AMERICAN JOURNAL OF PHARMTECH RESEARCH

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Synthesis and Antimicrobial Activity of *S*-[5-(Phenylamino)-1,3,4-Thiadiazole-2-Yl] Benzenecarbothioate /Ethanethioate

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ABSTRACT

In the present study, a series of *S*-[5-(phenylamino)-1,3,4-thiadiazole-2-yl] benzenecarbothioate and S-[5-(phenyl amino)- 1,3,4-thiadiazole-2-yl] ethanethioate were prepared by refluxing benzoyl chloride and acetyl chloride in presence of potassium carbonate with 5-(phenyl amino)-1,3,4-thiadiazole-2-thiol. 5-(Phenyl amino)-1, 3, 4-thiadiazole-2-thiol were prepared by cyclization of arylthiosemicarbazide with carbondisulphide. 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: thiadiazole, thiosemicarbazide, antibacterial, antifungal.

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Received 21 October 2011, Accepted 14 November 2011

Please cite this article in press as: Saqib M. *et al.*, Synthesis and Antimicrobial Activity of *S*-[5-(Phenylamino)-1,3,4-Thiadiazole-2-Yl] Benzenecarbothioate /Ethanethioate. American Journal of PharmTech Research 2012.

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 antifungal[1], antibacterial & 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-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 ^{1}H 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.

Antimicrobial Activity[9]:

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 $30^{0}C$ 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 37^0C for 24 hours for bacteria and 30^0C for 72hours for fungal inoculums. Sensitivity was recorded by measuring the clean zone of growth inhibition on agar surface around the disc.

Experimental:

Step-1: Synthesis of *N*-phenylhydrazine carbothioamide, (M1a-d):

i) From solid aryl amines:

Aromatic amines (0.1M) were dissolved in 25ml ethanol. Concentrated ammonia solution (30ml) was added drop by drop followed by addition of carbon disulphide (0.1M) with constant stirring. After complete dissolution of carbon disulphide the reaction mixture was allowed to stand for two hours. A solution of sodium chloroacetate (0.1M) was added followed by hydrazine hydrate (0.1M). The reaction mixture was then warmed on a water bath and filtered while hot. The filtrate was concentrated to half of its initial volume and allows standing overnight to give the product, which was collected, filtered, dried and recrystallized from ethanol.

ii) From liquid aryl amines:

Concentrated ammonia (0.1M) solution was added to aryl amines (0.1M) kept in 500 ml flask. The mixture was cooled below 30^0C and carbon disulphide (0.1M) was added gradually with constant stirring. Absolute ethanol (25ml) was added to the reaction mixture and the stirring is continued till carbon disulphide was completely dissolved. The reaction mixture was allowed to stand for two hours and is shaken afterwards with a solution of sodium chloroacetate (0.1M), followed by hydrazine hydrate (0.1M). The reaction mixture was then warmed on a water bath and filtered while hot. The filtrate is concentrated to half of its initial volume and allows standing overnight to give the product, which was collected, filtered, dried and recrystallized from ethanol.

M1-c IR (KBr cm^{-1}): 3292-3179 (NH; NH_2), 2941 (Ar-H), 134 (C-N), 1586-1491 (C=C), 1078 (C=S), 719 (C-Cl).**M1-d** IR (KBr cm^{-1}): 3292-3168 (NH, NH2), 3021 (Ar-H), 1618 (C-N), 1586-1448 (C=C), 1095 (C=S), 606 (C-Br).

Step-2: Synthesis of 5-(phenyl amino)-1, 3, 4-thiadiazole-2-thiol, (M2a-d):

A mixture of *N*-phenyl hydrazine carbothioamide (0.06M), carbon disulphide (0.06M) and absolute ethanol (25ml) was refluxed in a 250ml RBF on a water bath at 60-70^{0}C for two hours. Excess alcohol was distilled of under reduced pressure and residue after cooling the reaction mixture was poured in ice cold water. The solid was separated, filtered, washed well with water, dried and recrystallized from ethanol.

M2-c IR (KBr cm^{-1}): 3229 (NH), 3022 (Ar-H), 2613 (SH), 1602 (C=N), 1573-1489 (C=C), 720 (C-S-C), 631 (C-Cl)**. M2-d** IR (KBr cm^{-1}): 3231 (NH), 3021 (Ar-H), 2582 (SH), 1600 (C=N), 1570-1486 (C=C), 719 (C-S-C), 577 (C-Br).

Step-3: Synthesis of *S*-[5-(phenylamino)-1, 3, 4-thiadiazole-2-yl] benzenecarbothioate /ethanethioate, (M3a-d), (M4a-d):

A mixture of 5-(phenyl amino)-1, 3, 4-thiadiazole-2-thiol (0.01M), benzoyl chloride / acetyl chloride (0.01M) potassium carbonate (0.5gm) and dry acetone (20ml) was refluxed in a 100 ml RBF on a water bath for 3 hours. Excess of acetone was distilled off and after cooling the reaction mixture was poured in ice cold water. A light yellow mass was separated, which was collected by filtration, washed well with water, dried and recrystallized with ethanol.

M3-a IR (KBr cm^{-1}): 3261 (NH), 3050 (Ar-H), 1665 (C=O), 1601(C=N), 1547-1445 (C=C), 687 (C-S-C). **M3-b** IR (KBr cm^{-1}): 3304 (NH), 3034 (Ar-H), 2852 (C-H, in CH_3), 1665 (C=O),1600 (C=N), 1581-1447 (C=C),681 (C-S). ^{1}H-NMR (δ ppm): 1.25 (s, 3H, CH_3), 2.03 (s, 1H, NH), 7.13-8.01 (m, 9H, Ar-H). MASS (m/z). 327.7 (M+1) 100 %. **M4-a.** IR (KBr cm^{-1}): 3180 (NH), 3123 (Ar-H),2979 (C-H, in CH_3), 1680 (C=O), 1599 (C=N), 1568-1496 (C=C),693 (C-S-C). **M4-b.** IR (KBr cm^{-1}): 3230 (NH), 3113 (Ar-H), 3002 (C-H, in CH_3), 1681 (C=O), 1600 (C=N), 1510-1462 (C=C), 714 (C-S-C). **M4-c.** IR (KBr cm^{-1}): 3229 (NH2), 3112 (Ar-H), 2923 (C-H, in CH_3), 1689 (C=O), 1602 (C=N), 1571-1487 (C=C), 721 (C-S-C). **M4-d.** IR (KBr cm^{-1}): 3231 (NH2), 3114 (Ar-H), 2926 (C-H, in CH_3), 1689 (C=O), 1600 (C=N), 1563-1486 (C=C), 719 (C-S-C) 629 (C-Br).

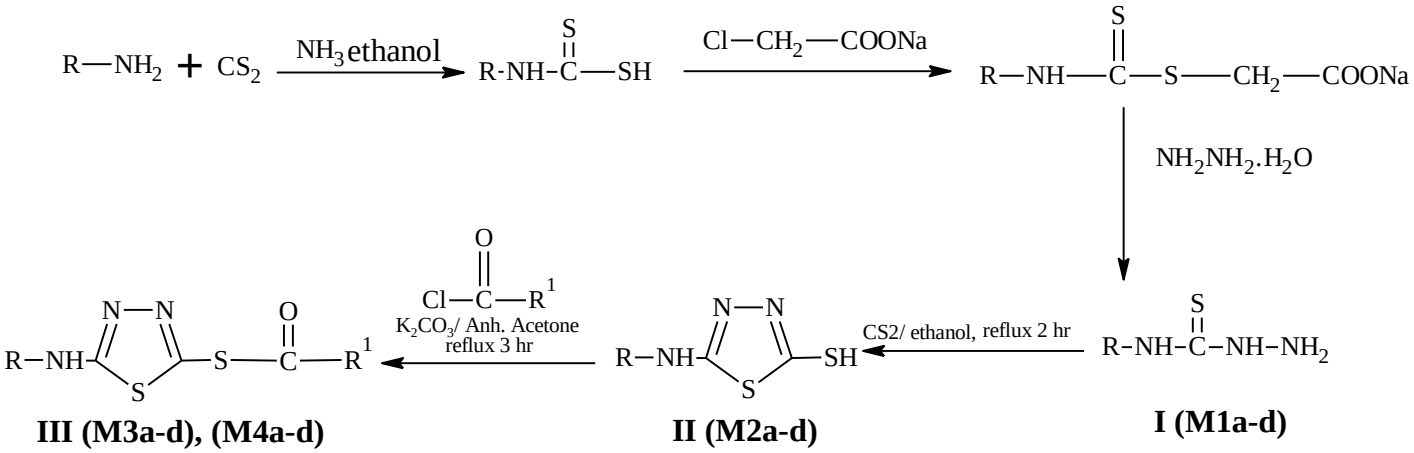
RESULTS AND DISCUSSION:

A series of *S*-[5-(phenyl amino)-1, 3, 4-thiadiazol-2-yl] benzene carbothioate / ethanethioate M3a-d and M4a-d were synthesized in good yield using the synthetic route outlined in Scheme. The starting material 5-(phenylamino)-1, 3,4-thiadiazole-2-thiol was prepared according to the literature procedure. Structure of the synthesized compounds was stabilized on the basis of IR, ^{1}H NMR, and Mass spectral data.

The IR spectrum of the intermediates M2c-d 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 M3a-b & M4a-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 1586-1445 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 [M3-b] showed the characteristic singlet around 1.25δppm for aryl CH_3 proton, a singlet at 2.35δppm for NH proton and multiplet around 7.13-8.01δppm for aryl proton. The mass spectra of compound [M3-a] showed an accurate molecular ion peak data at 327*m/z* [M+1].

Table-1 physicochemical data of *S*-[5-(phenyl amino)-1, 3,4-thiadiazol-2-yl] benzenecarbothioate / Ethanethioate(M3a-d), (M4a-d). Table-2 summarizes the *in vitro* antibacterial activity of synthesized compounds against some Gram-positive (*Staphylococcus aureus, Bacillus subtilis),* and Gram-negative *(E. coli, Pseudomonas aeruginosa)* bacteria. The antibacterial activity of test compounds was assessed in side-by-side with reference drug. The antibacterial data indicated that the 1,3,4-thiadiazole derivatives M3-b Showed significant activity again Gram-positive bacteria whereas compound M3-b, M4-b M4-c & M4-d showed better activity against Gram-negative bacteria. While other synthesized compounds of these series shown moderate activity.

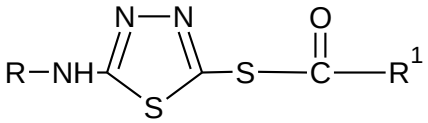
SCHEME



R = C_6H_5, *p*-$CH_3C_6H_4$, *p*-ClC_6H_4, *p*-Br C_6H_4

R^1 = C_6H_5, CH_3

Table-1: Physicochemical data of *S*-[5-(phenyl amino)-1, 3, 4-thiadiazol-2-yl] benzenecarbothioate / Ethanethioate(M3a-d), (M4a-d):



Sl. no.	Compound	R	R^1	Molecular Formula	Molecular Weight	M.P (^{0}C)	Yield (%)
1	M3-a	$-C_6H_5$	$-C_6H_5$	$C_{15}H_{11}N_3OS_2$	313.39	181-183	63%
2	M3-b	*p*-$CH_3C_6H_4$	$-C_6H_5$	$C_{16}H_{13}N_3OS_2$	327.42	188-190	80%
3	M3-c	*p*-ClC_6H_4	$-C_6H_5$	$C_{15}H_{10}ClN_3OS_2$	347.84	208-210	61%
4	M3-d	*p*-BrC_6H_4	$-C_6H_5$	$C_{15}H_{10}BrN_3OS_2$	392.29	213-215	87%
5	M4-a	$-C_6H_5$	$-CH_3$	$C_{10}H_9N_3OS_2$	251.32	193-195	63%
6	M4-b	*p*-$CH_3C_6H_4$	$-CH_3$	$C_{11}H_{11}N_3OS_2$	265.35	200-202	65%
7	M4-c	*p*-ClC_6H_4	$-CH_3$	$C_{10}H_8ClN_3OS_2$	285.77	208-210	60%
8	M4-d	*p*-BrC_6H_4	$-CH_3$	$C_{10}H_8BrN_3OS_2$	330.22	213- 215	69%

Table-2: Antibacterial activity of the synthesized compounds

Sl. No	Compound(200 µg/ml)	Zone of inhibition in mm			
		S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***
1	M3-a	12.39 ± 0.06	11.12 ± 0.55	11.21 ± 0.66	10.21 ± 0.36
2	M3-b	10.43 ± 0.88	12.57 ± 0.03	10.43 ± 0.78	13.11 ± 0.64
3	M3-c	13.31 ± 0.53	12.44 ± 0.08	11.43 ± 0.62	12.02 ± 0.22
4	M3-d	13.00 ± 0.40	15.20 ± 0.34	13.38 ± 0.01	15.00 ± 0.02
5	M4-a	10.43 ±0.08	09.18 ± 0.21	10.13 ± 0.07	13.00 ± 0.00
6	M4-b	12.40 ± 0.02	13.01 ± 0.21	15.25 ± 0.75	12.12 ± 0.88
7	M4-c	13.02 ± 0.08	13.90 ± 0.17	14.02 ± 0.48	15.13 ± 0.07
8	M4-d	12.42 ± 0.48	09.21 ± 0.66	15.13 ± 0.09	13.02 ± 0.00
9	**Ciprofloxacin**	**22.13 ± 0.99**	**22.22 ± 0.14**	**22.33 ± 0.09**	**24.33 ± 0.11**

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

Table-3: Antifungal activity of synthesized compounds

Sl. No	Compound (2000 µg/ml)	Zone of inhibition in mm	
		A. niger	***C. albicans***
1	M3-a	12.21± 0.34	11.06 ± 0.03
2	M3-b	15.43 ± 0.88	11.06 ± 0.07
3	M3-c	12.88 ± 0.00	10.87 ± 0. 76
4	M3-d	14.08 ± 0.88	10.12 ± 0.88
5	M4-a	11.46 ± 0.07	11.46 ± 0.07
6	M4-b	10.43 ± 0.08	10.38 ± 0.02
7	M4-c	12.39 ± 0.06	14.08 ± 0.88
8	M4-d	12.46 ± 0.11	13.80 ± 0.39
9	**Griseofulvin**	**18.13 ± 0.99**	**17.82 ± 0.14**

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

The same Compounds also screened for the antifungal activity. The data from Table 3 summarizes the *in vitro* antifungal activity of compounds M3-b, M3-d and M4-c showed significant activity. While other synthesized compounds of these series shows poor 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 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.

ACKNOWLEDGEMENT:

Authors are thankful to Dr. Kishore Singh Chatrapati Professor & HOD, Hariprasanna R. C. Principal & Professor of RMES's College of Pharmacy, for providing necessary facilities. They are also thankful to Oxygen Healthcare Research Pvt. Ltd**,** Ahmedabad and Central Salt & Marine Chemicals Research Institute**,** Bhavnagar for providing the Spectral Data.

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