9N_5S_2$ .Colour: cream

***N*-(3-(tetra-*O*-acetyl-β-D-glucopyranosyl)-1H(1,2,4 thiadiazolo(3,4-C)(1,2,4)thiadiazol-5(3H) ylidene) p-tolylamine (5c)**

IR (KBr) ν max cm^{-1}: 3312 (N-H), 3098 (C-H aromatic),1743(C=O),1602 (C=N),1419(C=C Aromatic ring stretch),1038 (C-O),890 (Glycosidic C-H deformation-β-anomer),742(C-S) .The molecular formula of 5c was established as C_{24} H $_{27}$ $O_9N_5S_2$ .Colour: cream

***N*-(3-(tetra-*O*-acetyl-β-D-glucopyranosyl)-1H(1,2,4 thiadiazolo (3,4-C)(1,2,4)thiadiazol-5(3H) ylidene)phenylamine (5d)**

IR (KBr) ν max cm^{-1}: 3400 (N-H), 3090 (C-H aromatic),1743 (C=O),1654 (C=N),888(Glycosidic C-H deformation-β-anomer) . The molecular formula of 5d was established as $C_{23}H_{25}O_9N_5S_2$ .Colour: cream

***N*-(3-(tetra-*O*-acetyl-β-D-glucopyranosyl)-1H(1,2,4 thiadiazolo (3,4-C)(1,2,4)thiadiazol-5(3H) ylidene) p-chlorophenylamine (5e)**

IR (KBr) ν max cm^{-1}: 3320 (N-H),3100 (C-H aromatic),1750 (C=O),1612 (C=N),1612 (C=N),1500 (C=C Aromatic ring stretch),1037 (C-O),893 (Glycosidic C-H deformation β-anomer),780(C-S) ; ^{1}H NMR ($CDCl_3$) δ ppm: 7.33-7.08 (m,4H,Ar-H), 5.77-4.85 and 4.28-3.69 (m,7H.,Glucosyl ring protons), 2.02 (m,12H,Acetyl protons),1.18(N-H) ; C^{13}-NMR($CDCl_3$) δ ppm :185.55 & 181.25 (2C, thiadiazolo ring carbon),169.67-169.09(4C,C=O of CH_3CO),138-119.54(6C,Aromatic ring carbons),99.99(1C, thiadiazolo ring carbon), 81.83-61.44(6C,Glucosyl ring carbons),20.47-20.15(4C,CH_3 of CH_3CO group);MS(m/z): 613(Cl^{35}) (M^+), 615(Cl^{37}) (M^+) both observed in 3:1 ratio.The molecular formula of 5e was established as $C_{23}H_{24}O_9N_5S_2Cl$.Colour:cream.

***N*-(3-(tetra-*O*-acetyl-β-D-glucopyranosyl)-1H(1,2,4 thiadiazolo (3,4-C)(1,2,4)thiadiazol-5(3H) ylidene) o-anisylamine (5f)**

The molecular formula of 5f was established as $C_{24}H_{27}O_{10}N_5S_2$.Colour: cream

***N*-(3-(tetra-*O*-acetyl-β-D-glucopyranosyl)-1H(1,2,4 thiadiazolo (3,4-C)(1,2,4)thiadiazol-5(3H) ylidene) p-anisylamine (5g)**

IR (KBr) ν max cm^{-1}: 3241 (N-H),3100 (C-H aromatic),1749 (C=O),1510 (C=N),1435 (C=C aromatic ring stretch),1036 (C-O),897 (Glycosidic C-H deformation β-anomer),748(C-S) ; ^{1}H NMR ($CDCl_3$) δ ppm: 11.34 (s,1H,NH), 7.22-6.76 (m,4H,Ar-H),5.75-4.94 and 4.25-3.81 (m,7H.,Glucosyl ring protons),3.73(Ar-OCH_3) 2.02 (m,12H,Acetyl protons); MS(m/z): 609 (M^+), 610 (M^++1) .The molecular formula of 5g was established as $C_{24}H_{27}O_{10}N_5S_2$..Colour: cream

***N*-(3-(tetra-*O*-acetyl-β-D-glucopyranosyl)-1H(1,2,4 thiadiazolo (3,4-C)(1,2,4)thiadiazol-5(3H) ylidene) o-chlorophenylamine (5h)**

The molecular formula of 5h was established as $C_{23}H_{24}O_9N_5S_2Cl$ Colour: cream.

3.4 Biological Screening of 5:

The compounds *N*-(3- tetra-*O*- acetyl β-D-glucopyranosyl)-1H (1,2,4-thiadiazolo (3,4-C) (1,2,4) thiadiazol-5(3H) ylidene) arylamine (5a to5h) synthesized here exhibited very good antibacterial (table 2) and antifungal activity (table 3). Inhibition zone record of the compounds showed that, the compounds 5a, 5b,5c,5e were highly sensitive against E.coli and S.aureus and other compounds were resistant.

Compounds 5b,5c, 5e and5g showed high sensitivity against both the fungus A.niger and C.albicans. Compound 5a showed high sensitivity against A.niger only and resistant to C.albicans. Other compounds were resistant to both the bacteria and fungus.

3.5 Table-2:- Antibacterial activity of *N*-(3- tetra O acetyl β-D-glucopyranosyl) -1H (1,2,4-thiadiazolo (3,4-C) (1,2,4) thiadiazol-5(3H) ylidene) arylamines (5a to 5h)

Organism	5a	5b	5c	5d	5e	5f	5g	5h
E.coli	+++ 30 mm	+++ 30 mm	+++ 60 mm	-	+++ 70 mm	-	-	-
S.aureus	+++ 30 mm	+++ 40 mm	+++ 70 mm	-	+++ 30 mm	-	-	-

(Diameter of inhibition zone in mm) (Concentration 500 μ g/ml)

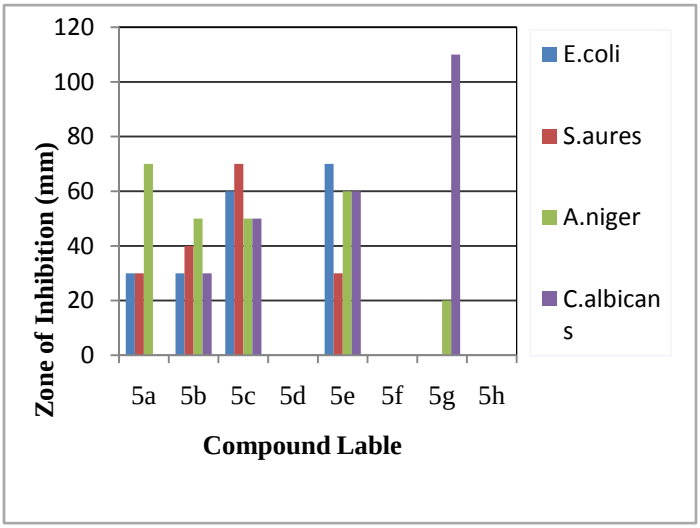
3.6 Table-3:- Antifungal activity of N-(3- tetra O acetyl β-D-glucopyranosyl) -1H (1,2,4-thiadiazolo (3,4-C) (1,2,4) thiadiazol-5(3H) ylidene) arylamines (5a to 5h)

Organism	5a	5b	5c	5d	5e	5f	5g	5h
A.niger	+++ 70 mm	+++ 50 mm	+++ 50 mm	-	+++ 60 mm	-	+++ 20 mm	-
C.albicans	-	+++ 30 mm	+++ 50 mm	-	+++ 60 mm	-	+++ 110 mm	-

(Diameter of inhibition zone in mm) (Concentration 500 μ g/ml)

3.7 Graph showing antibacterial and antifungal activity of N-(3- tetra O acetyl β-D-glucopyranosyl) -1H (1,2,4-

thiadiazolo (3,4-C) (1,2,4) thiadiazol-5(3H) ylidene) arylamine(5a to 5h)



4. EXPERIMENTAL

Melting points are uncorrected and were measured using electro thermal apparatus. FT-IR spectra were recorded using KBr disk on Perkin Elmer FT-IR KBR spectrophotometer recorded with ν max in inverse centimeters. ^{1}H NMR spectra were recorded on Bruker avance-II 400 NMR spectrometer at 400 MHz in DMSO/$CDCl_3$ as solvent. The spectra were recorded using tetramethylsilane as internal standard and chemical shifts being reported in parts per million (δ) relative to TMS. The mass spectra were obtained using Waters Q-TOF Micromass instrument. The progress of the reaction was monitored by TLC on Merck Silica Gel 60 F_{254} plates with detection by UV light and I_2 vapours as visualizing agent. The compounds were screened for their antibacterial and antifungal activities by the agar diffusion method.

The reagents required for the reaction carried out in this chapter were prepared as follows.

4.1. Aryl /Alkyl isothiocyanates (1)

Aryl isothiocyanates were prepared by the procedure described in "Vogel's text book of Practical Oganic Chemistry"[19].

4.2. Preparation of amidino thiocarbamides (2)

Amidino thiocarbamides [20] have been prepared as reported earlier.

4.3. Synthesis of 3-amino-5 arylimino-1,2,4 thiadiazolines (3a-h)

3-Amino-5 arylimino-1,2,4 thiadiazolines [21] have been prepared as reported earlier

4.4. Preparation of 2, 3, 4, 6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate.

The *N*-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate[22-24] was synthesized by method reported earlier.

4.5. Preparation of *N*-tetra-*O*-acetyl-β-D glucopyranosylimino chloromethane sulfenyl chloride(4)

The synthesis of *N*-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate and its dichloro derivative i.e *N*-tetra- *O*-acetyl-β-D-glucopyranosylimino chloromethane sulfenyl chloride [25] was carried out by the extension of already known methods.

4.6.Preparation of *N*-(3-(tetra-*O*-acetyl-β-D-glucopyranosyl)-1H(1,2,4 thiadiazolo (3,4-C) (1,2,4)thiadiazol-5(3H) ylidene) arylamine (5)

These have been prepared by the interaction of 3 amino-5 arylimino1,2,4 thiadiazolines and *N* glucosylimino chloromethane sulphenyl chloride or *N*- tetra-*O*-acetyl-β-D-glucopyranosyl-S chloro isothiocarbamoly chloride($TAGNCSCl_2$.)

The 3- amino-5 arylimino 1,2,4 thiadiazoline 0.30 g to 0.50 g (0.0014 to 0.0022 mole) were placed in 50 ml round bottom flasks. Chloroform 5 ml was added to it. The solution of and *N*- tetra-*O*-acetyl-β-D-glucopyranosyl-S chloro isothiocarbamoly chloride 0.6699 g to 1.0764 g (0.0014 to 0.00234 mole) in 5ml chloroform were added in R.B and the reaction mixtures were refluxed for 4h.The brisk reaction with evolution of hydrogen chloride gas was noticed. After the completion of reaction, the solvent was distilled off, when residues of 4 were isolated. They were crystalized from ethanol to afford cream coloured crystals of 4.

4.7Antibacterial: Initially, the stock cultures of bacteria were revived by inoculating in broth media (peptone-10g, NaCl-10 g and Yeast extract 5g, Agar 20g in 1000ml of distilled water) and grown at 37 °C for18 hours. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 hour old cultures (100 µl,10^{-4} cfu) and spread evenly on the plate. After 20 minutes, the wells were filled with the solution of compounds at different concentrations. The control wells with Gentamycin were also prepared. All the plates were incubated at 37 °C for 24 hours and the diameter of inhibition zone were measured[26]

4.8 Antifungal: Potato dextrose agar 250g of peeled potato were boiled for 20 minutes and squeezed and filtered. To this filtrate 20 g of dextrose was added and the volume was made up to 1000 ml by distilled water. Initially, the stock cultures of fungi were prepared and wells were made in the plate. Each plate was inoculated with 48 hour old cultures (100 µl,10^{-4}cfu)and spread evenly on the plate .After 20 minutes, the wells were filled with solution of compounds at different concentrations .The control plates with antibiotic Amphotericin were also prepared. All the plates were incubated at 27 °C for 48 hours and the diameter of inhibition zone were measured[27]

5. CONCLUSION:-

Synthesis of fused thiadiazolo-thiadiazole have been achieved by simple cyclo condensation reaction between thiadiazoline and *N*- tetra-*O*- acetyl β-D-glucopyranosylimino chloromethane sulphenyl chloride. Method is simple, efficient and useful for the introduction of carbohydrate moiety on heterocyclic nucleus.

ACKNOWLEDGEMENT

The authors acknowledge the help of SAIF Chandigarh for providing the spectral data. The authors are thankful to Dr.H.D.Juneja, Head and Dr.L.J.Paliwal Ex-Head Post Graduate Department of Chemistry, RTM Nagpur University for providing laboratory facilities. MRU is thankful to Raisoni management for encouragement.

REFERENCES

1.Y.Inaba,T.Kawakami,S.Aimoto,T.;Ikegami,T.Takeuchi,T.Nakazawa,S.Yano,Y.Mikata,*Carbohydr.res*, 344(5), pp 613-626, 2009.
2.Y.S.Prabhakar,S.;Solomen,V.R.Gupta,M.K.Top.*Heterocycl. Chem*. 4,pp161-249,2006.
3.C.Hansch,A.Leo,.Substituent constants for correlation analysis in chemistry and biology; Wiley, New York,1979.
4.H.Singh,L.D.S.Yadav,*Agri.Biol.Chem*,40(4),pp759-764,1976,
5.I.Chaaban,O.O.Oji,*J.Indian Chem.Soc*, ,61,523-525,1984,.
6.A.Mohsen,M.E.Omer,O.M.Aboulwafa,*J.Heterocycl.Chem*.,21,pp1415-1418, 1984
7.S.P.Hiremath,J.S.Birador,S.M.Kudari,*J.IndianChem.Soc*.,61, pp74-76,1984.
8.A.E.Abdel Rahman, A.M.Mahmoud,*Rev.Roum.Chim*.,27, 781, 1982
9.T.N.Komarova,A.E.Aleksandrova,A.S.Nakhmanovich,;R. A.Shehegoleva,T.I. Vinogradova,.*Khim.Farm*.Zh., 23,1481, 1989
10.M.Carmack,L.M.Weinstock, US Patent 3066147, *Ref.Zh.Khim*.5N26P,1965.
11.Y.Pokach,G.W. Reader, US Patent 4094986;*Ref. Zh.Khim*., 3O380P,1979.
12.H.K.Shukla,N.C.;Desai,R.R.Astik,K.A. Thaker,*J.Indian Chem.Soc*.,61,168, 1984
13.L.M.Weinstock,US Patent 3488360;*Ref.Zh.Khim*.4N12P, 1971.
14.T.E.Glotova,A.E.Aleksandrova,A.S.Nakhmanovich,T.I. Vinogradova,.*Khim.-Farm.Zh*.,24,No.11,48, 1990
15.T.Hassel,H.T.Muller,*Angew.Chem.Int.Ed.Engl*.,26,4,359-360,1987
16.G.M.Dyson,T.Harrington,*J.Chem.Soc*.,191-194,1940
17.M.G.Dhonde,S.P.Deshmukh,*Chem.Heterocycl.Compd*.,41,6 ,787-791,2005.
18. G.Ottman,H.Hook,*J.Org.Chem*.,31,838-841, 1966.
19.B.S.Furniss,A.J.Hannaford,P.W.G.Smith,A.R.Tatchell, ."Vogel's Text Book of Practical Organic Chemistry,5th edn',Longman,U.K.,1989.
20. M.R.Ugale, B.N.Berad **,**1st National Conference on"Recent Innovations in Science, Engineering & Technology" -2016, ISBN 978-93-5254-677-0, 35-39
21. M.R.Ugale, B.N.Berad **,** International Conference on Quality Up-gradation in Engineering, Science & Technology(ICQUEST)-2016, ISBN: 978-81-923623-1-1.
22.S.P. Deshmukh,M.G.Paranjpe,*J.Indian Chem.Soc*. ,62, 375-376, 1985**.**
23. S.P.Deshmukh,M.G.Paranjpe,Proceeding of the Annual Convention of Chemists. ,*Indian Chemical Society* .Calcutta. Paper No.ORG(P)-65,1981,.
24.K.B.Bama,K.B,K.G.Rajani,*Indian J.Chem*.,27B,1157-1158,1988.
25.S.A.Chavan,B.N.Berad, A.G.Ulhe,*Am.J.PharmTech Res*.,4(2),356-364, 2014.
26. E.J.Threlfall , I.S.T.Fisher , L.Ward , E.H.Tschape & P. Gerner-Smidt , Harmonization of antibiotic susceptibility testing for *Salmonella:* Results of a study by 18 national reference laboratories within the European Union-funded Enter-Net group. *Microb. Drug Resist*., 5,195–199,1999
27. R.D Walker, J.F.Prescott ,J.D.Baggot, Antimicrobial susceptibility testing and interpretation of results. In*: Antimicrobial Therapy in Veterinary Medicine*, IA, Iowa State University Press, Ames, 12–26, 2000.