*IOSR Journal of Applied Chemistry (IOSR-JAC)*
*e-ISSN: 2278-5736.Volume 6, Issue 6 (Jan. 2014), PP 01-07*
*www.iosrjournals.org*

# Synthesis and Biological Evaluation of Novel3, 5-Disubstituted 4h-1, 2, 4-Triazoles

L.NalandaSharada*, T.LakshmiViveka
*Osmania university, department of chemistry, Hyderabad, India*

***Abstract:*** *A general method was developed for the synthesis of novel unsymmetrical 3, 5-disubstituted 4*H*-1, 2, 4-triazoles with benzyl group in the 4-ring position (5a-j). A series of novel N-acyl amidrazone derivatives (4a-j) have been synthesized. The chemical structures of newly synthesis zed compounds were characterized by IR, $H^1$NMR,$C^{13}$NMR and mass. The newly synthesized compounds were screened for antimicrobial activity.*
***Keywords****: Acid chlorides-acyl amidrazones, benzyl amine, unsymmetrical 4H-1, 2, 4-triazoles.*

## I. Introduction:

1,2,4-Triazole and its derivatives are an important class of compounds which possess diverse agricultural, industrial and biological activities including anti-microbial, sedative, anticonvulsant, anticancer , anti-inflammatory, diuretic, anti bacterial, hypoglycemic, anti tubercular and antifungal. In recent years, the synthesis of these heterocyclic compounds has received considerable attention. This wide range of applications has been covered by more than sixty papers in the literature, many in the form of patents.

In the previous work ,synthesis of unsymmetrical 3,5-disubstituted 4*H*-1,2,4-tria-zoles (Ph, H or Ph, $CH_3$) with allyl or benzyl groups in the 4-ring position[1]. The reaction of the corresponding 3, 5-disubstituted 1, 3, 4-oxadiazoles with allylamine or benzyl amine gave the desired compounds. The oxadiazoles were prepared by heating at 100° *N, N'*-diacylhydrazines with phosphorus pentoxide.For avoiding high temperature condition another method was introduced.

A general method was developed for the synthesis of novel unsymmetrical 3, 5-disubstituted 4*H*-1, 2, 4-triazoles with benzyl group in the 4-ring position. The reaction of the corresponding N-acyl amidrazone derivatives on thermal cyclisation in t-butanol by the addition of acetic acid gave the desired compounds. The 1,2,4-triazoles were synthesized via of N-(E) benzyliminoacetohydrazidederivatives.these are prepared by amidation of benzyl amine with different acid chlorides and then chlorinated with PCl5 imidoyl chlorides are formed and these are trapped with acetic hydrazide.

## II. Materials And Methods:

Melting points of all synthesized compounds were determined in open capillary tubes on an electro thermal apparatus and are uncorrected. The progress of the reaction and purity of the compounds was checked by TLC on silica gel coated aluminium plates (Merck) as adsorbent and UV light as visualizing agent. IR spectra(KBr $\upsilon_{max}$ cm-1) were recorded on BRUKER FTIR spectrophotometer in the range of 4000-400cm-1.1H NMR spectra were recorded on BRUKER (400MHz) NMR spectrometer using CDCl3,DMSO-d6 as solvents and TMS as an internal standard(chemical shifts in δ ppm). Mass spectra were recorded on a MS using ESI mode positive ion trap detector.

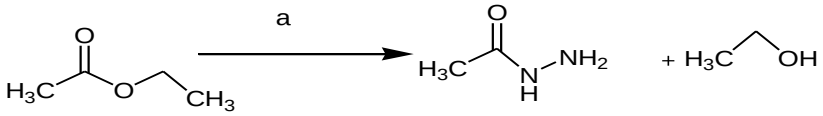
## III. Chemistry:

***Synthesis of Triazole*:**Initially acid chlorides prepared using thionyl chloride and amide formation takes places using benzyl amine, potassium carbonate and dry acetone at room temperature by **Schotten-Baumann reaction**. The amide was isolated and used without crystallization. The Triazole was synthesized in three steps, activation of amide as its corresponding imidoyl chloride followed by trapping with acetic hydrazide and acid catalyzed cyclisation to the Triazole (scheme)

As early investigations demonstrated that the imidoyl chloride decomposed on heating, the original chlorination conditions of phosphorus oxy chloride in refluxing chloroform were replaced by PCl5 in dichloro methane at o°c-5°c which resulted in the instantaneous formation of imidoyl chloride. Conversion of imidoyl chloride to the triazole proved to be more challenging. Addition of acetic hydrazide to a solution of imidoyl chloride resulted in the deposition of milky white gummy mass on the reaction vessel and was accompanied by 25-60% hydrolysis to the starting amide. Hydrolysis could be suppressed to less than 10-15%by azeotropic drying of acetic hydrazide in acetonitrile and t-butanol (3:1) ratio and gave clear conversion to intermediate(4)N-acyl amidrazone.an aqueous work up was introduced after the formation of N-acylamidrazone to remove HCl and phosphoric acid bi-product. A series of N-acylamidrazone derivatives were isolated and

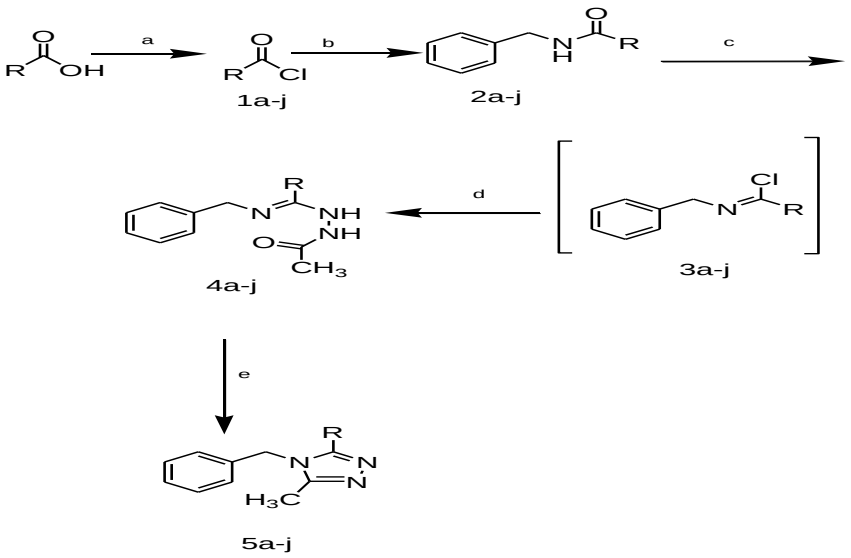
crystallized in acetone. Thermal cyclisation of N-acylamidrazone derivatives in t-butanol by the addition of acetic acid gave cleanest conversion of Triazole derivatives.

***Scheme 1:***




***Reagents and conditions***:( a) hydrazine hydrate, 75-80°c, 2hrs

***Scheme 2:***




***Reagents and conditions***:( a) thionyl chloride, at65-70°c; (b) benzyl amine, acetone, K2CO3,rt;(c)PCl5,DCM,0-5°c;(d)AcNHNH2,t-butanol;(e)aceticacid,t-butanol,80°c.

## IV. Experimental Section:

***General***All raw materials, reagents were purchased from AVRA and s.d. fine.chemicals.solventspurchased from commercial suppliers. All reactions were conducted in inert atmosphere. Reactions monitored for completion by TLC.

***Synthesis of N-benzyl amide derivatives (2a-j):***

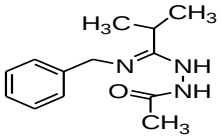
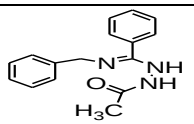
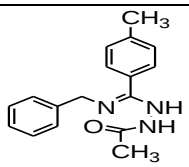
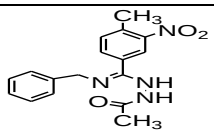
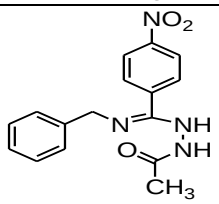
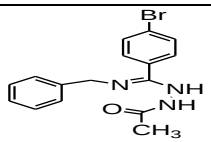
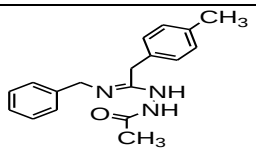
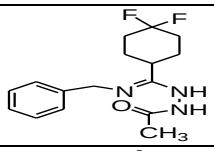
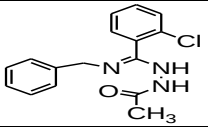
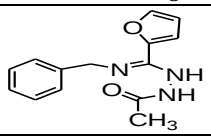
Benzyl amine (5g) diluted in acetone (25ml), charged anhydrous potassium carbonate (2eq) into r.b.f at rt.stirred for 10 mins. Cool to 0-5°c and add acid chloride(1.2eq)at 5°c.after the addition of acid chloride allow the reaction mass to get rt and maintain at rt for overnight stirring. Checked TLC if TLC is ok removed the solvent under vacuum and charged ice water50ml and maintained under stirring for 15 mins. Filter the product and finally washed the product with ether. Suck dry the product .dry the material in hot air oven**.**

***Synthesis of N-(E) benzyliminoacetohydrazide (N-acyl amidrazone) derivatives (4a-j):***

Charged PCl5 (1.3eq) and DCM (15ml) into r.b.f at rt. Cool the slurry to get 0°c .add amide solution to the PCl5 slurry at 0°c. maintained at rt for 2hrs .again cool the reaction mass to get 0°c .add azotropic acetic hyrazide solution to reaction mass at o°c.maintained at 0°c for 1/2hr.allowed the reaction mass to get rt .left the reaction mass at rt for 15hrs.checked TLC.if TLC is ok .cool to 0°c and charged water (100ml) to reaction mass for removing HCl and phosphoric acid bi-product. Adjust **ph** of reaction mass to get 9.0-9.5 using (10 N) NaOH solution. Separated the two layers and extracted the aqueous layer with DCM (3x25ml).combined the organic layer and washed with brine solution .dried the organic layer with anhy.sodiumsulphate. Filter and concentrated in vacuum. Isolated and crystallized the product in acetone.dry the product and analyzed with all spectral data.

***Synthesis of N-benzyl-3, 5-disubstituted-4H- 1, 2, 4-triazole derivatives (5a-j):***

The obtained 4a-j material(1.0g)and t-butanol(7ml) and 1ml acetic acid stirred at 80°c until get a clear solution. Checked TLC. If TLC is ok distiiled off the solvent in vacuum and charged water 15ml stirred for 30mins.filter the product and dried the material in hot air oven and analyzed the product .

| *S.NO* | *Name /code* | *structure* | *%yield* | **MR(°c)** |
|---|---|---|---|---|
| **4a** | **N-((E)-1-(benzylimino)-2-methylpropyl)acetohydrazide** |  | **71.2** | Viscous liquid |
| **4b** | **N-((E)-1-(benzylimino)(phenyl)methyl)acetohydrazide** |  | 70.3 | **140-144** |
| **4c** | **N-((E)-1-(benzylimino)(p-tolyl)methyl)acetohydrazide** |  | 67.5 | **152-158** |
| **4d** | **N-((E)-1-(benzylimino)(4-methyl-3-nitro phenyl)methyl)acetohydrazide** |  | **68.2** | **164-166** |
| **4e** | **N-((E)-1-(benzylimino)(4-nitro phenyl)methyl) acetohydrazide** |  | 67.32 | **162-165** |
| **4f** | **N-((E)-1-(benzylimino)(4-bromo phenyl)methyl) acetohydrazide** |  | **65.2** | **125-127** |
| **4g** | **N-((E)-1-(benzylimino)-2-p-tolylethyl)methyl) acetohydrazide** |  | **71.7** | ----- |
| **4h** | **N-((E)-1-(benzylimino)(4,4-difluoro cyclohexyl)methyl) acetohydrazide** |  | **55.8** | **84-88** |
| **4i** | **N-((E)-1-(benzylimino)(2-chloro phenyl)methyl) acetohydrazide** |  | 57.3 | **72-76** |
| **4j** | **N-((E)-1-(benzylimino)(furan-2-yl)methyl) acetohydrazide** |  | 68.2 | **156-160** |

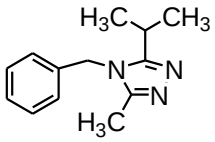
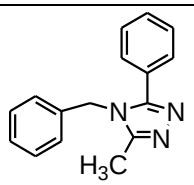
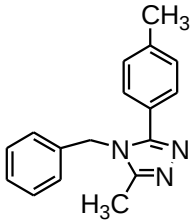
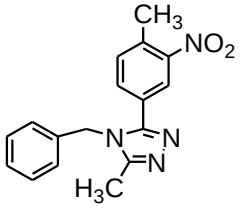
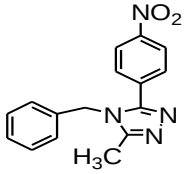
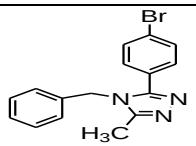
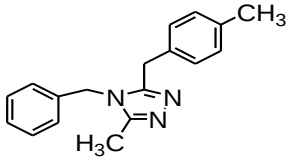
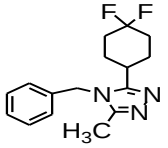
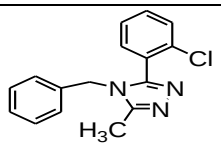
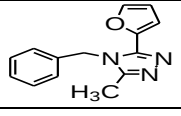
| *S.NO* | *Name /code* | *structure* | *%yield* | **MR(°c)** |
|---|---|---|---|---|
| *5a* | *4-benzyl-3-isopropyl-5-methy-4H-1,2,4-triazole* |  | *90.1* | **Viscous liquid** |
| *5b* | *4-benzyl-3-methyl-5-phenyl-4H-1,2,4-triazole* |  | *89* | **100-105** |
| *5c* | *4-benzyl-3-methyl-5-p-tolyl-4H-1,2,4-triazole* |  | *90.3* | **113-118** |
| *5d* | *4-benzyl-3-methyl-5-(4-methyl-3-nitro phenyl) 4H-1,2,4-triazole* |  | *87* | **82-85** |
| *5e* | *4-benzyl-3-methyl-5-(4-nitro phenyl)- 4H-1,2,4-triazole* |  | *92* | **176-181** |
| *5f* | *4-benzyl-3-(4-bromo phenyl)-5-methyl-4H-1,2,4-triazole* |  | *93* | **106-108** |
| *5g* | *4-benzyl-3-methyl-5-p-tolylethyl-4H-1,2,4-triazole* |  | *88* | **116-118** |
| *5h* | *4-benzyl-3-(4,4-difluoro cyclohexyl)-5-methyl-4H-1,2,4-triazole* |  | *82* | --- |
| *5i* | *4-benzyl-3-(2-chloro phenyl)5-methyl-4H-1,2,4-triazole* |  | *86.5* | ---- |
| *5j* | *4-benzyl-3-(furan-2-yl)-5-methyl-4H-1,2,4-triazole* |  | *88.3* | **158-160** |

Table-2:

## V. Spectral Data:

1. ***4-benzyl-3-isopropyl-5-methy-4H-1,2,4-triazole(5a):*** **$H^1$NMR(CDCl3)400MHz:***7.35-6.94(5H,m,Ar-H),5.06(2H,s,-CH2),2.93-2.83(1H,m,-CH),2.32(3H,s,-CH3),1.33-1.31(6H,d,2xCH3)*
   **$C^{13}$NMR (CDCl3)400MHz:** 159.06(-C=N), 151.03(-C=N), 134.5, 128.7, 127.8, 125.3(4c, benzene), 45.9(-CH2-N), 28.2(-CH), 21.0(-2xCH3), 10.48(-CH3).
   **IR(Cm$^{-1}$)**:2972(-assy-C-H-stre),3033,3064(-C=C-H-stre),1534.9,1516.4(C=C,-C=N-stre)
   **Mass:** 216.27(M+1), 217.27(M+2)
2. ***4-benzyl-3-methyl-5-phenyl-4H-1,2,4-triazole(5b):***
   **$H^1$NMR(CDCl3)400MHz:***7.55-6.94(10H,m,Ar-H),5.18(2H,s,-CH2), 2.42(3H,s,-CH3)*
   **$C^{13}$NMR (CDCl3)400MHz: 154.78** (-C=N), 152.44(-C=N attached to benzene ring), 134.5, 130.03, 129.02, 128.7, 128.4, 128.01, 126.4, 125.4(8c, benzene), 47.1(-CH2-N), 10.65(-CH3).
   **IR(Cm$^{-1}$)**:2961(-assy-C-H-stre),3025,3069(-C=C-H-stre),1578.1,1530.0(-C=C,-C=N-stre)
   **Mass:** 250(m+1)
3. **4-benzyl-3-methyl-5-phenyl-4H-1,2,4-triazole(5c):**
   **$H^1$NMR(CDCl3)400MHz:***7.43-6.97(9H,m,Ar-H),5.15(2H,s,-CH2), 2.38(3H,s,-CH3),2.09(-CH3-attached to benzene ring)*
   **$C^{13}$NMR (CDCl3)400MHz: 154.78** (-C=N), 152.44(-C=N attached to benzene ring), 134.5, 130.03, 129.02, 128.7, 128.4, 128.01, 126.4, 125.4(8c, benzene), 47.1(-CH2-N), 10.65(-CH3).
   **IR(Cm$^{-1}$)**:2961(-assy-C-H-stre),3025,3069(-C=C-H-stre),1543.5,1525.6(-C=C,-C=N-stre)
   **Mass:** 264.5(m+1)
4. **4-benzyl-3-methyl-5-(4-methyl-3-nitro phenyl) 4H-1,2,4-triazole(5d):**
   **$H^1$NMR(CDCl3)400MHz:***8.11-6.97(8H,m,Ar-H),5.19(2H,s,CH2),2.62(3H,s,-CH3),2.45(3H,s,-CH3attached to benzene ring)*
   **$C^{13}$NMR (CDCl3)400MHz:** 153.07(-C=N), 152.7(-C=N attached to benzene ring), 149.0, 135.1, 134.33, 133.3, 132.4, 129.2, 128.3, 126.3, 125.3, 124.3(10c, benzene), 47.3(-CH2-N), 20.14(-CH3 attached to benzene ring), 10.8(-CH3).
   **IR(Cm$^{-1}$)**:2930(-assy-C-H-stre),2999.15 (-C=C-H-stre),1543.89,1525.25(C=C,-C=N-stre)
   **Mass:** 309.3(m+1)
5. **4-benzyl-3-methyl-5-(4-nitro phenyl)- 4H-1,2,4-triazole(5e):**
   **$H^1$NMR(CDCl3)400MHz:***8.28-6.97(9H,m,Ar-H ),5.21(2H,s,CH2),2.45(3H,s,-CH3)*
   **$C^{13}$NMR (CDCl3)400MHz:** 153.07(-C=N), 153.17(-C=N attached to benzene ring), 148.5, 134.2, 133.3, 129.5, 129.3, 128.5, 125.38, 124.07(8c, benzene), 47.5(-CH2-N), 11.01(-CH3).
   **IR(Cm$^{-1}$)**:2927.5(-assy-C-H-stre),3093.9 (-C=C-H-stre),1567.59,1524.7(-C=C,-C=N-stre)
   **Mass:** 294.3(m/z)
6. **4-benzyl-3-(4-bromo phenyl)-5-methyl-4H-1,2,4-triazole(5f):**
   **$H^1$NMR (CDCl3)400MHz:** *7.69-6.96(9H,m,Ar-H),5.14(2H, s,-CH2), 2.39(3H,s,-CH3)*
   **$C^{13}$NMR (CDCl3)400MHz: 154.1** (-C=N), 152.7(-C=N attached to benzene ring), 134.7, 132,130.06, 129.3, 128.01, 126.1, 125.4, 124.5(8c, benzene), 47.1(-CH2-N), 10.65(-CH3).
   **IR(Cm$^{-1}$)**:2978.7(-assy-C-H-stre),3029,3080(-C=C-H-stre),1592.59,1531.5(-C=C,-C=N-stre)
7. **4-benzyl-3-methyl-5-p-tolylethyl-4H-1,2,4-triazoel(5g):**
   **$H^1$NMR(CDCl3)400MHz:***7.30-6.82(9H,m,Ar-H),4.83(2H,s,-CH2), 4.03(2H,s,-CH2)2.31(3H,s,-CH3),2.30(-CH3-attached to benzene ring)*
   **$C^{13}$NMR (CDCl3)400MHz: 153.59**(-C=N), 152.1(-C=N attached to benzene ring), 136.7,134.4,132.2,129.4,129.0,128.2,128.0,125.8(8c, benzene), 46.5(-CH2-N),31.3(p-tolyl-CH2),20.91(-CH3 attached to benzene)10.92(-CH3).
   **IR(Cm$^{-1}$)**:2951.3(-assy-C-H-stre),3027,3087(-C=C-H-stre),1535.8,1516.4(-C=C,-C=N-stre)
   **Mass:** 278.4(m+1)
8. **4-benzyl-3-(4,4-difluoro cyclohexyl)-5-methyl-4H-1,2,4-triazole(5h):**
   **$H^1$NMR(CDCl3)400MHz:**7.35-7.25(5H,m,Ar-H),4.45(2H.S,-CH2),2.35(3H,S.-CH3),2.2-1.69 (-9H, m.cyclohexyl ring protons)
   **IR(Cm$^{-1}$)**:3033(Aromatic-C-Hsrte),2945.6(aliphatic –C-H-stre),1637,1529(-C=C-,-C=N-)
9. **4-benzyl-3-(2-chloro phenyl)5-methyl-4H-1,2,4-triazole(5i):**
   **$H^1$NMR(CDCl3)400MHz:***7.55-6.94(10H,m,Ar-H),5.18(2H,s,-CH2), 2.42(3H,s,-CH3)*
   **$C^{13}$NMR (CDCl3)400MHz: 154.78** (-C=N), 152.44(-C=N attached to benzene ring), 134.5, 130.03, 129.02, 128.7, 128.4, 128.01, 126.4, 125.4(8c, benzene), 47.1(-CH2-N), 10.65(-CH3).
   **IR(Cm$^{-1}$)**:2961(-assy-C-H-stre),3025,3069(-C=C-H-stre),1578.1,1530.0(-C=C,-C=N-stre)
10. **4-benzyl-3-(furan-2-yl)-5-methyl-4H-1,2,4-triazole(5j):**
    **$H^1$NMR(CDCl3)400MHz:***7.51-6.52(8H,m,Ar-H),5.40(2H,s,-CH2),2.41(3H,s,-CH3)*

**$C^{13}$NMR(CDCl3)400MHz:152.27(**-C=N),146.89(-C=N attached to furan,143.6,142.15,111.62,111.5(furancarbons)134.78,128.9,128.0,125.96, (4c, benzene), 47.59(-CH2-N), 10.72(-CH3).
**IR(Cm$^{-1}$)**:2961.55(-assy-C-H-stre),3142,3098.83(-C=C-H stre),1576,1530.49(C=C,-C=N-stre)

## VI. Antimicrobial Activity:

All the synthesized compounds 5(a-j)were further evaluated for antibacterial activity against bacteria ***Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Escherichia Coli*** *following* Agar disc diffusion method. All the test and standard concentrations were prepared in **DMSO**. Gentamicin was taken as standard drug. Sterile filter paper discs were impregnated in sample or standard solutions. They were then carefully placed aseptically with a forceps on the surface of the Mueller-Hinton (MH) agar plates that were pre-inoculated with the 24 hr culture of bacteria and 0.1 ml spore suspension (1 x $10^5$ spores/ml). The control antibiotics disc containing **Gentamicinicin(40µg/ml)** was placed on each of the inoculated plates of nutrient agar. The plates were left on the bench undisturbedfor few minutes, after which the bacterial culture plates were incubated at 37° C for 24 h. The external diameters of visible zones of growth inhibition were measured after incubation

***Statistical analysis:*** Data collected in the study are expressed as the mean ± standard error of mean (S.E.M.) and the statistical analysis was carried out by using one-way analysis of variance (ANOVA) method. P value of less than 0.05 was considered to be statistically significant. All groups were compared with dimethyl sulfoxide treated control group.

## VII. Results And Discussion:

The anti bacterial activity results of 3,5-disubstituted 4H-1,2,4-triazoles in table 3. 5h, 5i, 5j exhibited good anti bacterial activity against all the tested bacterial strains than the other compounds of the series. Although the antibacterial activities of tested compounds are not comparable with the standard drug Gentamicin but they exhibited moderate to potent activity at high concentrations. 5b, 5c, 5d, 5e compounds show weakly active. The compounds 5a and 5g compounds show moderately active in all the bacterial strains.

***Conclusion:***

From the above investigation it could be concluded that the compounds 5h,5i,5j exhibited good antibacterial activity and compounds 5a and 5g exhibited moderatractivity.therefore these compounds considered as a new lead molecules for the development of newer class of antibacterial activity.

## Acknowledgement:

The authors are thankful to Dr.Y.Rambabu,Scientist-F,Flouro Organics Department,IICT,Hyderabad for the encouragement and for providing spectral data. Authors also thankful to K.Ramanjaneyulu, Deprtment of pharmacy,Visper, Vishnupur, Narsapur, Medak (Dist) for providing antibacterial activity and my sincere thanks to my friend Ch.Manikumar for supporting me to the entire work.

***Antibacterial activity of synthesized compounds***

| **Compound 1000 µg** | ***Diameter of zone of inhibition(mm) Mean*±SD** | | | |
|---|---|---|---|---|
| | ***Staphylococcus Aureus*** | ***B.Cereus*** | ***E-Coli*** | ***P.Aerugenosa*** |
| **5a** | **22±0.44** | **21±0.14** | **20±1.3** | **20±1.1** |
| **5b** | **17±1.8** | **18±1.2** | **13±1.1** | **10±0.9** |
| **5c** | **18±1.3** | **17±1.5** | **18.5±1.1** | **20±0.9** |
| **5d** | **19±1.5** | **21.2±0.9** | **17.3±1.5** | **14.5±1.1** |
| **5e** | **16.2±1.1** | **14±0.9** | **15±0.25** | **17±0.8** |
| **5f** | **23±0.5** | **21±0.7** | **18±0.8** | **14±0.9** |
| **5g** | **20±0.5** | **21.5±1.1** | **21.2±1.3** | **19.7±0.7** |
| **5h** | **24±1.1** | **22.3±0.4** | **20.2±1.3** | **21.5±1.2** |
| **5i** | **27±0.5** | **25.3±0.9** | **24.1±0.7** | **20.3±0.9** |
| **5j** | **24.5±0.8** | **22±0.3** | **20.5±0.4** | **21.2±1.3** |
| **Gentamicin 40 µg/ml** | **33.4±1.07** | **35±1.5** | **27.2±1.1** | **36.6±1.8** |
| **Values are expressed as mean + SEM AND analyzed by one way analysis of variance(ANOVA) followed by Dunnet's test** | | | | |

## References:

[1] PerH.J.Carlsen, kareB.Jorgensen,journal of hetero cyclic chemistry, volume 31,issue 4,pages 805-807.
[2] Pramod kumar,R.H.Udupiu and P.K.Dubey,International Journal of Pharm Tech Research,Vol.1,No.4,pp 1654-1662
[3] Olcay BEKIRCAN, Bahittin KAHVEC,Murat KUC'UK" ,Turk J Chem,30(2006),29-40.
[4] L. Nirmala Devi, Th. Nandita Devi, Kh. Biren Singh and M. Dhaneshwar Singh* , J. Chem. Pharm. Res., 2011, 3(6):631-638
[5] March, Jerry.Advanced organic chemistry reactions, mechanisms and structure, third addition. John wiley&sons,NY,NY.1985,page:370(schotten-baumann reaction)
[6] Journal of American chemical society,107,page:4249.
[7] Richard F.Smith,KarenJ.Coffman,SusanM.Geer,journal of hetero cyclic chemistry,voume 20,issue 1,page:69-71(1983)
[8] Ashraf A.Aly and Ahmed M.Nour-El-Din,functionality of amidines and amidrazones,ARKIVOC 2008(i)153-194
[9] Journal of organic chemistry,56,page:5482(1991)
[10] Tetrahedran letters,20,page:1725(1979)