Synthesis Characterization and biological evaluation of some 5-phenyl-N-(1-phenylethylidene)-1, 3, 4-thiadiazol-2-amine derivatives

Ugharatdar Siddik Ismail[1], Mohammad Saqib Usmani[2], Mohammed Shoaib Patel[1], Dr.Aejaz Ahmed[2]

1. Laxminarayandev College of Pharmacy , Bholav Bharuch Gujarat India
2. JIIU's Ali-Allana College of Pharmacy Akkalkuwa Nandurbar Maharashtra

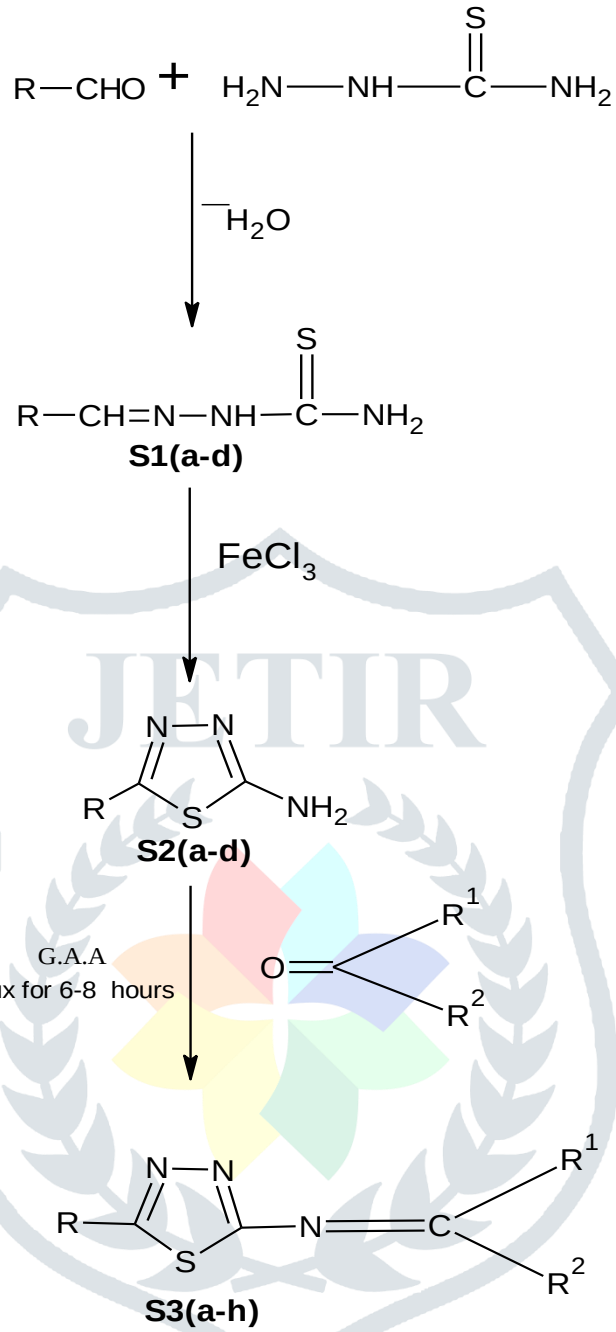
Abstract: Heterocyclic compounds like Thiadiazole plays a vital role owing to its wide range of therapeutic activities like antibacterial, antifungal, anti-inflammatory, anticonvulsant, antiviral, anticancer and antihistaminic. In the present work, an attempt was made to synthesize some of newer 5-phenyl-N-(1-phenylethylidene)-1, 3, 4-thiadiazol-2-amine derivative. The structure of new compounds prepared during present investigation have been authentically established by their IR, 1H NMR and Mass spectral studies. The antibacterial and antifungal activities of thidiazole derivatives also reported. Some of these derivatives exhibit significant antimicrobial activity

Key words: thiadiazole, thiosemicarbazide, antibacterial, antifungal

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 antifungal1, antibacterial & antimycobacterium2, anticonvulsant3, antitumor4, CNS depressants5, herbicidal6, antiviral7 and anti-inflammatory activity8. The thiadiazole system contains the following members the 1,2,3-thiadiazoles and their benzo derivatives the 1,2,4thiadiazoles 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-thiadiazolidines. 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 amines.

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 amines, ethanol, ammonia, Sodium bicarbonate, Hydrazine hydrate, 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 1H NMR of synthesized compounds were recorded in Bruker 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.

Experimental:



R = C6H5, *p*-CH3C6 H4, *p*-ClC6 H4,*p*-OCH3C6H4

R^1 = C6H5

R^2 = CH3

Step 1: Synthesis of thiosemicarbazones (S1a-d):

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.

S1-a IR: 3396 (NH2), 3157 (Ar-H) 3057, 1589 (C=N), 1537 (C=C), 1370 (C=S). S1-b IR: 3401 (NH), 3156 (Ar-H), 3025 (C-H, -CH3) 1598 (C=N), 1539 (C=C), 1370 (C=S) 768 (C-Cl).

Step-2: Synthesis of 5-phenyl-1, 3, 4-thiadiazole-2-amine (S2a-d):

Thiosemicarbazone (0.05 M) was suspended in 300 ml warm water, FeCl3 (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

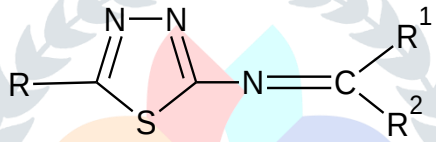
resulting mixture was divided into 4 parts and each part was neutralized separately with ammonia (10 %). The required amine separated out, filtered with suction, dried and recrystalized with appropriate solvent.
S2-b IR: 3281 (NH2), 3094 (Ar-H), 2959 (C-H, in CH3), 1634 (C=N) 1537-1470 (C=C). S2-c IR: 3273 (NH), 3088 (Ar-H), 1633 (C=N), 1596-1464 (C=C) 786 (C-Cl), 693 (C-S).

Step-3: Synthesis of 5-phenyl-N-(1-phenylethylidene)-1, 3, 4-thiadiazol-2-amine (S3a-h):

0.2 M compound 2, 0.2M carbonyl compound ($R_1R_2C=O$) and 2ml Glacial acetic acid were refluxed in 50ml methanol for 8 hrs. Solvent was distilled off and product recrystallized from mixture of benzene and chloroform (1:6 v/v).

S3-a IR (cm^{-1}): 3077 (Ar-H), 2935 (C-H, in CH3), 1671 (C=N), 1643 (C=C), 767 (C-S). S3-a ^1H NMR (δ ppm): 6.2-7.3 (m,13H,ArH), 2.9 (s,3H,CH3). S3-a Mass (m/z): 279.083 (M+1) 100 %
S3-b IR (cm^{-1}): 3041 (Ar-H), 2962 (C-H, in CH3), 166.42 (C=N), 1727 (C=C), 719 (C-S). S3-b ^1H NMR (δ ppm): 7.4-7.6 (m,15 H, ArH), 2.9 (s,3H,CH3). S3-b Mass (m/z): 303 (M+1) 100 %
S3-c IR (cm^{-1}):2923(Ar-H), 28.52.89(C-H, in CH3) 1607-1457(C=C) 1632 (C=N), 719 (C-S). S3-c ^1H NMR (δ ppm): 5.1-7.0 (m, 15H, Ar-H) 5.2,6.8(s,3H,CH_3) 3.8(dd,2H,CH_2). S3-c Mass (m/z): 297 (M+1) 100 %
S3-d IR (cm^{-1}): 2923 (Ar-H),1516.8, 2852 (C-H, in CH3), (C=C),1603(C=N) 770.80(C-S), S3-d ^1H NMR (δ ppm): 5.0-6.8 (m,16H,Ar-H) 5.3(s,3H,CH_3) 6.6(dd,2H,CH_2) S3-d Mass (m/z): 357.2 (M+1) 100 %

physicochemical data of 5-phenyl-N-(1-phenylethylidene)-1, 3, 4-thiadiazol-2-amine (S3a-h)



Sl. No	Compound Code	R	R^1	R^2	Molecular Formula	Molecular weight	MP (^0C)	Yield (%)
1	**S3-a**	$-C_6H_5$	$-C_6H_5$	$-CH_3$	$C_{16}H_{13}N_3S$	279.083	255-257	63%
2	**S3-b**	$-C_6H_5$	$-C_6H_5$	$-C_6H_5$	$C_{19}H_{15}N_3S$	317.083	285-287	67%
3	**S3-c**	*p*-$CH_3C_6H_4$	$-C_6H_5$	$-CH_3$	$C_{17}H_{15}N_3S$	293.083	290-292	67%
4	**S3-d**	*p*-$CH_3C_6H_4$	$-C_6H_5$	$-C_6H_5$	$C_{22}H_{17}N_3S$	355.083	252-254	73%
5	**S3-e**	*p*-ClC_6H_4	$-C_6H_5$	$-CH_3$	$C_{16}H_{12}ClN_3S$	313.083	264-266	81%
6	**S3-f**	*p*-ClC_6H_4	$-C_6H_5$	$-C_6H_5$	$C_{22}H_{14}ClN_3S$	375.083	244-246	77%
7	**S3-g**	*p*-$OCH_3C_6H_4$	$-C_6H_5$	$-CH_3$	$C_{17}H_{15}N_3SO$	294.083	285-287	67%
8	**S3-h**	*p*-$OCH_3C_6H_4$	$-C_6H_5$	$-C_6H_5$	$C_{22}H_{17}N_3SO$	371.083	265-267	78%

Antimicrobial Activity11:

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 370C 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

Antibacterial Activity of Synthesized Compounds

Sl. No	Compound code (200 µg/ml)	*S. aureus* (mm)	*B. subtilis* (mm)	*E. coli* (mm)	*P. aeruginosa* (mm)
1	**S3-a**	**15.79 ± 0.06**	10.12 ± 0.55	11.21 ± 0.66	12.21 ± 0.36
2	**S3-b**	12.43 ± 0.88	11.57 ± 0.03	12.43 ± 0.78	13.11 ± 0.64
3	**S3-c**	13.31 ± 0.53	**13.44 ± 0.08**	11.43 ± 0.62	12.02 ± 0.22
4	**S3-d**	13.90 ± 0.17	10.20 ± 0.34	11.38 ± 0.01	**15.00 ± 0.02**
5	**S3-e**	13.67 ± 0.40	09.18 ± 0.21	10.13 ± 0.07	13.00 ± 0.00
6	**S3-f**	12.40 ± 0.02	11.01 ± 0.21	10.25 ± 0.75	12.12 ± 0.88
7	**S3-g**	11.02 ± 0.08	11.90 ± 0.17	**14.02 ± 0.48**	12.13 ± 0.07
8	**S3-h**	12.42 ± 0.48	11.21 ± 0.66	12.13 ± 0.09	13.02 ± 0.00
9	**Ciprofloxacin**	**16.13 ± 0.99**	**13.90 ± 0.17**	**14.22 ± 0.14**	**15.13 ± 0.07**

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

Antifungal activity of synthesized compound (S3a-h)

Sl. No	Compound code (200 µg/ml)	*A. niger* (mm)	*C. albicans* (mm)
1	**S3-a**	14.21± 0.34	**13.76 ± 0.03**
2	**S3-b**	11.43 ± 0.88	11.06 ± 0.07

3	**S3-c**	**14.88 ± 0.00**	11.87 ± 0. 76
4	**S3-d**	11.08 ± 0.88	13.12 ± 0.88
5	**S3-e**	**14.96 ± 0.07**	11.46 ± 0.07
6	**S3-f**	10.43 ± 0.08	10.38 ± 0.02
7	**S3-g**	12.39 ± 0.06	11.08 ± 0.88
8	**S3-h**	13.46 ± 0.11	**13.06 ± 0.39**
9	**Griseofulvin**	**16.13 ± 0.99**	**14.22 ± 0.14**

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

RESULTS AND DISCUSSION:

A series of 5-phenyl-N-(1-phenylethylidene)-1, 3, 4-thiadiazol-2-amine (S3a-h) were synthesized in good yield using the synthetic route outlined in Scheme.

All the synthesized compounds were screened for antibacterial activity. The data in the table indicates that compounds S3-a, S3-c, S3-d**,** and S3-g were exhibited a significant antibacterial activity. While other synthesized compounds of this series shown moderate antibacterial activity.

All the synthesized Compounds also screened for the antifungal activity. The data in table indicates that the compounds S3-a, S3-c, S3-e and S3-h were exhibited a significant antifungal activity While other synthesized compounds of these series shown moderate antifungal activity.

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