DEVELOPMENT OF NEW ENTITIES OF 1,3,4-THIADIAZOLES FOR ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES.

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Abstract:
1, 3, 4- Thiadiazole is one of the heterocyclic compounds having different versatile activities such as anti-diabetic, anti-microbial, anti-convulsant, anti-inflammatory and anti-cancer. A new 1,3,4-Thiadiazole derivatives with Schiff base compound synthesized. The starting material N-(4-chlorophenyl)hydrazine carbothiamide synthesized by the reaction between aniline derivativesand carbondisulfide, hydrazine hydrate followed by synthesis of 5-(4-aminophenyl)-N-(4-chlorophenyl)-1,3,4-thiadiazole-2-amine. The chemical structures were confirmed by the elemental analysis IR, UV and H^1 NMR and Thin layer chromatography and perform pharmaceutical activity carried out against S aureus,E Coli, K. Pneumonia, M luteus and candida albicans and compared with the standards from which 4-NO_2, 4-Cl, 2-OH,-H derivatives shown promising antibacterial activity and 4-Cl derivative shown promising anti-fungal activity.

Keywords: 1, 3, 4- Thiadiazole, anti-bacterial activity, 4-Cl derivative, E Coli, Schiff base.

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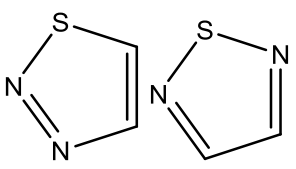
INTRODUCTION

Microbial infections are caused by many types of bacteria, viruses, and fungi, are one of the most common illnesses that kill millions of people throughout the world. The actions of microbes have a significant impact on humans. Microorganisms play a larger role in our lives than most of us realise. They have shaped our current environment, and their actions will have a significant impact on our future. Microorganisms should not be thought of as distinct from humans, but rather as an integral component of our lives. They are used in the production of dairy products, some meals, certain pharmaceuticals and therapeutic agents, as well as the production of other compounds.

Heterocyclic structures are constantly present in organic chemistry research and development. There are millions of heterocyclic structures with unique characteristics and biological significance. An acceptable synthetic approach was used to make a variety of thiadiazoles, which were then characterised using elemental analysis and spectrum data[1].

The ring contains multiple isomers of Thiadiazole: 1,2,3 Thiadiazole (1), 1,2,5 Thiadiazole (2), 1,2,4 Thiadiazole (3), 1,3,4 Thiadiazole (4), 1,3,4 Thiadiazole (5), 1,3,4 Thiadiazole (6), 1,3,4 Thiadiazole (7), 1,3,4 Thiadiazole (4)



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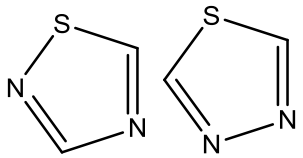


(3) **(4)**

"Fischer" initially described 1,3,4-thiadiazole in 1882, and "Busch" and his colleagues further improved it. Thiadia0zole has a five-membered ring structure with the chemical formula C2H2N2S, which comprises two carbon atoms, two hydrogen atoms, two nitrogen atoms, and one sulphur atom. A five-membered ring structure with two or more heteroatoms, one of which being nitrogen, is designated by the ending azole. Thiadiazoles are linked to a wide range of biological activities, most likely as a result of their – N=C-S - The heterocyclic nucleus of the 1,3,4-thiadiazole moiety contains sulphur at position -1 and two nitrogen atoms at positions 3 and 4. In the realm of medicinal chemistry, thiadiazole derivatives have a distinctive position. Thiadiazole's Sulphur atom improves lipid solubility and is meso ionic in nature. Pharmaceuticals, cyanide dyes, oxidation inhibitors and metal complexing agents are just a few of the uses for the 1,3,4-thiadiazole ring system. Thiadiazole is a multifunctional moiety with a wide range of biological activity. Acetazolamide, Methazolamide, Sulfamethazole, and other medicines containing the thiadiazole nucleus are available on the market. Thiadiazole can be used to substitute the thiazole molecule bio-isosterically. As a result, it behaves similarly to third and fourth generation cephalosporins and can be employed in antibiotic formulations. Thiadiazole derivatives have intriguing biological activity, which is likely due to the strong aromaticity of this ring structure, which results in excellent in vivo stability and low toxicity in higher vertebrates, including humans.

EXPERIMENTAL METHODOLOGY

N-(4-chlorophenyl) hydrazine carbothioamide is synthesised from 4-chloroaniline. Cyclization of N-(4-chlorophenyl) hydrazine carbothioamide with para amino benzoic acid in the presence of concentrated sulfuric acid is employed as a reactant in the synthesis of 5-(4-aminophenyl)-N-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine. After being treated with different substituted Aldehydes, it is further transformed to imines. The Staudinger Imines reaction of Schiff bases was used to synthesise the final compounds.

STEP-1: Synthesis of N-(4-chlorophenyl) hydrazine carbothioamide:

In a round bottom flask placed equimolar quantity of para chloro aniline (0.1mole), potassium hydroxide (0.1mole) and methanol (20ml) stirred on magnetic stirrer, maintained the temperature below 10^oC and carbon disulphide (0.1mole) added drop by drop, stirring continued for 2-3hrs at 0^oC and to it hydrazine hydrate (0.2mole) added drop by drop, stirring was continued at 0^oC for 1hr, then reflux the reaction mixture for 3-4hrs. The reaction mixture was poured into ice cold water then precipitation was formed. The product was collected by filtration, dried and purified with ethanol.

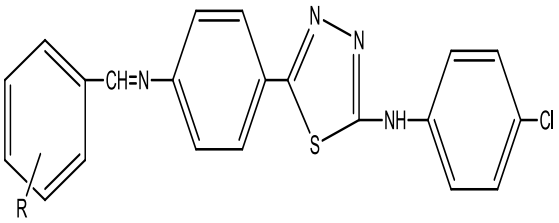
STEP-2: Synthesis of 5-(4-aminophenyl)-N-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine:

In a round bottom flask equimolar quantity of N-(4-chlorophenyl) hydrazine carbothioamide (0.1mole) and para amino benzoic acid(0.1mole) and sulfuric acid(4ml) in 20ml of methanol containing 2-3drops of glacial acetic acid were refluxed for 4-6hrs. The reaction mixture was poured in ice cold water and add 5%$NaHCO_3$ and the product was collected by filtration, dried and purified with methanol, crystalline product was obtained.

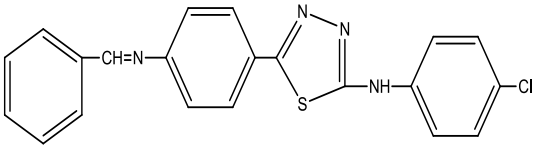
STEP-3: Synthesis of 5-(4-(benzylidene amino) phenyl)-N-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine:

In a round bottom flask placed 5-(4-aminophenyl)-N-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine (0.01mole) and benzaldehyde (0.01mole) containing 2-3 drops of glacial acetic acid and 20ml of ethanol were refluxed for 6 hrs at 20^oC. The reaction monitored by TLC (7:3 ethylacetate: Hexane). Then, the reaction mixture was cooled to room temperature and poured in to crushed ice. The product was separated by filtration and dried. It was purified recrystallise from methanol, crystalline product was obtained.

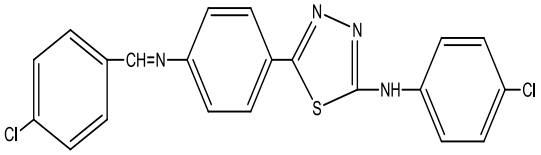
Compound III



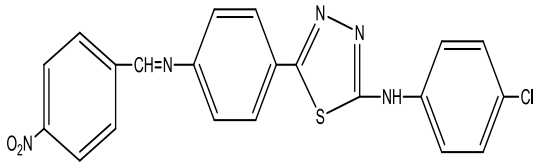
IV



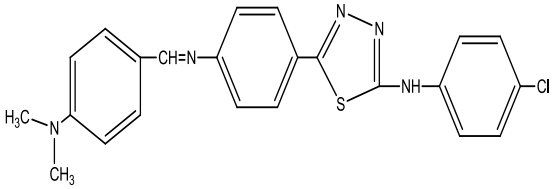
IVa



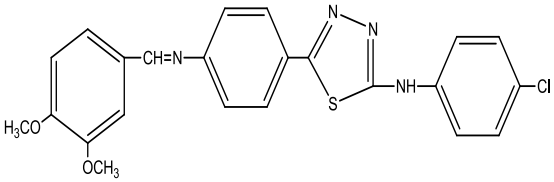
IVb



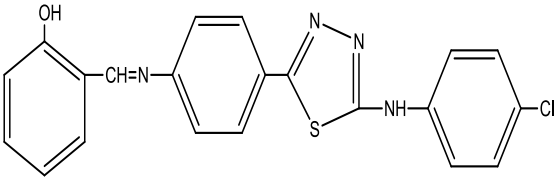
IVc



IVd



IVe



IVf

Physical data and spectral data of synthesised compounds:

S.no.	Compound	R	Mol. Formula	Mol. Weight	Melting point	% Yield
1	III	Intermediate	$C_{14}H_{11}ClN_4S$	302.78	120^oC	82%
2	IV-a	H	$C_{21}H_{15}ClN_4S$	390.89	110^oC	68.7%
3	IV-b	4-Cl	$C_{21}H_{14}Cl_2N_4S$	425.33	115^oC	73.5%

4	IV-c	4NO2	$C_{21}H_{14}ClN_5O_2S$	435.85	142°C	56.9%
5	IV-d	4-N, N- Dimethyl	$C_{23}H_{20}ClN_5S$	433.96	172°C	44.5%
6	IV-e	3,4-Di-methoxy	$C_{23}H_{19}ClN_4O_2S$	450.94	138°C	49.8%
7	IV-f	2-OH	$C_{21}H_{15}ClN_4OS$	406.89	85°C	67.8%

Spectral data of 5-(4-aminophenyl)-N-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine (III):(IR (KBr) cm^{-1}: 3422.59(-NH2), 3220.81(NH), 2984(Aromatic C-H), 1599 and 1492(C=C), 1039(C-N), 1634(C=N), 772.63(C-Cl).¹H-NMR (300 MHZ, DMSO) δ ppm: ¹H-NMR (300 MHZ, DMSO) δ ppm: 8.4(s, -1H, NH), 7.2– 8.2(m,8H, Ar-H), 4.65(s,2H, NH2). EI-MS: M+2 peak observed at 304.

Spectral data of 5-(4-(Benzylidene amino) phenyl)-N-(4-Chlorophenyl) -1,3,4-Thiadiazol-2-Amine (IV): IR (KBr) cm^{-1}: 3283.81(NH), 2984(Aromatic C-H), 1634(C=N), 784.42(C-Cl),.¹H-NMR (300 MHZ,DMSO)δ ppm: 8.45(s,-1H,NH), 7.40– 8.18(m,13H,Ar-H), 4.39(d,2H,CH). EI-MS: M+2 peak observed at 437.

Spectral data of (E)-5-(4-((4-chlorobenzylidene)amino)phenyl)-N-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine (IV-b) : (IR (KBr) cm^{-1}: 3422.59(-NH2), 3220.81(NH), 2984(Aromatic C-H), 1599 and 1492(C=C), 1039(C-N), 1634(C=N), 772.63(C-Cl). ¹H-NMR (300 MHZ, DMSO) δ ppm: ¹H-NMR (300 MHZ, DMSO) δ ppm: EI-MS: M+2 peak observed at 425.

Spectral data of N-(4-chlorophenyl)-5-(4-((4-nitrobenzylidene)amino)phenyl)-1,3,4-thiadiazol-2-amine (IV-C) :): IR (KBr) cm^{-1}: 3283.81(NH), 2984(Aromatic C-H), 1634(C=N), 784.42(C-Cl), 1358& 1537(C-NO2).¹H-NMR (300 MHZ,DMSO)δ ppm: 8.45(s,-1H,NH), 7.40–8.18(m,13H,Ar-H), 4.39(d,2H,CH). EI-MS: M+2 peak observed at 392.

Spectral data of N-(4-chlorophenyl)-5-(4-((4-(dimethylamino)benzylidene)amino)phenyl)-1,3,4-thiadiazol-2-amine (IV-d) : IR (KBr) cm^{-1}: 3283.81(NH), 2984(Aromatic C-H), 1634(C=N), 784.42(C-Cl) ,1335-1250(C-N).¹H-NMR (300 MHZ,DMSO)δ ppm: 8.45(s,-1H,NH), 7.40–8.18(m,13H,Ar-H), 4.39(d,2H,CH). EI-MS: M+2 peak observed at 435.

Spectral data of N-(4-chlorophenyl)-5-(4-((3,4-dimethoxybenzylidene)amino)phenyl)-1,3,4-thiadiazol-2-amine (IV-e) : IR (KBr) cm^{-1}: 3283.81(NH), 2984(Aromatic C-H),2835(C-H of OCH_3), 1634(C=N), 784.42(C-Cl),.¹H-NMR (300 MHZ,DMSO)δ ppm: 8.45(s,-1H,NH), 7.40–8.18(m,13H,Ar-H), 4.39(d,2H,CH). EI-MS: M+2 peak observed at 452.

Spectra data of 2-(((4-(5-((4-chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)phenyl)imino)methyl)phenol (IV-f) :): IR (KBr) cm^{-1}: 3283.81(NH), 2984(Aromatic C-H),3345(C-OH), 1634(C=N), 784.42(C-Cl).¹H-NMR (300 MHZ,DMSO)δ ppm: 8.45(s,-1H,NH), 7.40– 8.18(m,13H,Ar-H), 4.39(d,2H,CH). EI-MS: M+2 peak observed at 408.

PHARMACOLOGICAL EVALUVATION OF SYNTHESIZED COMPOUNDS:

Antibacterial activity: By the Agar Well Diffusion technique, roughly 25ml of nutritious agar medium was sterilised (autoclaved at 120oC for 20 minutes) and put into sterile Petri dishes, which were set aside for solidification. Bacterial lawns were created by using a series L-shaped glass rod to disseminate the bacterial suspension (1ml/100ml, or cu/ml) across the surface of the agar plates. Using a sterile borer, wells were punched in the agar plates (6mm). The prepared concentrations (100g/ml, 500g/ml, 1000g/ml) of the test sample, as well as the control and standard, were aseptically placed into 50l wells. To allow optimal diffusion of the solution into the nutrient agar medium, the plates were left undisturbed in the refrigerator for at least 2 hours. The plates were then incubated at 371°C for 24 hours. After the incubation time, the diameter of each well's zone of inhibition was manually measured and tabulated using a millimetre scale. Whole experiment was carried out in triplicate. Simultaneously dimethyl sulfoxide was maintained as control to observe the solvent effect on bacteria. Compounds IVa, IVf shows 7mm zone of inhibition against Klebsiella pneumoniae at 1000µg/ml the standard drug shows 10mm, in case of Micrococcus luteus compounds IVa, IVb, IVc shows 8,9,8mm zone of inhibition respectively, the standard drug ciprofloxacin shows 14mm at concentration of 1000µg/ml, in Case of Staphylococcus aureus compound IVb shows 3mm but the standard ciprofloxacin shows 6mm of zone of inhibition. In case of E coli compound IVb shows 2mm but the standard shows 3mm zone of inhibition.

Antifungal activity: By agar well diffusion method. 1,3,4 thiadiazole derivatives having less active towards the fungi. In case of ***Candida albicans*** the compounds IVa, IVc, IVd shows 4mm zone of inhibition but the standard drug shows 14 mm at the concentration of 1000µg/ml.

Anti-oxidant activity: By using the free radical scavenging property of DPPH, The IC**50** values of all synthesized test compounds were found between 11.3µM to 96. 34µM.All the compounds were tested at 1nM to 1mM concentrations and results were compared with standard drug (Ascorbic acid) at the same concentrations. Compound IV-a (R= 5-H) & IV-b (R= 4-Cl) showed (**21.6µM and 22.8µM)** effective antioxidant activity. And IV-e (R= 4-N, N-Dimethyl) comparatively weak **(96.34µM)** antioxidant activity.

CONCLUSION:

New 1,3,4-Thiadiazole derivatives showed promising antimicrobial activity. Compounds **R= 4-Cl, R= 4-NO_2, R=2-OH, R= H** were found to be the more potent antibacterial activity respectively towards gram positive (*Micrococcus luteus*) and gram negative (*klebsiella pneumonia*) and compound **R= 4-Cl** found to be more potent antifungal activity towards *Candida albicans* and *Aspergillus Niger.* New 1,3,4-Thiadiazole derivatives showed promising antioxidant activity. Compounds IV-a **(R=H)**& IV-b **(R= 4-Cl)** were found to be more potent antioxidant compounds among the all test compounds.

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CONFLICT OF INTEREST

All the authors declare that they have no conflict of interest.

ABBREVIATION USED

All the abbreviations used have been described in the text.

REFERENCES:

1. GeorgetaSerban et al, "2-Amino-1,3,4-thiadiazole as a potential scoffold for promising antimicrobial agents", *Drug Des DevelTher*, 2018, *NCBI (National Center for Biotechnology Information)* Vol.12, pg.no. 1545-1566.
2. Krishnakant T. Waghmode and vishal U. Jadhav, "synthesis, characterization and antibacterial study of thiadiazole derivatives", *International journal of pharm bio sciences*, 2017, Vol.8(4), pg.no. 119-123.
3. Shingade S. G. and Shirodkar S.S., "Synthesis and Antimicrobial Screening of ThiadiazoleDerivatives",*Journal of Advanced Chemical Sciences*, 2016, Vol.2(3), pg.no. 309–312.
4. Lincy Joseph, Mathew George and Prabha Mathews, "A Review on Various Biological Activities of 1,3,4- Thiadiazole Derivatives", journal *of Pharm Chem BiolSci*, 2015, Vol. 3(3) pg.no. 329-345.
5. Rohit Bhatia and Amandeep Kaur, "Synthesis, spectral studies and antimicrobial activity of some imidazo [2,1-b] [1,3,4] thiadiazole derivatives", Der *Pharma Chemica*, 2014, Vol.6 (6), pg.no.114-120.
6. Baghel U. S, Chawla A and Dhawan R. K," Synthesis and antimicrobial evaluation of thiadiazole derivatives", *Der Pharma Chemica*, 2014, Vol.6(2) pg.no. 66-69.
7. Mahendrasinh M Raj, Hemul V Patel, Lata M Raj, and Naynika K Patel, "Synthesis, Characterization and Antimicrobial Evaluation of Some 5(Substituted)-2-Amino-Thiadiazoles", *Research Journal of Pharmaceutical, Biological and Chemical sciences*, 2013, Vol. 4(3), pg.no. 721-727.
8. Adediji Johnson F, Adebayo Matthew A, AjayiYewande O, and Yusuf Latifat A., "Novel mixed ligand of 2,5-diamino-1,3,4-thiadiazole schiff base incorporating benzoic acid: Synthesis and antimicrobial activity", *Journal of Chemical and Pharmaceutical Research*, 2012, Vol. 4(3), pg.no. 1501-1504.
9. VenuMadhav. N, Srinivas naik. A, Venkashwarrao. J and sarangapani. M, "Synthesis of some new 1,3,4-thiadiazoles as antimicrobial agents", journal *of pharmacy research*, 2011, Vol. 4(5), pg.no. 1386-1397.
10. SanjeevaR.cherkupally, Chandrashekar R. Dasari, YakubVookanti and NagrajAdki, "Synthesis and antimicrobial study of bis-[thiadiazol-2-yl-tetrahydro-2H-pyrazolo[3,4-d] [1,3] thiazole] methanes", *Organic communications*, 2010, Vol. 3(3), pg no. 57-69.
11. Sabir Hussain, Jyothi Sharma and Mohd. Amir, "Synthesis and Antimicrobial Activities of 1,2,4-Triazole and 1,3,4-Thiadiazole Derivatives of 5-Amino-2-Hydroxybenzoic Acid", *E-Journal of Chemistry, 2008, Vol. 5(4), pg.no. 963-968.*
12. Sunny Jalhan, Anil Jindal, Avneet Gupta, Hemraj, "Synthesis, biological Activities and

chemistry of thiadiazole derivatives and Schiff's base", *Asian Journal of Pharmaceutical and Clinical Research*, 2012, Vol. 5(3), pg.no. 199-208.

13. Hatice N. Dogan,a, ArzuDuran,aSevimRollas,a Go kselSener, b Meral K. Uysalb and DumrulGulenc "Synthesis of New 2,5-Disubstituted-1,3,4-thiadiazoles and Preliminary Evaluation of Anticonvulsant and Antimicrobial Activities" ,*Bioorg. Med. Chem*., (2002), vol.10, 2893–2898.
14. Jitendra Kumar Gupta, Rakesh Kumar Yadav, Rupesh Dudhe, Pramod Kumar Sharma " Recent Advancements in the Synthesis and Pharmacological Evaluation of Substituted 1, 3, 4Thiadiazole Derivatives" *Int.J. PharmTech Res.* 2010,2(2), 1494-1507.
15. NadjetRezki, Amjad M. Al-Yahyawi, Sanaa K. Bardaweel, Fawzia F. Al-Blewi and Mohamed R. Aouad, "Synthesis of Novel 2,5-Disubstituted-1,3,4-thiadiazoles Clubbed 1,2,4-Triazole, 1,3,4-Thiadiazole, 1,3,4-Oxadiazole and/or Schiff Base as Potential Antimicrobial and Antiproliferative Agents" Molecules 2015, 20, 16048-16067.
16. Alya'aJabbar Ahmed "Pharmacological activity of 1,3,4-thiadiazole derivatives and its complexes" *Int.Res. J. Pharm*., 2018, 9and (10).
17. Hugo and Russell's "pharmaceutical Microbiology", edited by stepen P. Denyer, Norman A. Hodges and sean P. Gorman, 6th edition
18. Thoraya A. Farghaly, Magda A. Abdallah and Mohamed R. Abdel Aziz "Synthesis and Antimicrobial Activity of Some New 1,3,4-Thiadiazole Derivatives", Molecules 2012, vol. 17, p.g.no.14625-14636.
19. Sunny Jalhan, Anil jindal, Avneetgupta, Hemraj "Synthesis, Biological activities and chemistry of thiadiazole derivatives and Schiff bases", *Asian J Pharm Clin Res*, Vol 5, Issue 3, 2012, 199-208.
20. Nesrinsener, Mahmut, "Novel 1,3,4-thiadiazole compounds derived from 4-phenylbutyric acid: Synthesis, characterization and DFT studies", BAUN *Fen Bil. Enst. Dergisi*, 20(1),2018, 145-155.
21. Sevgikarakus, Sevimrollas, "Synthesis and antimycobacterial activity of some 2-(4-aminophenyl)5-substituted amino-1,3,4-thiadiazole derivatives and their coupling products",*Marmara Pharmaceutical Journal* 20, 199-206, 2016.
22. Vinit Raj, AmitRai, Mahendra Singh, Ram Kumar, Arvind Kumar, Vinod Kumar and S.K Sharma, "Recent Update on 1,3,4-Thiadiazole Derivatives: As Anticonvulsant Agents",*American Research Journal of Pharmacy*, Volume 1(1), 2380-5706, 2015.
23. HibaIbraheem, Yasmeen AI-Majedy, Ahmed AI-Amier, "4-Thiadiazole: The Biological Activities",*Systematic Reviews in Pharmacy*, 9(1), 2018, 36-40.
24. Prasanna A DatarandTejashreeADeokule, "Design and Synthesis of Thiadiazole Derivatives as Antidiabetic Agents", *Medicinal chemistry*, 4(4), 390-399, 2014.
25. HarinderKaur, HarmandeepKaur, AmitChawla, U.S. Baghel and R.K. Dhawan, "Thiadiazole: An amazing anticancer moiety", *International Journal of Pharmaceutical Chemistry*, 04(1), 2249-734X (Online), 2014.
26. Harika.M, Sudha.B, "Synthesis & characterization of N-thiadiazo lylthiazo lidi none derivatives", *International Journal of Pharmaceutical Sciences*, 4(2), 13–16 2014.
27. Adil A, Otham, MebroukKihel, Sarah Amara, "review on 1,3,4-thiadiazole, 1,3,4-oxadiazole and 1,2,4-triazole derivatives as potential antibacterial agents", *Arabian journal of Chemistry*, 1878-5352, 2014.
28. Abhishek Kumar Jain, Simant Sharma, AnkurVaidya, VeerasamyRavichandran and Ram Kishore Agrawal, "1,3,4-Thiadiazole and its Derivatives: A Review on Recent Progress in Biological Activities", *ChemicalBiology and drug design*, 81, 557–576, 2013.
29. HooshangVahedi,JalilLari, AbolfazlHousaini, Mohammad Habibi, "Synthesis of 4,5-Dihydro 1,3,4-Oxadiazoles And 1,3,4-Thiadiazoles Carrying Isatin Moiety", *International Journal of ChemTech Research*, .4(4), 1379-1382, 2012.
30. MasiHasmin. H., Gajjar A. K., SavjaniJ.K., MasiInayat A., "Synthesis and Anticonvulsant Activity of Novel 2,5-Disubstituted -1,3,4 - Thiadiazole Derivatives", *International Journal of PharmTech Research*, 3(4),2017-2024, 2011.
31. JitendraSainy, Ganesh P. Mishra, Rajesh Sharma, Subhash C. Chaturvedi. "Synthesis of novel 2-amino 5-Sulfanyl- 1, 3, 4-thiadiazole for Anti Inflammatory and Analgesic agents" *Pharmaceutical Chemistry Journal*, 43[1]:19-24,2009.
32. Chinnagiri T. KeerthiKumar, JathiKeshavayya, Tantry N. Rajesh Sanehalli K. "Synthesis, Characterization and Biological Activity of 5-Phenyl-1, 3, 4-thiadiazole 2amine". International *Journal of Chemistry*., 6[12], 2013.
33. Hatice N. Dogan, Arzu Duran, SevimRollas, GokselSener, Meral K. Uysal and

DumrulGulen,"Synthesis of New 2,5-Disubstituted-1,3,4-thiadiazoles and Preliminary Evaluation of Anticonvulsant and Antimicrobial Activities", *Bioorganic & Medicinal Chemistry*, 10, 2893–2898, 2002. NJ M

34. RobertM. Silverstein, FrancisX. Webster. Sixth edition. Wiley publishing houses; (212-234), 1916.
35. Subhas S. karki, Vivekrana, Ramjith U. Sivan, Sujeethkumar, Vinayakumarrenuka, Sureshbabu A. Ramareddy, Prasanna G. Subbarao and Sudam C. "Synthesis and Antiinflamma toryactivity of someImidazo[2,1-b] [1,3,4] Thiadiazolede - rivatives" , Acta*pol oniae pharmaceutica-Drug Research*,72(5), 931-936, 2015.
36. Anamaria Cristina, DenisaLeonte, Laurian Vlase, LászlóCsabaBencze, Silvia Imre, Gabriel Marc, BogdanApan, Cristina Mogosan and ValentinZaharia, "Synthesis, Characterization and Biological Evaluation of Imidazo[2,1-b] [1,3,4] Thiadiazole Derivatives as Anti-Inflammatory Agents" *Molecules*, 23, 2425, 2018.
37. Blios,M.S.〞Anti-oxidant Determination by the Use of Stable free Free Radical" *Nature*.,26(1958),1199-1200.