IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

SAR AND ANTIMICROBIAL ACTIVITY OF DESIGNED, SYNTHESIZED -1, 3-DISUBSTITUTED UREA ANALOGUES

Sushama S. Kadam[*1], Suryawanshi S.B[2]., G.M.Nazeruddin[3]

[1]Department of Chemistry, Faculty of Science, Dnyaneshwar Gramonnati Mandal's Hon. Balasaheb Jadhav Arts, Comm. & Sci. College, Ale, Tal-Junnar, Dist- Pune. 412411, Maharashtra, India.*

[2]Department of Chemistry (P.G. Centre, Faculty of Science, Hutatma Rajguru Mahavidyalaya, Rajgurunagar.Tal-Khed, Dist-Pune.410505 Maharashtra, India.

[3] Ex-Principal, Head, Dept. of Chemistry (P.G. Centre), Poona College of Arts, Science and Commerce. Camp, Pune. Maharashtra, India.

ABSTRACT

A series of 2, 5-disubstituted 1, 3, 4-thidiazole molecules were synthesized and modified free 2-NH_2 into their derivatives to enhance their biological activity. And among these synthesized analogues 1, 3, di substituted urea analogues were studied separately to highlight their application on basis of antimicrobial activity. 5-alkyl/aryl-1, 3, 4-thidiazole-2-amine as lead molecules were synthesized applying a new method. A strategic plan was made to synthesize their various derivatives by using amino acids and primary amine-cyclopentyl amine. The chemical structures of all new derivatives were established by IR, ^{1}HNMR, and mass spectra data. In bloom, the in vitro antibacterial and antifungal properties were tested against some human pathogenic microorganisms by employing the Kirby Bauer disc diffusion technique. All synthesized analogues of urea showed activity against the entire strain of microorganisms. In SAR study the relationship between the functional group variation and the biological activity of the evaluated compounds were well discussed. Based on the results obtained, all analogues were found to be very active compared to the standard drug chloramphenicol and basic moiety 5-phenyl-1, 3, 4-thidiazole-2-amine.

Keywords: 5-alkyl/aryl-1,3,4-thidiazole-2-amine, SAR, chloramphenicol, antibacterial and antifungal, Kirby Bauer disc diffusion technique.

ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
HUMAN

INTRODUCTION

Now a day's microbial infections and threat of antimicrobial resistance are a growing problem in contemporary medicine. To battle the infectious diseases use of antibiotics is unavoidable. The world sales of antibiotics are generally higher when compared to other drugs which are prescribed. Abnormality and mortality due to enteric bacterial infection is most common around the world and in specific regions such as the Indian subcontinent, part of South America and tropical part of Africa are worst affected[1.] Thus the deal with the global problem of bacterial dissemination remains a challenging therapeutic problem. In contravention of this many antibiotics and chemotherapeutics available, the emergence of old and new antibiotic resistant bacterial strains in the last decades constitutes a massive need for new classes of antibacterial agents.[2] Moreover a spread of resistance among the common respiratory pathogens was recognized as one of the three major areas of concern by the Infectious Diseases Society of America which creates a need for the development of newer antibiotics.[3] Literature survey reveals that urea and thiourea derivatives showed a broad spectrum of biological Activities as anti-HIV, antiviral, HDL- elevating antibacterial, analgesic properties. [4-7] Urea, which is a naturally occurring compound, was the first organic compound which has synthesized in lab by Wohler in 1928, and played important physiological and biological roles in animal kingdom. Synthesis of urea became a remarkable step in the history of synthetic organic chemistry [8] Some of the urea derivatives are thio-urea, phenyl urea, sulphur urea etc have been found to exhibit a potent inhibiting effect on HIV-1 protease enzyme and as an anticancer, anticonvulsive and sedative-hypnotic activities. They are also served as an extensive application as agrochemicals, resin precursors and synthetic intermediates. [9]

In this research paper we tried to emphasis on the 1,3-disubstitued urea analogues synthesis by ease method with good yield.5-(substituted)-1,3,4-thidiazole-2-amine acts as lead molecules which were easily synthesized. Past decade literature survey exhibit various pharmacological activities of this parent molecule. By taking account of all these and advantage of free 2-amine of 1, 3, 4-thidiazole various analogues were prepared by carbamate formation and then nucleophilic substitution.

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

MATERIALS AND METHODS

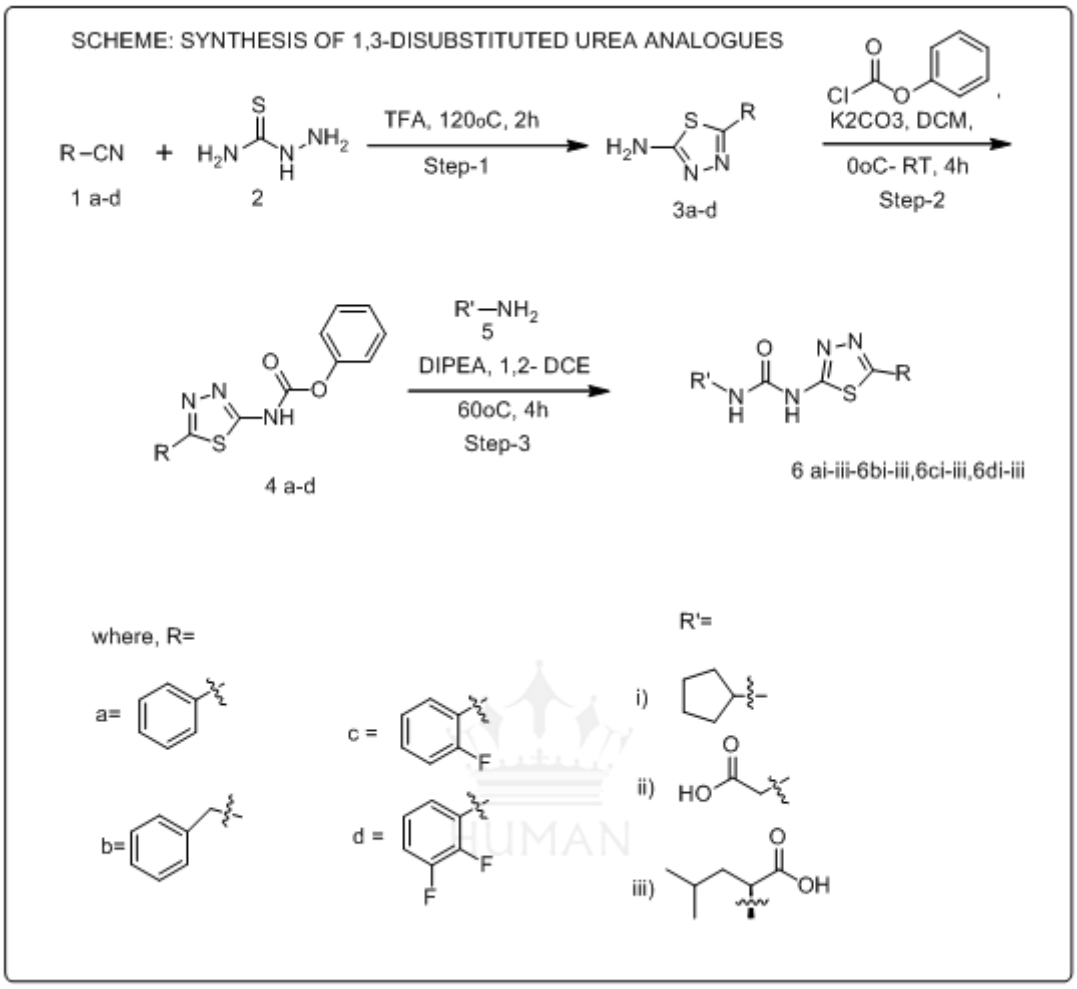
Material: Chemicals and reagents

All chemicals and reagents used in this study were purchase from Aldrich Chemicals and were used without further purification. Laboratory chemicals were supplied by Vijay Chemicals Ltd. Pune.

EXPERIMENTAL

All melting points were taken in open capillary tube and are uncorrected. The purity of the compounds was checked by TLC on pre coated SiO_2 gel (HF254, 200 meshes) readymade aluminium plates. Products were purified by column chromatography using solvent system pet ether: ethyl acetate (1:1/as per requirements) visualized in UV chamber to identify desired product spot. R_f values of the synthesised compounds were recorded. FTIR spectra using KBr pallets in the range of 4000-400 cm^{-1} were recorded with Perkin Elmer-838 spectrophotometer. The ^{1}HNMR spectra were determined with Brucker 400 MHz FT-IR spectrometer and mass spectra by HRMS. Elemental analyses of the entire compounds were the evaluated values.

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN



Step-I Synthesis of 5-alkyl/aryl-mono,di-fluro substituted phenyl-1,3,4-thidiazole-2-amine[10,11]

A mixture of alkyl/aryl-mono,di-fluro substituted phenyl nitrile (Aa-d) and thiosemicarbazide (B) in equimolar quantities taken in glass bottle dissolved in trifluoroacetic acid and sealed it using teflon tape and make it as glass bomb which kept in oil bath refluxed at 120^0C for 2hrs. The resultant mixture was slowly cooled to room temperature and poured on to crushed ice, stirred for 5 minutes. The solid separates out was filtered and crude products formation were confirmed by measuring R_f value and similarly purified by column chromatography using selective solvent system i.e. pet ether: ethyl acetate (80:20) as mobile phase moderate yield was obtained ,m.p. was determined and confirmed by ^{1}H NMR and FT-IR method.

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Step –II carbamate formation:

5-(substituted)-1,3,4-thidiazole-2-amine (1gm, 0.052 mole) in RB flask and DCM mixed with dry K_2CO_3 (2.2gm 0.155mole) stirred the reaction 0-5^oC, phenyl chloroformate (1.21gm, 0.077mole) was added slowly and continue stirring at room temp. The progress of reaction was monitored by TLC silica gel 60 F254. The resultant reaction mixture was extracted with DCM washed with water, brine, concentrated on rotary vacuum evaporator. A solid was separated, dried and purified by column chromatography using pet ether: ethyl acetate (80:20) as mobile phase. The desired product was obtained confirmed by TLC and directly used for next step synthesis.

Step-III Synthesis of 1, 3-disubstituted urea analogues (nucleophilic substitution): 6ai-iii, bi-iii, 6ci-iii, 6di-iii

A synthesized product of second step reacted with primary amines in solvent 1, 2- DCE and added DIPEA the solution was heated at 60^0C for 4 hour. The progress of reaction was monitored by TLC. After completion of the reaction, the reaction mixture was concentrated using rotary vacuum evaporator and obtained residue was purified by column chromatography using DCM: ethyl alcohol (80:20) as mobile phase. R_f values, melting points, elemental analysis of final target molecules were recorded and analyzed using IR, ^1H NMR and HRMS.

Spectral analysis of synthesized analogues of urea by IR, ^1H NMR:

6ai) *1-Cyclopentyl-3-(5-(2-phenyl)-1, 3, 4- thiadiazol-2-yl) urea:*

IR (KBr pallets)

3383.26 cm-1 (-NH-stretch –C=O -Amide) 3196.15 cm-1 (C-H stretch, aromatic) 2956.97 cm-1 (CH stretch) 1707cm-1 (C=O), 1635 cm-1(C=N) 1534 cm- 1 (C=C, aromatic), 1419 cm-1 (C-C stretch, aromatic), 1238.34 cm-1 (C-N stretch) 1238 cm-1 (C-O stretch),1093 cm-1, 990.44 cm-1 (C-H), 761 cm-1 (C-H stretch).

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

^{1}H NMR ($CDCL_3$, 200 MHz)

δ 1.79-1.57(m, 6H, Cyclopentyl), 2.09-2.04(m, 2H), 4.30-4.24(m, 1H), 6.04-6.01(d, 1H, *J*=6.25), 7.48-7.45(m, 3H, Aromatic), 7.89-7.85(m, 2H, Ar), 12.90 (bs, 1H, NH).

6aii) ***5-(2-Phenyl)-1,3,4-thiadiazol-2-yl)carbamoyl)glycine:***

IR (KBr pallets)

3386 cm-1 (-NH-stretch –C=O) 3075.60 cm-1 (C-H stretch, aromatic) 2759.19 cm-1 (C-H stretch) 1719cm-1 (C=O), 1938 cm-1(C=N) 1522 cm-1 (C=C, aromatic), 1415 cm-1 (C-C stretch, aromatic), 1142.34 cm-1 (C-N stretch) 1230 cm-1 (C-O), 1090 cm-1, 991.44 cm-1 (C-H), 821.35 cm-1, 751 cm-1 (C-H stretch).

^{1}H NMR (DMSO-d6, 200 MHz)

ɗ 3.89-3.87(d, 2H, *J*= 5.6Hz), 7.03-7.00(d, 1H, aromatic), 7.54-.47(m, 3H, aromatic), 7.91-7.86(m, 2H, aromatic), 11.39(bs, 1H, NH).

6aiii) ***(5-(2-Phenyl)-1, 3, 4-thiadiazol-2-yl)carbamoyl)-L-leucine:***

IR (KBr pallets)

3298 cm-1 (-NH-stretch –C=O) 3274 cm-1 (O-H stretch carboxylic acid) 2759 cm-1 (C-H stretch), 2959.25 cm-1(=C-H), 1938 cm-1 (C=N)1712cm-1 (C=O), 1651 cm-1546 cm-1 (C=C, aromatic), 1437cm-1 (C-H alkane), 1291.34 cm-1 (C-N stretch) 1321cm-1 (C-O stretch), 1090cm-1 (=C-H), 991.44 cm-1 (C-H) , 751 cm-1 (C-H bend).

^{1}H NMR (DMSO-d6, 200 MHz)

δ 0.94- 0.89 (m, 6H 2- methyl), 1.64 - 1.55 (m, 3H), 4.26 - 4.23 (m, 1H), 6.99-6.95 (d, *J* = 7.7 Hz, 1H), 7.54 - 7.49 (m, 3H), 7.91-7.86(m, 2H).

6bi) ***1-Cyclopentyl-3-(5-(2-benzyl)-1,3,4-thiadiazol-2-yl)urea:***

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

IR (KBr pallets)

3392.18 cm-1 (-NH-stretch –C=O) 3180.60 cm-1 (C-H stretch Ar-H) 2951.19 cm-1 (C-H stretch alkane) 1707cm-1 (H-C=O), 1635 cm-1(C=N) 1534 cm-1 (C=C, Ar), 1419 cm-1 (C-C stretch Ar-H), 1224.34 cm-1 (C-N stretch) 1238 cm-1 (C-O stretch), 1059 cm-1 (=C-H bend), 990.44 cm-1 (C-H) 761 cm-1 (C-H stretch).

^{1}H NMR (DMSO-d6, 200 MHz)

d ̂ 1.37-1.34(m,2H), 1.61-1.53(m,4H,Cyclopentyl), 1.83-1.80(m,2H), 3.96-3.86(m,1H), 4.26(s,2H), 6.60-6.56(d,1H,*J*=7 Hz), 7.38-7.22(m,5H, Ar-H), 10.40(bs,1H, NH).

6bii) ***(5-(2-Benzyl)-1,3,4-thiadiazol-2-yl) carbamoyl)glycine:***

IR (KBr pallets)

3379.40 cm-1 (carboxylic O-H) 3310.18 cm-1 (-NH-stretch –C=O) 3070.60 cm-1 (C-H stretch Ar-H) 2851.19 cm-1 (C-H stretch) 1701cm-1 (C=O), 1661 cm-1(C=N) 1528 cm-1 (C=C, Ar), 1418 cm-1 (C-C stretch Ar), 1228.34 cm-1 (C-N stretch) 1235 cm-1 (C-O stretch), 1166 cm-1, 991.44 cm-1 (C-H) , 755 cm-1 (C-H stretch).

^{1}H NMR (MeOD, 200 MHz)

δ 3.95 (s, 2H,-CH2), 4.25 (s,2H), 7.16 (bs, 2H, NH), 7.29 - 7.23 (m, 5H, aromatic), 11.62 (bs, 1H, OH).

6biii) ***(5-(2- Benzyl)-1, 3, 4-thiadiazol-2-yl) carbamoyl)-L-leucine:***

IR (KBr pallets)

3325.15 cm-1 (-NH-stretch –C=O) 3059.69 cm-1 (O-H stretch carboxylic acid) 2956.82 cm-1 (C-H stretch), 2959.25 cm-1(=C-H), 1701cm-1 (C=O), 1651 cm-1(C=N) 1537 cm-1 (C=C, Ar), 1437cm-1 (C-H alkane), 1220.99 cm-1 (C-N stretch) 1313cm-1 (C-O stretch), 1057 cm-1 (=C-H), 991.44 cm-1 (C-H), 751 cm-1 (C-H bend).

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

^{1}H NMR (DMSO-d6, 200 MHz)

ḋ 0.93-0.87(m, 6H, 2 CH3), 1.66-1.52(m, 3H), 4.20-4.17(m, 1H), 4.30(s, 2H), 7.10-7.06(d, 1H, J=4.5 Hz), 7.38-7.28(m, 5H), 11.42 (bs, 1H, OH).

6ci) ***1-cyclopentyl-3-(5-(2-fluorophenyl)-1, 3, 4-thiadiazol-2-yl) urea:***

IR (KBr pallets): 3385.18 cm-1 (-NH-stretch –C=O) 3190.60 cm-1 (C-H stretch, aromatic) 2951.19 cm-1 (C-Hstretch) 1707cm-1 (C=O), 1635 cm-1(C=N) 1534 cm-1 (C=C, aromatic), 1419 cm-1 (C-C stretch ,aromatic), 1238.34 cm-1 (C-N stretch) 1238 cm-1 (C-Ostretch),1093 cm-1, 990.44 cm-1 (C-H) ,831.35 cm-1 (C-F stretch) 761 cm-1 (C-H stretch).

^{1}H NMR (DMSO-d6, 200 MHz) δ 1.43 - 1.49 (m, 2Hcyclopentyl),

1.54 - 1.72 (m, 4H cyclopentyl), 1.85 -1.91 (m, 2H-cyclopentyl), 3.93 - 4.03 (m, 1H), 6.69 (d, *J* = 8.1 Hz, 1H NH-cyclopentyl), 7.34 - 7.59 (m, 3H,aromatic), 8.14 - 8.22 (m, 1H-cyclopentyl), 10.73 (bs, NH)

6cii) ***5-(2-fluorophenyl)-1,3,4-thiadiazol-2-yl)carbamoyl)glycine*** **:**

IR (KBr pallets): 3315.18 cm-1 (-NH-stretch –C=O) 3072.60 cm-1 (C-H stretch ,aromatic) 2861.19 cm-1 (C-H stretch) 1710cm-1 (C=O), 1651 cm-1(C=N) 1522 cm- 1 (C=C, Ar), 1415 cm-1 (C-C stretch aromatic), 1218.34 cm-1 (C-N stretch) 1230 cm-1 (C-O stretch),1090 cm-1, 991.44 cm-1 (C-H) ,821.35 cm-1 (C-F stretch) 751 cm-1 (C-H stretch).

^{1}H NMR (DMSO-d6, 400 MHz): δ 3.88 (d, *J*= 5.6 Hz, 2H, -CH2), 7.01 (bs, 1H), 7.36 - 7.46 (m, 2H, Ar-H), 7.54 - 7.59 (m, 1H, Ar-H), 8.19 (t, J = 4.8 Hz, 1H),

11.42 (bs, 1H ,NH)

6ciii) ***(5-(2-fluorophenyl)-1, 3, 4-thiadiazol-2-yl) carbamoyl)-L-leucine:***

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

IR (KBr pallets):

3325.15 cm-1 (-NH-stretch –C=O) 3059.69 cm-1 (O-H stretch carboxylic acid) 2871.82 cm-1 (C-H stretch),2959.25 cm-1(=C-H), 1712cm-1 (C=O), 1651 cm-1(C=N) 1546 cm-1 (C=C, aromatic), 1437cm-1 (C-H alkane), 1291.34 cm-1 (C-N stretch)

1243cm-1 (C-O stretch), 1090 cm-1 (=C-H), 991.44 cm-1 (C-H), 825.35 cm-1 (C-F stretch) 751 cm-1 (C-H bend).

^{1}H NMR (DMSO-d6, 200 MHz):

δ 0.88 - 0.93 (m, 6H 2- methyl), 1.52 - 1.68 (m, 3H), 4.22 - 4.29 (m, 1H), 7.08 (d, *J* = 7.7 Hz, 1H), 7.35 - 7.59 (m, 3H), 8.19 (d, J =8.0 Hz, 1H)

6di) ***1-cyclopentyl-3-(5-(2,3-difluorophenyl)-1,3,4-thiadiazol-2-yl)urea:***

3180.60 cm-1 (C-H stretch Ar-H) 2951.19 cm-1 (C-H stretch alkane) 1707cm-1 (H-C=O), 1635 cm-1(C=N) 1534 cm-1 (C=C, Ar), 1419 cm-1 (C-C stretch Ar), 1224.34 cm-1 (C-N stretch) 1238 cm-1 (C-O stretch),1059 cm-1 (=C-H bend), 990.44 cm-1 (C-H) ,831.35 cm-1 (C-F stretch) 761 cm-1 (C-H stretch).

^{1}H NMR (CDCl3, 200 MHz):

δ 1.57 -1.77 (m, 6H, cyclopentyl), 2.00 - 2.10 (m, 2H ,cyclopentyl), 4.25 -4.31 (m, 1H-cyclopentyl), 5.90 (d, J = 6.8 Hz, 1H, NH near to cyclopentyl), 7.15 - 7.34 (m, 2H, aromatic), 7.86 - 7.91 (m, 1H, aromatic), 12.91 (bs, 1H, NH-)

6dii) ***((5-(2,3-difluorophenyl)-1,3,4-thiadiazol-2-yl) carbamoyl)glycine:***

IR (KBr pallets):

3310.18 cm-1 (-NH-stretch –C=O) 3070.60 cm-1 (C-H stretch Ar-H) 2851.19 cm-1 (C-H stretch) 1715cm-1 (C=O), 1661 cm-1(C=N) 1528 cm-1 (C==C, Ar), 1418 cm-1 (C-C stretch Ar), 1228.34 cm-1 (C-N stretch) 1235 cm-1 (C-O stretch),1090 cm-1, 991.44 cm-1 (C-H) ,831.35 cm-1 (C-F stretch) 755 cm-1 (C-H stretch).

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

^{1}H NMR (DMSO-d6, 200 MHz):

δ 3.88 (s, 2H,-CH2), 7.16 (bs, 1H, NH), 7.37 - 7.39 (m, 1H, aromatic), 7.53 - 7.66 (m, 1H, aromatic), 7.93 - 8.00 (m, 1H, aromatic), 11.62 (bs, 1H, OH)

6diii) *((5-(2, 3-difluorophenyl)-1, 3, 4-thiadiazol-2-yl) carbamoyl)-L-leucine:*

3059.69 cm-1 (O-H stretch carboxylic acid) 2956.82 cm-1 (C-H stretch),2959.25 cm-1(=C-H), 1701cm-1 (C=O), 1651 cm-1(C=N) 1537 cm-1 (C=C, Ar), 1437cm-1 (C-H alkane), 1220.99 cm-1 (C-N stretch) 1313cm-1 (C-O stretch),1057 cm-1 (=C-H), 991.44 cm-1 (C-H) ,881.35 cm-1 (C-F stretch) 751 cm-1 (C-H bend).

^{1}H NMR (DMSO-d6, 400 MHz):

δ 0.88 - 0.93 (m, 6H, 2 CH3), 1.62 - 1.68 (m, 3H), 4.21 (bs, 1H), 7.38 (m, 2H), 7.59 - 7.61 (m, 1H), 7.97 - 7.99 (m, 1H), 11.42 (bs, 1H, OH)

Table-01 Data of physical constants, HRMS and elemental analysis

Entry	M.P ^{0}C	R_f	Yield (%)	Molecular Formula	Mol.Wt (exact mass)	Observed Mass by (HRMS)	Elemental analysis calculated %				
							C	H	F	N	S
6ai	285	0.66	65	$C_{13}H_{14}N_4O_2S$	290.0837	291.0919 (M+H)	53.78	4.86	11.02	19.43	11.14
6aii	287	0.55	62	$C_{14}H_{16}N_4OS$	288.1045	289.1130 (M+H)	58.31	5.59	5.55	20.13	11.52
6aiii	185	0.65	60	$C_{11}H_{10}N_4O_3S$	278.0474	279.0562 (M+H)	47.48	3.62	17.25	16.73	9.59
6bi	163	0.50	63	$C_{15}H_{18}N_4O_3S$	334.11	335.1178 (M+H)	53.88	5.43	14.35	18.53	10.60
6bii	280	0.72	72	$C_{14}H_{16}N_4O_2S$	304.0994	305.1079 (M+H)	55.25	5.30	10.51	19.17	10.97
6biii	282	0.65	65	$C_{15}H_{18}N_4OS$	302.1201	303.1288 (M+H)	59.58	6.00	5.29	16.08	9.20
6ci	175	0.52	73	$C_{12}H_{12}N_4O_3S$	292.063	293.0708 (M+H)	49.31	4.14	16.42	18.29	10.46
6cii	180	0.75	64	$C_{16}H_{20}N_4O_3S$	348.1256	349.1342 (M+H)	55.16	5.79	13.78	18.91	10.82
6ciii	160	0.80	73	$C_{15}H_{17}FN_4O_3S$	352.1005	353.1077 (M+H)	51.13	4.86	5.39	15.90	9.10
6di	270	0.58	75	$C_{14}H_{14}F_2N_4OS$	324.0856	325.0934 (M+H)	51.84	4.35	11.71	17.27	9.88
6dii	185	0.72	73	$C_{11}H_8F_2N_4O_3S$	314.0285	315.0368 (M+H)	42.04	2.57	12.09	17.87	10.20
6diii	162	0.78	50	$C_{15}H_{16}F_2N_4O_3S$	370.0911	371.0987 (M+H)	48.64	4.35	10.26	15.13	8.66

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Table No.02 In vitro antimicrobial activity of synthesized Derivatives (6ai-iii, 6bi-iii,6ci-iii,6di-iii)

Entry	Antibacterial data in zone of inhibition(mm)				Antifungal data in zone of inhibition (mm)	
	Gram + Ve Bacteria		Gram-Ve Bacteria		Fungi	
	S. aureus A*	*B. subtilis* B*	*E. coli* C*	*E.aerogenus* D*	*A.niger* E*	*P.chrysogenum* F*
6ai	13.4	14.4	16.2	16.8	12.5	….
6aii	14.4	13.6	15.2	15.2	11.5	….
6aiii	10.2	17.6	8.2	11.6	11.5	10
6bi	..	…	12.8		13	7.25
6bii	…	…	…		12	9.5
6biii	…	8.6	14.2		9.2	8.5
6ci	13.4	---	9.6	13.2	7	8.25
6cii	10.6	8	10.6	14.6	8.7	11.5
6ciii	13.2	--	14.6	16.4	8	--
6di	10.6	12.6	14	16	14	7
6dii	12.4	12.4	14.2	11.6	12.5	9.75
6diii	11.2	---	12	16.6	10	--
STD	10	10	10	10	10	10
TM	10.8	10.4	9.8	10	15.2	15.25

Highly active = (inhibition zone > 16 mm) moderately active = (inhibition zone 10 - 16mm)

Table no.03 Minimum inhibition concentration of selected derivatives

Sr. No.	Bacteria	Concentration(ug/ml) MIC FOR TGA				MIC FOR 6biii				MIC FOR 6aiii			
		50	100	150	200	25	50	100	200	25	50	100	200
1	A*	---	++	++	++	+	+	+	+	_	+	+	+
2	B*	__	++	++	++	_	+	+	+	_	_	+	+
3	C*	__	++	++	++	+	+	+	+	_	+	+	+
4	D*	__	++	++	++	+	+	+	+	+	+	+	+
5	E*	++	++	++	++	+	+	+	+	+	+	+	+
6	F*	++	++	++	++	_	+	+	+	_	+	+	+

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

6bii-*((5-(2, 3-difluorophenyl)-1, 3, 4-thiadiazol-2-yl)carbamoyl)glycine* **6aii-** ***(5-(2-fluorophenyl)-1, 3, 4-thiadiazol-2-yl) carbamoyl)glycine***

RESULTS AND DISCUSSION

The synthesis of the urea analogues was practised in three steps and the synthetic strategy is outlined in scheme. It has been noted that electron withdrawing group present in the phenyl system of the parent molecule gave more percentage yield than that of unsubstituted phenyl ring. To study the lead compound and find out the parts of the molecule are important to activity so that they can be retained in new analogues (i.e. structure activity relationship)

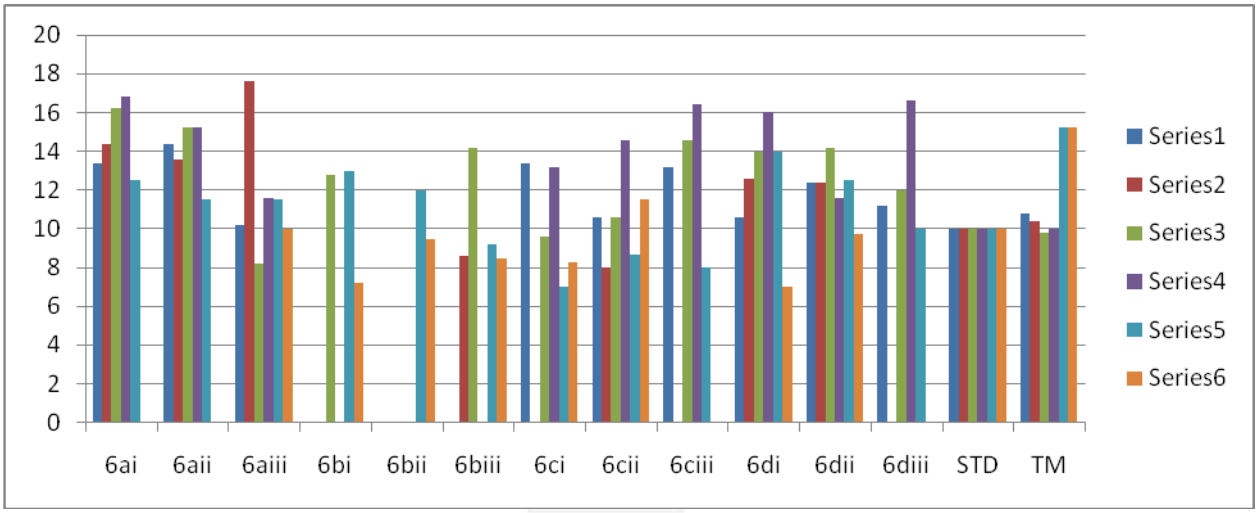
Antibacterial and Antifungal activity:-

Antibacterial and antifungal activity of newly twelve synthesized analogues of urea (6ai-iii 6bi-iii, 6ci-iii,6di-iii) were done by using Kirby Bauer disc diffusion method using antibiotic chloroamphenicol as a standard . The medium used for the maintenance of bacterial culture was Nutrient agar and for Fungal cultivation Potato Dextrose Agar. For zone inhibition experiment the culture medium used was Muller Hinton Medium. All medium were of HI-Media. The synthesized analogues were dissolved in DMF and antimicrobial activities were carried out at a concentration of 25-200µg/ml (minimum inhibition concentration) the lowest conc. of an antimicrobial that will inhibit the visible growth of microorganism after overnight incubation.

Table No.3 showed that antifungal activity of urea analogues were excellent in lower concentration while bacterial strain inactive at small concentration 50µg/ml otherwise all microorganism were active. From table no.02 bar graph is plotted to explain SAR based on effect of EWG substituent connected to phenyl showed hydrophobic part but activates the moiety to enhance antimicrobial activity.

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Bar Graphs: To show the zone of inhibition (mm) activity of various synthesized analogues of 1, 3-di substituted urea against human pathogenic bacteria and fungal strains *(A* Staphylococcus aureus*, *B* Bacillus subtilis* (Gram positive bacteria), C* ***Escherichia coli , D* Enterobacter aerogenes*** (Gram negative bacteria) **E* - *Aspergillus niger* F*- *Penicillium chrysogenum*** (Fungus).**TM- *5-Phenyl-1, 3, 4-Thidiazole-2-Amine* STD-Chloramphenicol.**



From this bar graph clear idea regarding SAR study i.e. alkyl substituted 1,3,4-thidiazole moiety showed nil effect against *S.aureus* due to hydrophobic character of substituent next when substituent were changed i.e hydrophobic group with electron group withdrawing para phenyl, mono, di fluro substituted fluorine connected to moiety observed increase in antimicrobial property. The phenyl substituted Alkyl substituted analogues remained weak due to hydrophobic character .With literature survey such types of synthesized molecules remain useful to microbial infection and may be used in medicine after several clinical trial such as treatment of urinary tract infections, eye lotion, treatment of infections of mucous membrane, treatment of gut infection.

CONCLUSION

1, 3-di-substituted urea analogues were remained good substituent for early antimicrobial agents used in medicines. Variation in side chain of linkage urea alters the antimicrobial

activity. To extend the study of these molecules as protein kinase inhibitor in future is aim to this research.

ACKNOWLEDGEMENT

The authors would like to express gratitude and thanks to guide Dr. G. M. Nazeruddin, Dr. Naveen Patel for his valuable guidance in research work. Very much thankful to Chairman and all members of Dnyaneshwar Grammonati Mandal Education Institute, Ale, Dist-Pune, Maharashtra for their encouragement. Thanks to Principal and Head of Chemistry Department of my college for their help. Our sincere thanks to NCL, Pune, IISER. Pune Savitribai Phule Pune University, Vishal Institute of Pharmacy, Ale, Junnar for spectral analysis of samples.

REFRENCES

1. Qadri F. Svennerholm A.M. Faruque A.S.G.,Sack R.B.(2005) Enterotoxigenic Eschericia coli in developing countries: Epidemiology,microbiology, clinical Features, treatment, and prevention.Clin. Microbiol. Rev. 18, 465–483.
2. Chopra I. Schofield C. Everett,M. Oneill A. Miller K.,Wilcox M. Frere J.M, Dawson M. Czaplewski L. Urleb U. Courvalin,P. (2008). Treatment of health care associated infections caused by Gram negative bacteria: A consensus statement. Lancet Infect. Dis. 8, 133–139
3. Spellberg B. Guidos R. Gilbert D. Bradley J. Boucher H. Scheld W T. Bartlett J. Edwards J. (2008). The epidemic of antibiotic resistant infections: A call to action for the medical community from the infectious diseases society of america. Clin. Infect. Dis. 46,155–164
4. Struga M, Kossakowski J, Kedzierska E, Fidecka S, Stefańska J (2007) 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 (Tokyo) 55, 796-799.
5. Venkatachalam T K, Mao C, Uckun F M (2004) Effect of stereochemistry on the anti-HIV activity of chiral thiourea compounds. Bioorg Med Chem 12, 4275-4284.
6. Bloom J D, Dushin R G, Curran K J, Donahue F, Norton E B, et al. (2004) Thiourea inhibitors of herpes viruses. Part 2: N-Benzyl-N'-arylthiourea inhibitors of CMV. Bioorg Med Chem Lett 14, 3401-3406.
7. Lee J, Lee J, Kang M, Shin M, Kim J M, et al. (2003) N-(3-acyloxy-2-benzylpropyl)-N'-[4-(methylsulfonylamino)benzyl]thiourea analogues: novel potent and high affinity antagonists and partial antagonists of the vanilloid receptor. J Med Chem 46, 3116-3126.
8. Perveen S, Mustafa S, Khan M A, Dar A, Khan K M, et al. (2012) Substituted urea derivatives: a potent class of antidepressant agents. Med Chem 8, 330-336.
9. Viana G M, Aguiara L C, Ferrão J A, Simas A BC, Vasconcelos M G (2013) The use of aqueous potassium dichloroiodate for the synthesis of ureas. Tetrahedron Letters 54, 936-940
10. Sushama S. Kadam, G. M. Nazeruddin synthesis of 5-aryl/alkyl-1,3,4-thidiazole-2-amine & their derivatives using aryl /alkyl nitriles and study of their antimicrobial activity World Journal of Pharmaceutical Research 5(2), 2016 1456-1466.

ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
HUMAN

11. Sushama S. Kadam, G. M. Nazeruddin (2015) synthesis, characterization and antimicrobial activity of 5-flurosubstituted phenyl - 1, 3, 4-thiadiazol-2-amine and their derivatives Int. J. Curr. Res. Chem. Pharma. Sci. 2(12), XX–XX