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Synthesis and Antimicrobial Activity of some 1, 3, 4-Thiadiazole Derivatives

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ABSTRACT

The structural and therapeutic diversity coupled with commercial viability of small heterocyclic molecules has fascinated organic and medicinal chemist. So, a great deal of research was carried out in the field of heterocyclic containing sulphur and nitrogen, because of their immense biological importance. The thiadiazole derivatives posses' different pharmacological and biological activity. Hence, the search for never effective antimicrobial agents is imperative. It focuses on the problems of cross resistance and better activity against variety of infections. The majority of 1, 3, 4-thiadiazoles are based on the cyclisation of thiaosemicarbazide derivative incorporating this basic structural unit. The structure of new compounds prepared during present investigation has been authentically established by their IR, ^{1}H NMR and Mass spectral studies. The antimicrobial activity of thiadiazole derivatives also reported some of these derivatives exhibit significant and broad spectrum antimicrobial activity. All the synthesized compounds were screened for antibacterial activity against *Bacillus subtilis, Bacillus pumilus, E coli and Pseudomonas aureginosa* by Disc diffusion method using ciprofloxacin as a standard against Gram positive and Gram negative bacteria.

Key Words: Thiadiazole, Thiosemicarbazide, Antimicrobial, Cyclization, Screening

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INTRODUCTION

Antimicrobial agent can be found in sewage effluents especially in place where they are used extensively such as hospital, pharmaceutical production plant and near forms where animal feed containing antimicrobial agents is used. So these drugs are employed mainly for the prevention and use of bacterial infections in man. The clinical value of antimicrobial agents cannot be assumed simply because they inhibit or kill microbial pathogens in vitro. Pharmacodynamic studies, which show how pharmaceutical agents interact with their therapeutic targets, have found that the success of antimicrobial agents at eliminating bacterial pathogens is affected by the local milieu of infection.

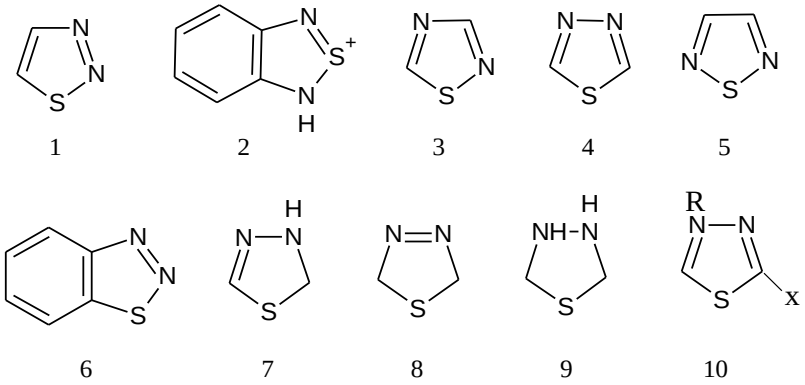


Figure 1: The thiadiazole system contains the following members the 1, 2, 3-thiadiazoles

During recent years there has been a large investigation on different classes of thiadiazole compounds, many of which were found to possess an extensive spectrum of pharmacological activity such as antifungal[1], antimicrobacterial activity[2], antibacterial[3], antimycobacterium[4,5,11.], anticovulsant and Antimicrobial activities[6-7], antitumor[8], CNS depressants[10], herbicidal[12], antiviral[13], Antitrypanosomal activity [14,15] and anti-inflammatory activity[16]. The thiadiazole system contains the following members the 1,2,3-thiadiazoles(**1**) and their benzo derivatives (**2**) the 1,2,4-thiadiazoles (**3**) the 1,3,4-thiadiazoles (**4**) and the 1,2,5-thiadiazole (**5**) and their benzo derivatives (**6**). Most of the published work, by far, is on 1, 3, 4-thiadiazoles. Between 1967 and March 1, 1982 chemical abstracts lists 724 references for this ring system. This includes the 1, 3, 4-thiadiazolines (**7**) and (**8**) and the 1, 3, 4-thiadiazolidines (**9**). 1, 3, 4-thiadiazoles were first described in 1882 by Fischer and further developed by Busch and his co-workers. The advent of sulphur drugs and the later discovery of mesoionic compounds greatly accelerated the rate of progress in the field of thiadiazole. The 1, 3, 4-thiadiazoles are divided into three subclasses. Aromatic systems which include the neutral thiadiazole (**4**) and constitute a major part of this review. Mesoionic systems (**10**) which are defined as five membered heterocyclic which are not

covalent or polar and possess a sextet of electrons in association with the five atoms comprising the ring. Non-aromatic systems such as the 1, 3, 4-thiadiazolines (**7**) and (**8**) and the tetra hydro 1, 3, 4-thiadiazolidines (**9**). In the partially reduced systems the positions of double bond is denoted by prefix Δ with (**7**) being a Δ^2-1, 3, 4-thiadiazoline.

MATERIALS AND METHODS:

The chemicals and reagents used in the present project were of AR grade and LR grade, purchased from Lancaster, Sigma, Qualigens, NR Chem., Rolex, S.D.Fine Chem. Ltd., Merck, Loba and Hi-media. Melting points were determined by using Toshniwal apparatus in open capillaries and are uncorrected. The purity of the compound was checked by TLC on silica gel G plates using Methanol: chloroform (7:3) solvent system and U.V lamp using as a visualizing agent. IR spectra were recorded using KBr pellets on a JASCO FT/IR-5300 spectrophotometer. 1HNMR spectra on a Varian EM-200 spectrophotometer using DMSO and CDCl3 solvent and TMS as standard (chemical shift values expressed in δ ppm) Mass spectra were recorded by LCMS technique.

Analytical Techniques

Physical data:

Melting points of the synthesized compounds were determined using Thiele's melting point Apparatus and were found uncorrected.

Thin Layer Chromatography (TLC):

Purity of the compounds was checked by thin layer chromatography using silica gel G as Stationary phase and various combinations of chloroform and methanol are used as mobile Phase. The spots resolved were visualized as brown colored spots by using iodine chamber.

Instrumentation:

The techniques employed for the characterization of the synthesized compounds were IR, ^{1}HNMR and Mass spectra.

Infrared spectra:

The IR spectra of the synthesized compounds were recorded using dry KBr pellets in range of 4000-400 cm-1 on a BRUKER α-Fourier transforms IR spectrometer at Sipra labs ltd. Hyderabad and frequencies were recorded in wave numbers.

^{1}H NMR magnetic resonance spectra:

1H-NMR spectra were recorded in BRUKER (200 MHz) using $CDCl_3$ as solvent at Sipra Labs Ltd. Hyderabad.

Mass spectra: Mass spectra were done by LC-MS technique at Sipra labs pvt.ltd Hyderabad.

Antimicrobial Activity[9]:

The compounds synthesized during the present investigation were screened for their antibacterial activity. The antibacterial tests were conducted on four common microorganism such as Bacillus subtilis, Bacillus pumilus, Escherichia coli and Pseudomonas aeruginosa that are the representative type of gram positive and gram negative organism respectively. The antibacterial activity of the compounds was assessed by Disc-diffusion method.

BIOLOGICAL ACTIVITY

Preparation of nutrient broth

Peptone (Bacteriological): 20 g Beef extract (Bacteriological): 05 g Sodium chloride: 05 g Distilled water up to: 1000 ml Nutrient broth is prepared by dissolving all these and steam and steam for about 2 hours adjust the reaction mixture pH to about 7.2 and autoclave at 15 lbs pressure for 20 minutes. One day prior to the testing, the organisms obtained from the laboratory stock were sub-cultured in to sterile nutrient broth and incubated at 37 ^{0}C for 24 hours. The culture growth thus obtained was used as inoculums for the antibacterial testing.

Preparation of nutrient agar media:

The nutrient agar media was prepared by using the following ingredients

Peptone (Bacteriological)	:20 g
Beef extract (Bacteriological)	:05 g
Sodium chloride	: 05 g
Agar (Bacteriological)	:20 g
Distilled water up to	: 1000 ml

Weighed quantities of peptone, beef extract were dissolving in distilled water by gentle warming, and then specified amount of agar was dissolve by heating on cooling water bath. The pH of the above solution is adjusted by adding sodium chloride and the volume of final solution is made up to 1000ml with distilled water. Then the above prepared nutrient agar media is sterilized by autoclave at 120 ^{0}C for 20minutes at 15lbs/ in2 pressure.

Biological activity

Preparation of test solution

10 mg of the test compound in 10 ml of DMSO. From this 1ml of solution was taken and diluted to 10 ml with DMSO. Now the concentration of the test is 100µg/ml. These sample solution were made in suitably labeled sterilized test tubes.

Preparation of standard solution

The standard drug uses for the testing is Ciprofloxacin It is water soluble, concentration of this drug is adjusted so as to contain 100µg/ml.

Preparation of discs

Discs of 6-7 mm in diameter were punched from no.1 whattmann filter paper with sterile cork borer of same size. These discs were sterilized by keeping in oven at 130 ^{0}C for 60 minutes. The standard and test solution were added to each discs and discs were air-dried.

Methods of testing

The sterilized was cooled to 45 ^{0}c with gentle shaking to bring about to uniform cooling and then inoculated with 18 to 24 hours old culture under aseptic conditions, mixed well by gentle shaking. This was poured into sterile Petri dishes and allowed the medium to set. After solidification all the Petri dishes were transferred to laminar air flow unit then the discs which were previously prepared were carefully kept on the solidified media by using sterilized foreseps. These Petri dishes were kept as it is for 1hr for diffusion at room temperature and then incubation at 37 ^{0}c for 24hr in an incubator. The extent diameter of inhibition after 24 hours was measured as the zone of inhibition in mm and the results were shown in table no 4.

Experimental:

Step 1: Synthesis of thiosemicarbazones (MY1a-c):

Aromatic aldehyde (0.2 M) in warm alcohol (300 ml) and thiosemicarbazide (0.2 M) in warm water (300 ml) were mixed slowly with continuous stirring. The product separated immediately on cooling which was filtered with suction, dried and recrystalized in 75% ethanol to yield thiosemicarbazone.

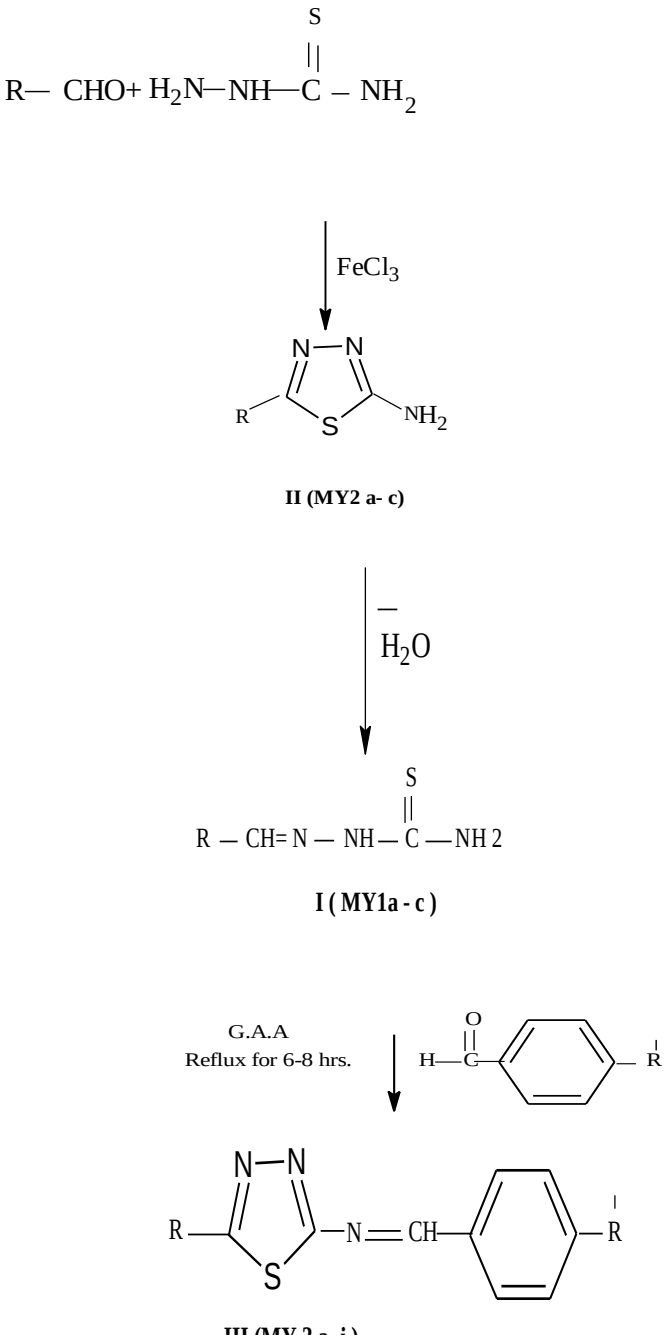
Step-2: Synthesis of 5-phenyl-1, 3, 4-thiadiazole-2-amine (MY2a-c):

Thiosemicarbazone (0.05 M) was suspended in 300 ml warm water, $FeCl_3$ (0.15 M) in 300 ml water was added quantitatively, slowly with constant stirring. The contents were heated at 80-90 ^{0}C for 45 min. Solution was filtered while hot and then citric acid (0.11 M) and sodium citrate (0.05M) were added. The resulting mixture was divided into 4 parts and each part was neutralized separately with ammonia (10%). The required amine separated out, filtered with suction, dried and recrystallized with appropriate solvent.

Step-3: Synthesis of N-benzylidene-5-phenyl-1, 3, 4-thiaadiazole -2-amine (MY3ai)

To a stir solution of compound (0.02mol, 5g) in methanol (50 ml) containing glacial acetic acid (2 ml) was added appropriate aromatic aldehyde (0.02 mol) and the mixture refluxed for 6-8 hours on a water bath the separated solvent was distilled off at reduced pressure and the resulting

solids was cooled, dried and recrystallised from benzene-chloroform mixture to give the compound.



SCHEME 1

R = C_6H_5, *p*-ClC_6H_4 *p*-$CH_3C_6H_4$,

R^1 = C_6H_5, *p*-ClC_6H_4 *p*-$CH_3C_6H_4$,

RESULTS AND DISCUSSION

All the synthesized compounds were screened for antibacterial activity. The data in the table**.** No. 4 indicates that compounds **MY3-b, MY3-c, MY3-d,** and **MY3-I** were exhibited a broad spectrum antibacterial activity. While other synthesized compounds of this series shown poor antibacterial activity.

All the synthesized compounds were screened for antibacterial activity against Bacillus subtilis, bacillus pumilus, Escherichia coli and Pseudomonas aureginosa by disc diffusion method using Ciprofloxacin as a standard against gram +ve and Gram – ve bacteria. The synthesized Compounds **MY3-b, MY3-c, MY3-d** and **MY3-I** were exhibited a broad spectrum antibacterial activity. While other synthesized compounds of this series shown poor antibacterial activity.

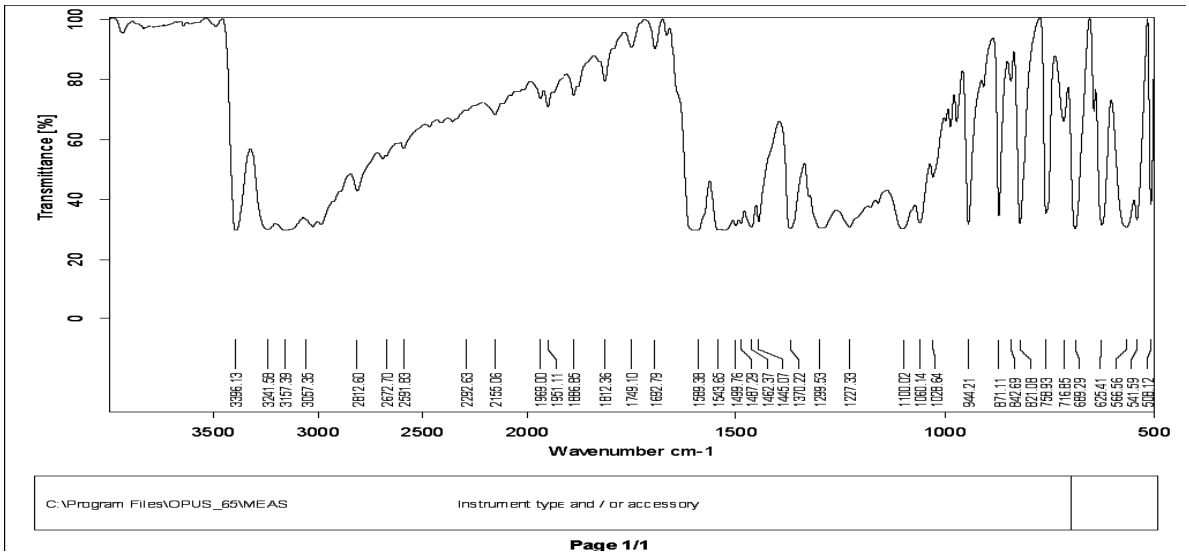


Figure 2: Thiosemicarbazones (MY1-a)

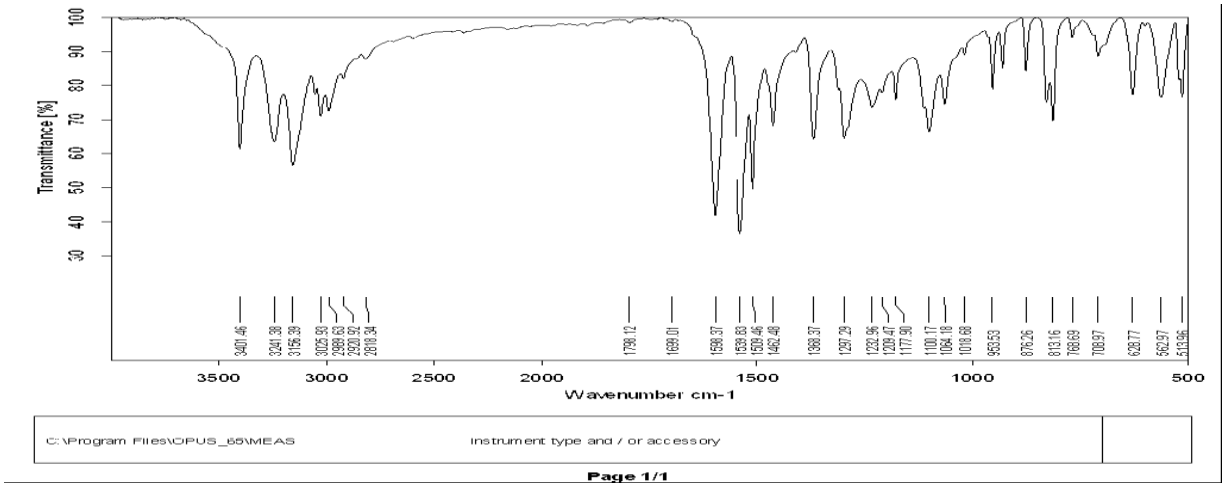


Figure 3: Thiosemicarbazones (MY1-c)

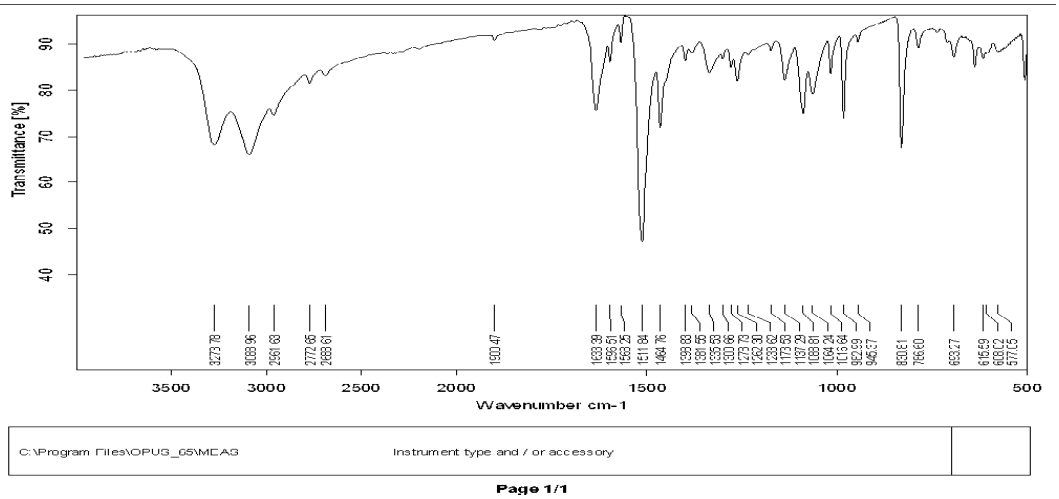


Figure 4: 5-Phenyl-1, 3, 4-thiadiazole-2-amine (MY2-b)

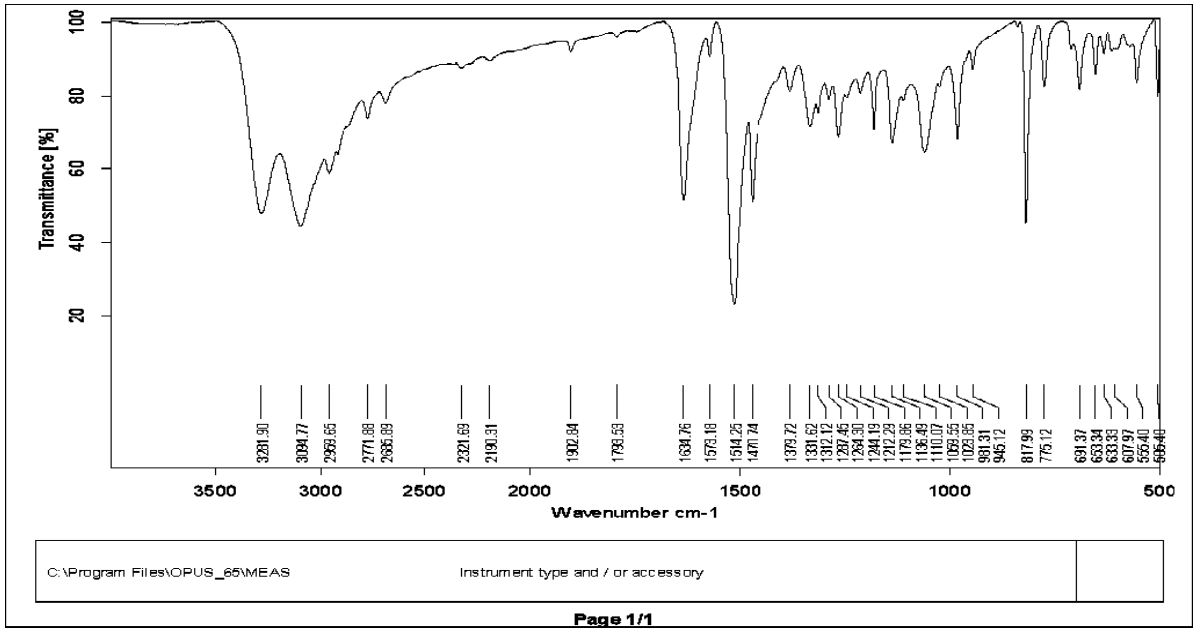


Figure 5: 5-Phenyl-1, 3, 4-thiadiazole-2-amine (MY2-c)

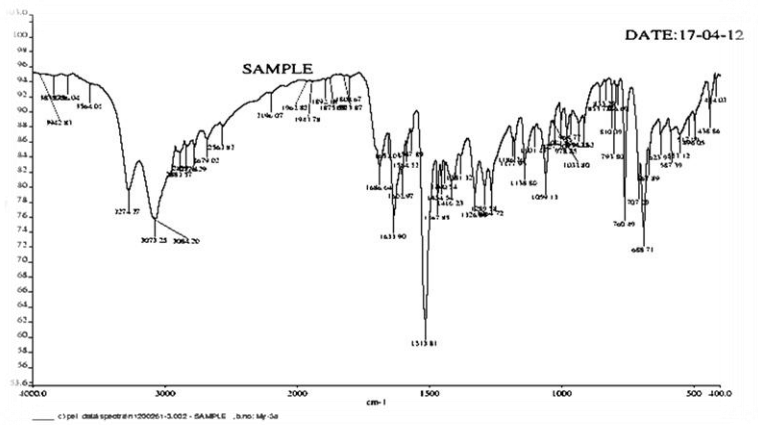


Figure 6: N-benzylidene-5-Phenyl-1, 3, 4-thiadiazole-2-amine (MY3-a)

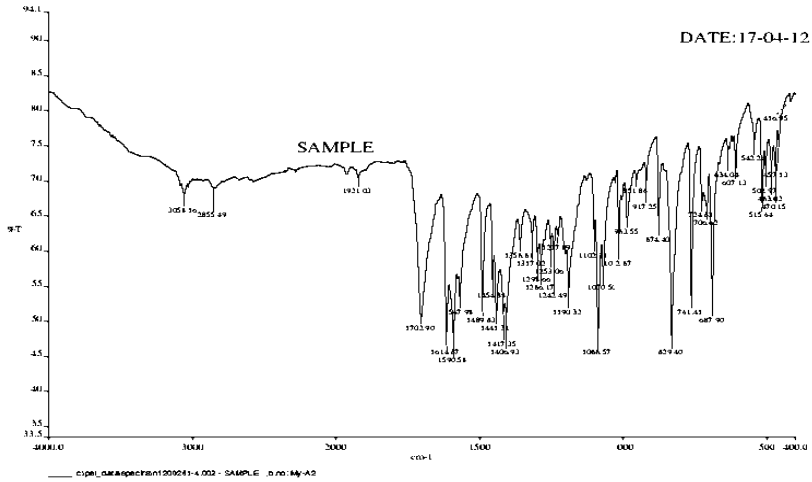


Figure 7: N-benzylidene-5-Phenyl-1, 3, 4-thiadiazole-2-amine (MY3-b)

MY1-a:3396-3241 (NH, NH_2), 3057 (Ar-H), 1599 (C-N) 1543-1499 (C=C), 1100 (C=S)

MY1-c:3401-3156 (NH, NH2), 3025 (Ar- H) 2920 (C-H, in CH3), 1598 (C=N) 1539-1462 (C=C), 1100 (C=S)

MY1-a:3396-3241 (NH, NH_2), 3057 (Ar-H), 1599 (C-N) 1543-1499 (C=C), 1100 (C=S)

MY1-c:3401-3156 (NH, NH2), 3025 (Ar- H) 2920 (C-H, in CH3), 1598 (C=N) 1539-1462 (C=C), 1100 (C=S)

MY3-a:3274 (NH), 3073 (Ar-H) 1686 (C=N), 1633(C=C) 688 (C-S)

MY3-b:3058 (NH), 2855 (Ar-H), 1702 (C=N), 1665 (C=O), 1590-1614 (C=C), 681 (C-S)

MY3-c: 3276 (NH), 3061 (Ar-H) 2964 (C-H, in CH_3) 1686-1687 (C=N), 1633 (C=C) 688 (C-S)

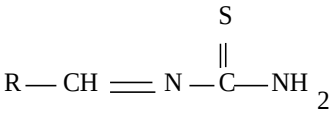
(MY3-b)- ^{1}H-NMR (δ ppm): -7.26-7.98 multiplet for (9H, Ar-H)

Mass spectra (m/z): 300 (M+1 100 %)

(MY3-c)- ^{1}H-NMR (δ ppm): - 2.45-2.7Singlet (3H of CH_3) 7.26-8.96 (9H of Ar-H)

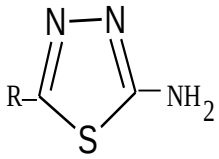
Mass spectra (m/z): 280(M+1 100%)

Table -1: Physicochemical data of thiosemicarbazones (A1a-c):



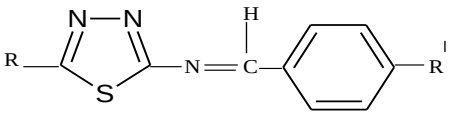
Sl No.	Compound	R	Molecular Formula	Molecular Weight	M.P(^{O}C)	Yield(%)
1	MY1-a	-C_6H_5	$C_8H_8N_2S$	164.2	158-160	92%
2	MY1-b	*p*-ClC_6H_4	$C_8H_7ClN_2S$	178.25	169-160	95%
3	MY1-c	*p*-$CH_3C_6H_4$	$C_9H_{10}N_2S$	198.67	205-207	90%

Table-2: Physicochemical data of 5-phenyl-1, 3, 4-thiadiazol-2-amine (A2a-c):



Sl No.	Compound	R	Molecular Formula	Molecular Weight	M.P (^{o}c)	Yield (%)
1	MY2-a	- C_6H_5	$C_8H_7N_3S$	177.2	222-224	65%
2	MY2-b	*p*-ClC_6H_4	$C_8H_6ClN_3S$	191.25	214-216	62%
3	MY2-c	*p*-$CH_3C_6H_4$	$C_9H_{10}N_3S$	211.67	226-228	70%

Table- 3:Physicochemical data of *N*-benzylidene-5-phenyl-1, 3, 4-thiadiazol-2-amine (A3a-i):



Sl No.	Compound Code	R	R^1	Molecular Formula	Molecular weight	MP (^{O}C)	Yield (%)
1	MY3-a	-C_6H_5	-C_6H_5	$C_{15}H_{11}N_3S$	266	255-257	63%
2	MY3-b	- C_6H_5	p-ClC_6H_4	$C_{15}H_{10}ClN_3S$	299	285-287	67%
3	MY3-c	- C_6H_5	p-$CH_3C_6H_4$	$C_{16}H_{13}N_3S$	279	290-292	67%
4	MY3-d	*p*-ClC_6H_4	-C_6H_5	$C_{15}H_{10}ClN_3S$	299	252-254	90%
5	MY3-e	*p*-ClC_6H_4	*p*-ClC_6H_4	$C_{15}H_9C_{l2}N_3S$	334	264-266	94%
6	MY3-f	*p*-ClC_6H_4	*p*- $CH_3C_6H_4$	$C_{16}H_{12}ClN_3S$	313	244-246	88%
7	MY3-g	*p*- $CH_3C_6H_4$	-C_6H_5	$C_{16}H_{13}N_3S$	279	285-287	67%
8	MY3-h	*p*- $CH_3C_6H_4$	*p*-ClC_6H_4	$C_{16}H_{12}ClN_3S$	313	265-267	88%
9	MY3-i	*p*- $CH_3C_6H_4$	*p*- $CH_3C_6H_4$	$C_{17}H_{15}N_3S$	293	264-267	94%

Table -4: Antibacterial activity of synthesized compound (MY3a-i)

Sl No.	Compound Code	zone of inhibition diameter in mm			
		B.subtilis	B.pumillis	E.coli	P.aeriginosa
1	MY3-a	6	7	10	9
2	MY3-b	12	14	13	13
3	MY3-c	11	12	13	12
4	MY3-d	12	14	13	13
5	MY3-e	10	11	9	11
6	MY3-f	9	8	10	9
7	MY3-g	10	10	9	9
8	MY3-h	9	9	10	8
9	MY3-i	11	12	13	14
10	**Ciprofloxacin**	17	17	18	17
11	DMSO	-	-	-	-

Standard: ciprofloxacin

*Each value is an average of three independent determination + Standard deviation.

Note: '-' denotes no activity, 8-12mm poor activity, 13-17mm moderate activity, 18-20 mm Good activity.

CONCLUSION

From the data of the Table No-4 of antibacterial it is clearly concluded that the synthesized compounds are significant and moderate antibacterial agents. The substituted 1,3,4-thiadiazole moieties are already known for different Biological activities. Here we have synthesized some novel 1, 3, 4-thiadiazole analogues combining with different substituted aromatic and aliphatic system with view to get a good antibacterial and antifungal agents with less toxic and side effects. From the above results one can establish that the synthesized substituted 1, 3, 4 thiadiazole can be rich source for the exploitation. Therefore in search of new generation of the active compounds, it may be worthwhile to explore the possibility in this area by introducing different functional groups or by cyclization as substitutions. Which may results into better pharmacological agents.

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