WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 5, Issue 2, 1456-1466. Research Article ISSN 2277– 7105

# SYNTHESIS OF 5-ARYL/ALKYL-1,3,4-THIDIAZOLE-2-AMINE&THEIR DERIVATIVES USING ARYL /ALKYL NITRILES AND STUDY OF THEIR ANTIMICROBIAL ACTIVITY

**Sushama S. Kadam[1*] and G. M. Nazeruddin[2]**

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

[2]Principal, Head, Dept. of Chemistry (P.G. Center), Poona College of Arts, Science and Commerce. Camp, Pune. Maharashtra, India.

Article Received on
15 Dec 2015,

Revised on 05 Jan 2016,
Accepted on 25 Jan 2016

***Correspondence for Author**
**Sushama S. Kadam**
Department of Chemistry, Dnyaneshwar Gramonnati Mandal's Hon. Balasaheb Jadhav Arts, Comm. & Sci. College, Ale, Tal-Junnar, Dist- Pune. 412411, Maharashtra, India.

## ABSTRACT

Thiadiazole is a versatile moiety that exhibits a wide variety of activity due to the presence of N=C-S present 1,3,4-thidiazole in the ring. They have become an important class of heterocyclic compounds of great interest of researches because of their broad types of biological activity. In the present research work reported an efficient way to synthesize of 5-phenyl/benzyl-1,3,4-thidiazole-2-amine and their derivatives from aryl/ alkyl nitriles. The structures and elemental analysis of novel synthesized derivatives were confirmed by various ways of spectral analysis. Study of antimicrobial property was done by Kirby Bauer Disc diffusion method using standard antibiotic chloroamphenicol.

**KEYWORDS:** Heterocyclic compounds, 5-phenyl/benzyl-1,3,4-thidiazole-2-amine, chloroamphenicol, antimicrobial etc.

## INTRODUCTION

The fight against bacterial infections over the last 70 years has been one of the great success stories of medicinal chemistry, yet it remains to be seen whether it will last bacterial infection is still major cause of death in developing world. For example the World Health Organization estimated that in year 2002, 1.9 million children died worldwide of respiratory infections with 70% of these deaths occurring in Africa and Asia Bacteria such as *Staphylococcus aureus* have the worrying ability to gain resistance to known drugs and so the search for new

drug is never ending.[1] A survey of the literature revealed that differently substituted 1,3,4-thiadiazoles and annelated 1,3,4-thiadiazoles have wide range of pharmacological activities such as antibacterial, antifungal, antituberculosis, antihepatitis B viral, antileishmanial, anti-inflammatory analgesic, CNS depressant, anticancer, antioxidant, antidiabetic, molluscicidal, antihypertensive, diuretic, analgesic, antimicrobial, antitubercular, and anticonvulsant activities.[2–12] In our research work we would like synthesized novel derivatives of 5-phenyl/benzyl-1,3,4-thidiazole-2-amine with the aim of new antibacterial and antifungal drugs development. In our previous study, one of thiadiazole derivatives, namely 5-(2,3-difluorophenyl)-1,3,4-thiadiazol-2-yl) carbamoyl) glycine was reported potent antimicrobial property[13]. This observation had an impact on our further work on the synthesis and it promotes to continue search for some novel derivatives of thiadiazoles with the antimicrobial activity. Previously synthesized and antimicrobial activity evaluated 5-mono&di-fluro-substituted phenyl-1,3,4-thidiazole-2-amine and their derivatives are compared with newly synthesized and anti microbial property of 5-mono&di-fluro-substituted phenyl-1,3,4-thidiazole-2-amine and their derivatives. The structures of the synthesized compounds were elucidated using UV, IR, $^{1}$H NMR, mass spectroscopy and elemental analysis.

## 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.

## ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

Antibacterial and antifungal activity of newly eight synthesized derivatives ($6a_{i\text{-}iv}$ $6b_{v\text{-}viii}$) of 5-phenyl/benzyl-1,3,4-thidiazole-2-amine were done by using Kirby Bauer Disc Diffusion method using antibiotic chloroamphenicol as a standard antibiotic.

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.

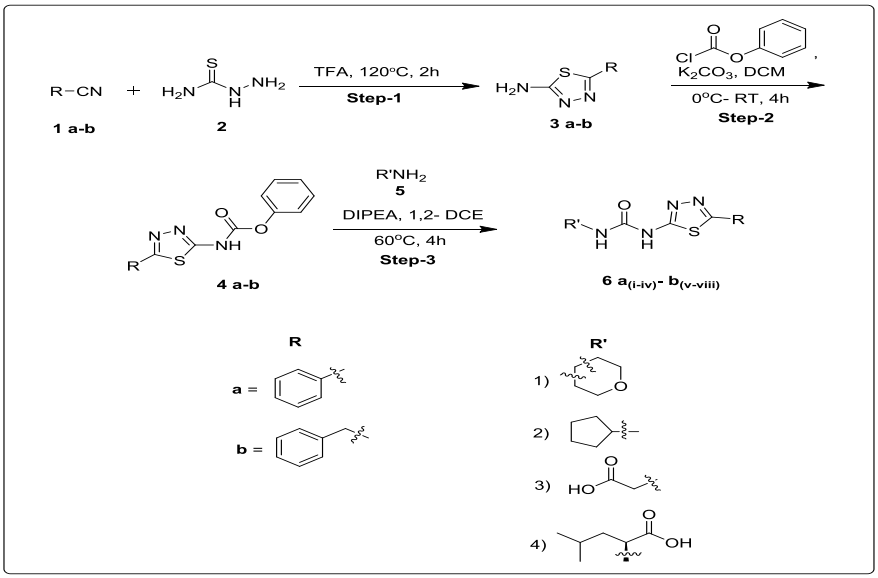
The antibacterial activity tested against microorganism used as *Staphylococcuc aureus, Bacillus subtilis,* (Gram positive bacteria) *Escherichia coli and Enterobacter aerogenes* (Gram negative bacteria).

The antifungal activity tested against microorganism used as *Aspergillus niger* and *Penicillium chrysogenum.*

The synthesized compounds were dissolved in DMF and antimicrobial activities were carried out at a concentration of 25-200µg/ml (minimum inhibition concentration- MIC) the lowest conc. of an antimicrobial that will inhibit the visible growth of microorganism after overnight incubation.

## EXPERIMENTAL

All melting points were taken in open capillary tube and are uncorrected. The purity of the compounds was checked by TLC on precoated $SiO_2$ gel (HF254, 200 meshes) readymade aluminium plates (E Merck). Products were purified by column chromatography using solvent system Pet Ether: Ethyl Acetate (1:1/as per requirements) visualized in UV chamber to identify it. $R_f$ 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 newly synthesized compounds were performed on Carlo Erba 1108analyzer. Elemental analysis of the entire compounds were in agreement with the calculated values.




**Scheme for Synthesis of -phenyl/benzyl-1,3,4-thidiazole-2-amine and Their Derivatives.**

**Synthesis of 5-phenyl/benzyl-1,3,4-thidiazole-2-amine from phenyl/benzyl nitrile (3a-b)[13]**

A mixture of unsubstituted phenyl nitrile (Ia-b) and Thiosemicarbazide (II) 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 $120^0$C for 2 hours on Hot Plate with magnetic stirrer apparatus. 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 using readymade TLCs Silica gel 60 F254 by selective solvent system purified by column chromatography using pet ether: ethyl acetate (80:20) as mobile phase. Yield was 85%. M.P. $245^0$C confirmed by $^1$H NMR and FT-IR method.

**Synthesis of 5-phenyl/benzyl-1,3,4-thidiazole-2-phenyl acetate(4a-b) (carbamate formation) [13]**

5-phenyl/benzyl-1,3,4-thidiazole-2-amine (Compound-III 1gm, 0.052 mole) in RB flask and DCM mixed with dry $K_2CO_3$ (2.2gm 0.155mole) stirred the reaction 0-5$^o$C, phenyl chloroformate (1.21gm, 0.077mole) was added slowly and continue stirring with the help of microsyringe overnight 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 synthesis.

**Synthesis of N-(5-(unsubstituted phenyl)-1, 3, 4-Thiadiazol-2-yl) N-containing compound-4-carboxamide (Nucleophilic Substitution)[13] $6a_{i\text{-}iv}b_{v\text{-}viii}$**

A mixture of 5-(unsubstituted phenyl) -1, 3, 4- Thidiazol-2-phenyl acetate and N- containing molecules such as (morpholine, cyclopentyl amine, Glycine and L-leucine) one by one from $6a_{i\text{-}iv}b_{v\text{-}viii}$ dissolved in 1, 2- dichloroethane and added DIPEA (N, N-Di-isopropylethylamine) the solution was heated at $60^0$C for 4 hour. The progress of reaction was monitored by TLC using TLC Silica gel 60 F254. during completion of the reaction, the reaction mixture was concentrated using rotary vacuum evaporator and obtained residue was purified by column chromatography using pet ether: ethyl acetetate (80:20) as mobile phase. Rf values, melting points, elemental analysis of final target molecules were recorded and analyzed using- IR, $^1$H NMR and HRMS.

**Table no. 01: Physical constants and micro analytical data.**

| Comp No | M.P $^0$C | $R_f$ | Yield (%) | Molecular Formula | Molecular Weight (exact mass) | Observed Mass by (HRMS) | Elemental analysis calculated % | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | C | H | O | N | S |
| **$6a_i$** | 285 | 0.66 | 85.30 | $C_{13}H_{14}N_4O_2S$ | 290.0837 | 291.0919 (M+H) | 53.78 | 4.86 | 11.02 | 19.30 | 11.04 |
| $6a_{ii}$ | 287 | 0.55 | 72.62 | $C_{14}H_{16}N_4OS$ | 288.1045 | 289.1130 (M+H) | 58.31 | 5.59 | 5.55 | 19.43 | 11.12 |
| $6a_{iii}$ | 185 | 0.65 | 60.60 | $C_{11}H_{10}N_4O_3S$ | 278.0474 | 279.0562 (M+H) | 47.48 | 3.62 | 17.25 | 20.13 | 11.52 |
| $6a_{iv}$ | 163 | 0.50 | 63.30 | $C_{15}H_{18}N_4O_3S$ | 334.11 | 335.1178 (M+H) | 53.88 | 5.43 | 14.35 | 16.76 | 9.59 |
| $6b_v$ | 280 | 0.72 | 72.12 | $C_{14}H_{16}N_4O_2S$ | 304.0994 | 305.1079 (M+H) | 55.25 | 5.30 | 10.51 | 18.41 | 10.53 |
| 6bvi | 282 | 0.65 | 65.10 | $C_{15}H_{18}N_4OS$ | 302.1201 | 303.1288 (M+H) | 59.58 | 6.00 | 5.29 | 18.53 | 10.60 |
| 6bvii | 175 | 0.52 | 73.78 | $C_{12}H_{12}N_4O_3S$ | 292.063 | 293.0708 (M+H) | 49.31 | 4.14 | 16.42 | 19.17 | 10.97 |
| 6bviii | 165 | 0.68 | 60.15 | $C_{16}H_{20}N_4O3S$ | 348.1256 | 349.1342 (M+H) | 55.16 | 5.79 | 13.78 | 16.08 | 9.20 |

**THE PHYSICAL AND SPECTRAL DATA OF THE NOVEL SYNTHESIZED DERIVATIVES [6a(i-iv) –6b(v-viii)]**

**6ai)** ***N-(5-(2-Phenyl)-1, 3, 4-thiadiazol-2-yl) morpholine-4-carboxamide***

White solid, Yield 85 %, M.P 285$^0$C, M.F $C_{13}H_{14}N_4O_2S$, Mol. Wt. (expected) 290.0837, Mol .Wt. (observed) 291.0919 (M+H) (M+H) by HRMS.

**IR (KBr pallets)**

3429 cm$^{-1}$(-NH-stretch –C=O) 3201 cm$^{-1}$ (C-H stretch, aromatic) 2968cm-1 (=C-H) 1795cm$^{-1}$ (C=O), 2357 cm$^{-1}$(C=N) 1534 cm$^{-1}$ (C=C, aromatic), 1419 cm$^{-}$ (C-C stretch aromatic), 1247 cm$^{-1}$ (C-N stretch) 1541 cm$^{-1}$ (C-O stretch), 997.44 cm$^{-1}$ (C-H).

**$^1$H-NMR** ($CDCl_3$, 200 MHz): δ 3.84-3.83 (m, 8H, morpholine), 7.48 - 7.45 (m, 3H aromatic), 7.89-7.85 (m, 1Hp-aromatic), 11.98 (bs, 1H, NH).

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

White solid, Yield 73 %, M.P 287$^0$C, M.F $C_{14}H_{16}N_4OS$, Mol. Wt. (expected) 288.1045 Mol .Wt. (observed) 289.1130 (M+H) by HRMS.

**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) 1707$cm^{-1}$ (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).

**$^1$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,Aromatic), 12.90 (bs, 1H, NH).

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

White solid, Yield 61 %, M.P 185$^0$C, M.F $C_{11}H_{10}N_4O_3S$, Mol. Wt. (expected) 278.0474 Mol .Wt. (observed) 279.0562 (M+H) by HRMS.

**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)** ď 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).

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

White solid, Yield 63 %, M.P 163$^0$C, M.F.$C_{15}H_{18}N_4O_3S$, Mol. Wt (expected) 334.11 Mol .Wt. (observed) 335.1178 (M+H) by HRMS.

**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 )1712$cm^{-1}$ (C=O), 1651 cm-1546 $cm^{-1}$ (C=C, aromatic), 1437$cm^{-1}$ (C-H alkane), 1291.34 $cm^{-1}$ (C-N stretch) 1321$cm^{-1}$ (C-O stretch), 1090$cm^{-1}$ (=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).

**6bv)** ***N-(5-(2-Benzyl)-1, 3, 4-thiadiazol-2-yl) morpholine-4-carboxamide***

White solid, Yield 72 %, M.P 280$^0$C, M.F $C_{14}H_{16}N_4O_2S$, Mol. Wt. (expected) 304.0994 Mol .Wt. (observed) 305.1079 (M+H) by HRMS.

**IR (KBr pallets)**

3325.09cm$^{-1}$ (-NH-stretch –C=O) 3024.48 cm$^{-1}$ (C-H stretch aromatic) 2914.54 cm$^{-1}$ (=C-H) 1717cm-1 (C=O), 1630 cm-1(C=N) 1530 cm-1 (C=C, sp2 aromatic), 1419 cm$^{-1}$ (C-C stretch aromatic), 1318 cm$^{-1}$ (C-N stretch) 1228 cm$^{-1}$ (C-O stretch), 991.44 cm$^{-1}$ (C-H ), 765 cm$^{-1}$ (C-H stretch).

**$^1$H NMR (CDCl3, 200 MHz)**

δ 3.75 – 3.74 (m, 8H morpholine), 4.28 (s, 2H),7.40- 7.26 (m, 5H aromatic), 11.77 (bs, 1H, NH).

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

White solid, Yield 65 %, M.P 282$^0$C, M.F $C_{15}H_{18}N_4OS$, Mol. Wt. (expected) 302.1201 Mol .Wt. (observed) 303.1288 (M+H) by HRMS.

**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).

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

White solid, Yield 74 %, M.P 175$^0$C, M.F $C_{12}H_{12}N_4O_3S$ Mol. Wt. (expected) 292.063, Mol .Wt. (observed) 293.0708 (M+H) by HRMS.

**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).

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

White solid, Yield 60 %, M.P 165$^0$C, M.F $C_{16}H_{20}N_4O_3S$ Mol. Wt. (expected) 348.1256, Mol .Wt. (observed) 349.1342 (M+H) by HRMS.

**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), 1701$cm^{-1}$ (C=O), 1651 $cm^{-1}$(C=N) 1537 $cm^{-1}$ (C=C, Ar), 1437$cm^{-1}$ (C-H alkane), 1220.99 $cm^{-1}$ (C-N stretch) 1313$cm^{-1}$ (C-O stretch), 1057 $cm^{-1}$ (=C-H), 991.44 $cm^{-1}$ (C-H), 751 $cm^{-1}$ (C-H bend).

**$^1$H NMR (DMSO-d6, 200 MHz)**

d 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).

**Table no. 02: Antibacterial and Antifungal activity of synthesized Derivatives: ($6a_{I-IV}b_{V-VIII}$).**

| Comp. No. | Antibacterial data in zone of inhibition(mm) | | | | Antifungal data in zone of inhibition (mm) | |
|---|---|---|---|---|---|---|
| | Gram + Ve Bacteria | | Gram-Ve Bacteria | | | |
| | *S. aureus* A* | *B. subtilis* B* | *E. coli* C* | *E.aerogenus* D* | *A.niger* E* | *P.chrysogenum* F* |
| 6ai | 9.8 | 11.8 | 8 | 9 | -- | -- |
| 6aii | 13.4 | 14.4 | 16.2 | 16.8 | 12.5 | *9.25* |
| 6aiii | 14.4 | 13.6 | 15.2 | 15.2 | 11.5 | -- |
| 6aiv | 10.2 | 17.6 | 8.2 | 11.6 | 11.5 | -- |
| 6bv | 7.2 | 11.4 | 11.2 | 14.4 | 11.5 | *10* |
| 6bvi | --- | -- | 12.8 | 13 | 12.7 | 7.25 |
| 6bvii | --- | -- | --- | 12 | 10.5 | 9.5 |
| 6bviii | --- | 8.6 | 14.2 | 9.2 | 11 | 8.5 |
| TGA | 10.8 | 10.4 | 9.8 | 10 | 15.2 | 15.25 |
| STD | 10 | 10 | 9 | 10 | 15 | 15 |

**Table no. 03: Minimum inhibition concentration (MIC) of selected Derivatives of 5-phenyl/benzyl-1,3,4—thiadiazol-2-amine.**

| Sr. No | microorganisms | Minimum inhibition concentration in µg/ml | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MIC of TGA | | | | MIC FOR (6aiii) | | | | MIC (6bv) | | | |
| | | 50 | 100 | 150 | 200 | 25 | 50 | 100 | 200 | 25 | 50 | 100 | 200 |
| 1 | A* | --- | ++ | ++ | ++ | _ | + | + | + | _ | + | _ | + |
| 2 | B* | __ | ++ | ++ | ++ | _ | + | + | + | + | + | + | + |
| 3 | C* | __ | ++ | ++ | ++ | + | + | + | + | + | + | + | + |
| 4 | D* | __ | ++ | ++ | ++ | + | + | + | + | + | + | + | + |
| 5 | E* | ++ | ++ | ++ | ++ | _ | _ | + | + | _ | _ | + | + |
| 6 | F* | ++ | ++ | ++ | ++ | _ | _ | _ | _ | _ | _ | _ | _ |

**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).

**TGA- 5**-*Phenyl-1, 3, 4-Thidiazole-2-Amine,* STD-Chloroamphenicol.

**6aiii** - *5-(2-phenyl)-1, 3, 4-thiadiazol-2-yl)carbamoyl)glycine.*

**6bv**- *N-(5-(2-benzyl)-1, 3, 4-thiadiazol-2-yl) morpholine-4-carboxamide.*

## RESULTS AND DISCUSSION

The series of novel derivatives of 5-phenyl/benzyl-1,3,4—thiadiazol-2-amine were synthesized and in vitro antimicrobial screening of these derivatives (6ai-6bviii) carried out using culture of four bacteria species namely, *S.Aureus, B.Subtilis* (Gram positive), *E.C-oli, E.aerogenes* (Gram negative) and Culture of two fungal strain including *A.niger and P chrysogenum.* Chloroamphenicol used as standard antibiotic to evaluate the potency of the tested compounds under the same condition. Potency of these newly synthesized molecules were compared with target synthesized molecule in first step acts as moiety 5-Phenyl-1, 3, 4-Thidiazole-2-Amine.

The result of antimicrobial activities for **6ai, 6aii, 6aiii, 6aiv** derivatives showed promising effect against antibacterial and antifungal agents. Phenyl group at 5-position of 1, 3, 4-Thidiazole-2-Amine moiety leads to increase in activity**. 6bvi, 6bvii** derivatives has no activity against *S.Aureus, B.Subtilis* (Gram positive) bacteria. Only 6bviiii is inactive against Escherichia *coli* (Gram-negative bacteria).

Antifungal activity of newly synthesized derivatives such as 6ai and 6aii showed inactive property. 6aiii, 6aiv showed inactive antifungal property against *P.Chrysogenum*. 6bv-6bviii

showed excellent antifungal property compare to moiety 5-Phenyl-1, 3, 4-Thidiazole-2-Amine and standard refrence antibiotic drug Chloroamphenicol.

The minimum inhibitory concentration (MIC) of the synthesized novel derivatives against highly inhibited organism is reported in Table no.03. **6aiii**-*5-(2-phenyl)-1, 3, 4-thiadiazol-2-yl)carbamoyl)glycine and* **6bv**- *N-(5-(2-benzyl)-1, 3, 4-thiadiazol-2-yl) morpholine-4-carboxamide* showed maximum efficacy. It means that at conc. 25 µg/ml-200 µg/ml of drug gives maximum effect at lesser concentration with fewer side effects. It was observed that at conc. 25 µg/ml **6aiii** is inactive against *Staphylococcus aureus* and B*- *Bacillus subtilis (*Gram-positive bacteria) In presence of *Aspergillus niger* at lowest conc. 25-50 **µ**g/ml concentration **6aiii** and **6bv** showed inactive inhibition. *Penicillium chrysogenum* remained inactive throughout conc. of derivatives- 6aiii **and 6bv**. Overall MIC of synthesized molecule is good against gram negative bacteria *E.coli* and *E.aurogenus*.

## CONCLUSION

Novel derivatives of 5-phenyl/benzyl-1,3,4-thidiazole-2-amine were synthesized easily using aromatic nitriles, thiosemicarbaxide using TFA as solvent with good yield and in short time. We can conclude that it is very efficient way of synthesis of 1, 3, 4-Thidiazole-2-Amine as moiety which is very important in medicinal chemistry. All the novel derivatives were evaluated in vitro antimicrobial activity. The result showed some of them posses' strong antibacterial and antifungal activities. In previous research study it was observed that Fluro substituted Phenyl ring connected to 1,3,4-Thidiazole moiety enhance the antimicrobial property. In future we will try to report on the anticancer activity of these derivatives and trying to synthesis several derivatives by substituting phenyl with electron donating and electron withdrawing group.

## ACKNOWLEDGEMENT

The authors would like to express their gratitude and thanks to Hon. Chairman and all members of Dnyaneshwar Gramonnati Mandal, Principal and Head, Dept. of chemistry for providing research and laboratory facilities. Authors also wish to thanks Dr. Mrs. Minal Joshi, Bhide Foundation Institute, Pune for evaluation of antimicrobial activity. Our sincere thanks to IISER, NCL and SP Pune University for spectral analysis. Last but not least would like to express my thanks to Dr. G.M. Nazeruddin, Dr. Navin Patel, Dr. Suryawanshi S. Dr. Pravin Mahske for their valuable guidance and continue encouragement in research.

**REFRENCES**

1. An Introduction to Medicinal Chemistry fourth Edition Graham L. Patrick
2. Siddiqui N, Ahuja P, Ahsan W, Pandeya SN, Alam MS. Thiadiazoles: progress report on biological activities. J Chem Pharm Res., 2009; 1: 19–30.
3. Singh AK, Mishra G, Jyoti K. Review on biological activities of 1, 3, 4-thiadiazole derivatives. J Appl Pharm Sci., 2009; 1: 44–9.
4. Kamal M, Shakya AK, Jawaid T. 1,3,4-Thiadiazole as antimicrobial agent: a review. Int J Biomed Res., 2011; 2: 41–61.
5. Mishra G, Singh AK, Jyoti K. Review article on 1,3,4- thiadiazole derivatives and its pharmacological activities. Int J Chem Tech Res., 2011; 3: 1380–93.
6. Kamal M, Shakya AK, Jawaid T. 1, 3, 4-Thiadiazole as antimicrobial agent: a review. Int J Biomed Res., 2011; 2: 1–4.
7. Kushwaha N, Kushwaha SKS, Rai AK. Biologial activities of thiadiazole derivatives: a review. Int J Chem Tech Res., 2011; 4: 517–31.
8. Gupta JK, Dudhey R, Sharma PK. Synthesis and pharmacological activity of substituted 1, 3, 4-thiadiazole derivatives. Medichemonline, 2010; 1: 1–9.
9. Singh AK, Mishra G, Jyoti K. Review on biological activities of 1,3,4-thiadiazolederivatives. J Appl Pharm Sci., 2011; 1: 44–9.
10. Ahmad T, Singh AK, Jaiswal N, Singh D. Synthesis and pharmacological activity of 1,3,4-thiadiazole derivatives: a review. Int Res J Pharm., 2012; 3: 70–82.
11. Bhuva H, Sahu D, Shah B, Modi DC, Patel MB. Biological profile of thiadiazole. Pharmacol Online, 2011; 1: 528–45.
12. Kushwaha N, Kushwaha SKS, Rai AK. Biologial activities of thiadiazole derivatives: a review. Int J Chem Tech Res., 2012; 4: 517–31.
13. Sushama Kadam, G.M. Nazeruddin 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., 2015; 2(12): 51-57.