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Synthesis and Antimicrobial Activity of *N*-(5-Phenyl-1, 3, 4-Thiadiazole-2-yl) Benzamide/ Acetamide

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ABSTRACT

In the present study, a series of *N*-[5-(phenyl)-1,3,4-thiadiazole-2-yl] benzamide and N-[5-(phenyl)-1,3,4-thiadiazole-2-yl] acetamide were prepared by refluxing with benzoyl chloride and acetyl chloride with 5-phenyl-1,3,4-thiadiazole-2-amine. 5-phenyl-1,3,4-thiadiazole-2-amine were prepared by oxidative cyclization of thiosemicarbazone (through condensation of aromatic aldehyde and thiosemicarbazide). The structure of new compounds prepared during present investigation have been authentically established by their IR,^{1}H-NMR and Mass spectral studies. The antibacterial and antifungal activities of thiadiazole derivatives also reported. Some of these derivatives exhibit significant antimicrobial activity.

Key words: Thiosemicarbazone, Thiadiazole, antibacterial, antifungal.

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INTRODUCTION

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 antibacterial[1], & antimycobacterium[2], anticonvulsant[3], antitumor[4], CNS depressants[5], herbicidal[6], antiviral[7] and anti-inflammatory activity[8]. The thiadiazole system contains the following members the 1,2,3-thiadiazoles and their benzo derivatives the 1,2,4-thiadiazoles the 1,3,4-thiadiazoles and the 1,2,5-thiadiazole and their benzo derivatives. Most of the published work, by far, is on 1,3,4-thiadiazoles. Between 1967 and March 1982 chemical abstracts lists 724 references for this ring system. This includes the 1,3,4-thiadiazolines and the 1, 3, 4-thiadiazolines. Generally, in pharmaceutical field new drugs are discovered by molecular modification of the lead compound of established activity. So an attempt was made to synthesize, new substituted 1,3,4-thiadiazoles compounds as antimicrobial agents. Hence synthesis of different derivative of 1,3,4-thiadiazoles was carried out along with other substituted aromatic aldehyde..

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 Himedia. Substituted aromatic aldehyde, thiosemicarbazide, Benzoyl chloride, acetyl chloride etc were used in this work.

The completion of reactions was monitored by TLC technique using Silica gel-G (for TLC) using suitable solvent. Determination of melting point was done by open capillary tube method using paraffin bath and are uncorrected. Recrystallization was done by suitable solvent. The ^{1}H-NMR of synthesized compounds were recorded in Br FT-NMR (400MHz & 200MHz) as TMS as internal standard and IR-spectra were recorded in Bruker alpha FT-IR using KBr pellets. The Mass spectra were recorded on Shimadzu LCMS with ESI source.

a) Antimicrobial Activity[11]:

Clinically isolated four bacterial strains namely *Staphylococcus aureus, Bacillus subtilis, E. coli, Pseudomonas aeruginosa* and two different fungal strains namely, *Aspergillus niger,* and *Candida albicans* were collected from Department of Microbiology, M. R. Medical College, Gulbarga, India. The bacterial strains were grown in Mac Conkey agar plates at 37^{0}C and maintained on nutrient agar slants, while fungi were grown at 30°C and maintained in Saboraud glucose agar slants. The test was performed by disc diffusion assay as per NCCLS, 1993. The

nutrient agar plates containing an inoculums size of 106cfu/ml for bacteria and 2×105 spores for fungi on Saboraud glucose agar plates were used. Previously prepared compound impregnated disc (6mm in diameter) at the concentrations of 200µg/ml for bacterial and 200µg/ml for fungal strains were placed aseptically on sensitivity plates with appropriate controls Ciprofloxacin (200µg/ml) and Griseofulvin (200µg/ml) were used as standard antibacterial and antifungal antibiotics respectively. Plates were incubated at 37°C for 24hrs for bacteria and 30°C for 72hrs for fungal inoculums. Sensitivity was recorded by measuring the clean zone of growth inhibition on agar surface around the disc.

b) Experimental:

Step 1: Synthesis of thiosemicarbazones (A1a-c):

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

A1-a IR: 3396 (NH2), 3157 (Ar-H) 3057, 1589 (C=N), 1537 (C=C), 1370 (C=S).

A1-b IR: 3401 (NH), 3156 (Ar-H), 3025 (C-H, -CH_3) 1598 (C=N), 1539 (C=C), 1370 (C=S) 768 (C-Cl).

Step-2: Synthesis of 5-phenyl-1, 3, 4-thiadiazole-2-amine (A2a-c):

Thiosemicarbazone (0.05mol) was suspended in 300ml warm water, FeCl3 (0.15mol) in 300ml water was added quantitatively, slowly with constant stirring. The contents were heated at 80-90°C for 45min. Solution was filtered while hot and then citric acid (0.11mol) and sodium citrate (0.05mol) 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.

A2-b IR: 3281 (NH2), 3094 (Ar-H), 2959 (C-H, in CH_3), 1634 (C=N) 1537-1470 (C=C).

A2-c IR: 3273 (NH), 3088 (Ar-H), 1633 (C=N), 1596-1464 (C=C) 786 (C-Cl), 693 (C-S).

Step-3: Synthesis of *N*-(5-phenyl-1, 3, 4-thiadiazole-2-yl) benzamide /acetamide (A3a-c), (A4a-c):

To a solution of 5-phenyl-1,3,4-thiadiazol-2-amine (0.005mol), in dry acetone (15ml) and benzoyl chloride/ acetyl chloride (0.005mol), was added drop by drop at 0-5°C. The reaction mixture was stirred for 2hrs and refluxed for 6hrs. Excess of acetone was distilled off and after cooling the reaction mixture was poured in ice cold water. A light pinkish mass was separated, which was collected by filtration, washed well with water, dried and recrystallized with ethanol.

A3-b IR: 3150 (NH), 3004 (Ar-H) 2917 (CH, in CH_3), 1662 (C=O), 1601 (C=N), 1579-1449 (C=C), 696 (C-S-C). ^{1}H-NMR (δ ppm): 1.21 (s,3H,CH_3), 2.43 (s,1H,NH)7.03-8.33, (m,9H,Ar-H). Mass (m/z). 295.8 (M+1) 100 % A4-b; IR 3254 (NH), 3019 (Ar-H),2916 (C-H, in CH_3), 1626 (C=O),1563 (C=N), 1533-1445 (C=C),704 (C-S-C). A4-c IR 3268 (NH), 3052 (Ar-H), 2907 (C-H, in CH_3), 1692 (C=O), 1626 (C=N), 1597-1485 (C=C), 704 (C-S-C)

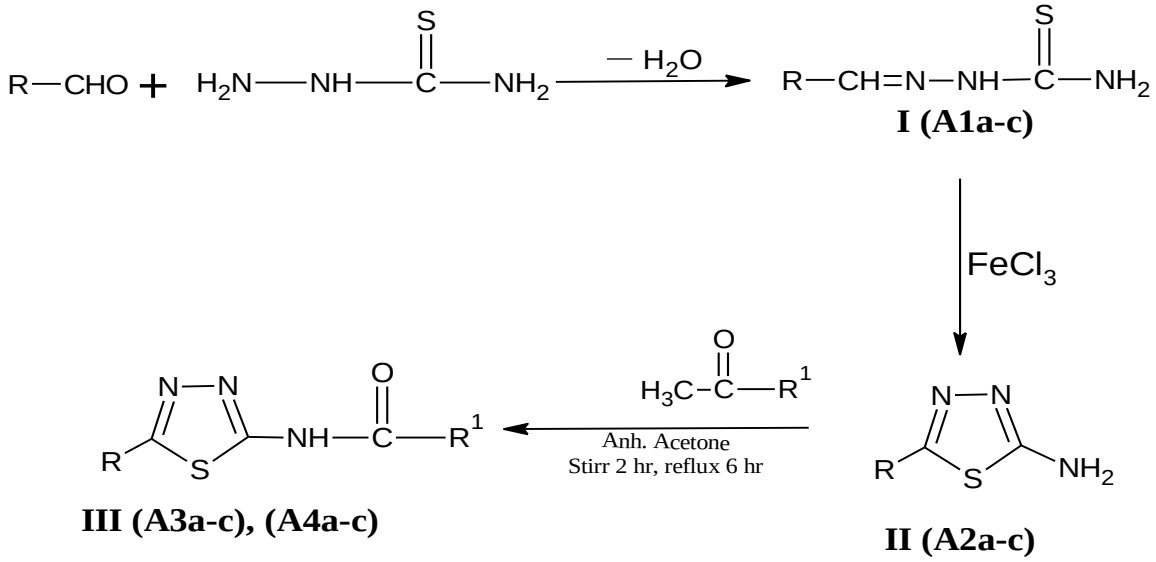
RESULTS AND DISCUSSION:

The synthesis of *N*-(5-phenyl-1, 3, 4-thiadiazole-2-yl) benzamide/ acetamide is accomplished is figure was synthesized by 3-step procedure. Whereas *N*-(5-phenyl-1, 3, 4-thiadiazole-2-yl) benzamide/ acetamide obtained by refluxing equimolar amount of 2-amino-5-aryl-1,3,4-thiadiazole and various carbonyl compound. The structures of the new compounds were elucidated by analytical and spectroscopic measurement.

IR spectrum of the intermediates [A2b-c] Showed absorption band around 3281- 3229cm^{-1} for NH, and NH2, 1639-1600cm^{-1} for C=N, and several peaks around 1573-1464cm^{-1} for C=C. The title compounds [A3-b] and [A4c-d] displayed absorption bands ranging from 3304-3150cm^{-1} for NH, while distinguishing broad absorption peak for C=O was observed in the range 1689-1626, 1601-1563cm^{-1} for C=N and several peaks in between 1586-1445cm^{-1} for C=C in their respective IR spectra. These compounds also exhibited appropriate peaks at corresponding δ ppm in their ^{1}H-NMR spectra which were in conformity with their assigned structures. ^{1}H-NMR spectrum of compound [A3-b] showed the characteristic singlet around 1.21δ for aryl CH_3 proton, a singlet at 2.43δ for NH proton and multiplet around 7.03-8.33δ for aryl proton. The mass spectra showed an accurate molecular ion peak data at 295*m/z* [M+1] for compound [A3-a**]**. All the compounds give satisfactory chemical analysis.All the synthesized compounds were screened for antibacterial activity. The data in the Table-2 indicate that compounds, A3-a, A3-b and A4-b were exhibited significant antibacterial activity. While other synthesized compounds of these series shown moderate activity. The same Compounds also screened for the antifungal activity. The data from Table-3 summarizes the *in vitro* antifungal activity of compounds A4-a **&** A4-c were exhibited significant antifungal activity. While other synthesized compounds of these series shown moderate activity.

CONCLUSION:

The objective of the present work is to synthesize some novel 1,3,4-thiadiazole derivatives and to study their antibacterial, antifungal activity, thus an attempt has been made in this direction. As expected 1,3,4-thiadiazole derivatives exhibited significant and moderately antibacterial and



SCHEME 1

R = $C6H_5$, *p*-$CH_3C_6H_4$, *p*-ClC_6H_4, R^1 = C_6H_5, CH_3

Table-1:Physicochemical data of *N*-(5-phenyl-1, 3, 4-thiadiazol-2-yl) benzamide\ acetamide (A3a-c), (A4a-c):

Sl. no	Compound Code	R	R^1	Molecular Formula	Molecular weight	MP (^{0}C)	Yield (%)
1	**A3-a**	-C_6H_5	-C_6H_5	$C_{15}H_{11}N_3OS$	281.33	255-257	63%
2	**A3-b**	*p*-$CH_3C_6H_4$	-C_6H_5	$C_{16}H_{13}N_3OS$	295.35	285-287	67%
3	**A3-c**	*p*-ClC_6H_4	-C_6H_5	$C_{15}H_{10}ClN_3OS$	315.77	290-292	67%
4	**A4-a**	-C_6H_5	-CH_3	$C_{10}H_9N_3OS$	219.26	252-254	90%
5	**A4-b**	*p*-$CH_3C_6H_4$	-CH_3	$C_{11}H_{11}N_3OS$	233.28	264-266	94%
6	**A4-c**	*p*-ClC_6H_4	-CH_3	$C_{10}H_8ClN_3OS$	253.70	244-246	88%

Table-2:Antibacterial Activity of Synthesized Compound

Sl. No	Compound code (200µg/ml)	*S. aureus* (mm)	*B. subtilis*(mm)	*E. coli* (mm)	*P. aeruginosa* (mm)
9	A3-a	13.42 ± 0.08	14.03 ± 0.09	15.03 ± 0.07	12.70 ± 0.01
10	A3-b	15.70 ± 0.10	11.21 ± 0.66	12.02 ± 0.08	13.22 ± 0.14
11	A3-c	14.21 ± 0.34	12.39 ± 0.06	12.21± 0.30	13.65 ± 0.00
12	A4-a	12.44 ± 0.01	14.00 ± 0.01	15.00 ± 0.00	12.19 ± 0.88
13	A4-b	09.99 ± 0.01	11.68 ± 0.31	09.00 ± 0.14	15.40 ± 0.11
14	A4-c	11.27 ± 0.99	12.00 ± 0.00	10.02 ± 0.40	14.66± 0.10
15	**Ciprofloxacin**	**16.13 ± 0.99**	**14.22 ± 0.14**	**15.33 ± 0.09**	**16.95 ± 0.11**

All values are expressed as mean ± S.E.M. of three replications

Table-3:Antifungal Activity of Synthesized Compounds

Sr. No	Compound code(200µg/ml)	*A. niger*(mm)	*C. albicans*(mm)
1	A3-a	12.21 ± 0.66	08.01 ± 0.94
2	A3-b	12.08 ± 0.59	10.01 ± 0.43
3	A3-c	11.06 ± 0.07	09.39 ± 0.06
4	A4-a	12.78 ± 0.07	14.39 ± 0.00
5	A4-b	08.80 ± 0.01	07.88 ± 0.23
6	A4-c	14.08 ± 0.88	12.10 ± 0.76
7	**Griseofulvin**	**16.13 ± 0.99**	**14.22 ± 0.14**

All values are expressed as mean ± S.E.M. of three replications

antifungal activity when compared with standard drugs respectively. Further the detailed structural activity relationship studies are required along with the molecular manipulation i.e. molecular modeling may give better drugs.

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