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SYNTHESIS, ANTIMICROBIAL AND ANTICANCER ACTIVITIES OF 5-(4-SUBSTITUTED-PHENYL)-1,3,4-THIADIAZOLE-2-AMINES

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ABSTRACT

A series of 5-(4-substituted phenyl)-1,3,4-thiadiazole-2-amines **(4a-h)** were prepared from dehydrocyclization of 4-substituted benzoyl thiosemicarbazides **(3a-h)** using concentrated sulphuric acid. The chemical structures of compounds were elucidated by Infra-red, NMR, Mass spectral and elemental analyses. Antibacterial activities of these compounds against *Staphylococcus aureus, Bacillus substilis, Eschereria coli* and *Pseudomonas aeruginosa* using Ciprofloxacin while antifungal activities against *Aspergillus niger* and *Candida albicans* using Fluconazole as standard drugs were performed. Minimum inhibitory concentrations and anticancer activity of potent compounds was determined.

The results indicate that compounds **4(a), 4(b)** and **4(c)** possess significant antibacterial activity whereas compounds **4(f)** and **4(g)** possess significant antifungal activity among the series of synthesized thiadiazoles comparing with standard drugs. Further, the results of anticancer studies on breast cancer cell line suggested that some potent antimicrobials i.e. **4(c), 4(f)** and **4(g)** associated with moderate to good anticancer activity. Thus, it may be found that synthesized compounds have good antibacterial activity and moderate antifungal activity. However, these compounds could be further screened for the antibacterial activities against a battery of bacteria to achieve their broad spectrum profile and also used to screen cytotoxic effect against other cancer cell lines.

Keywords: Synthesis, thiadiazole, antimicrobial, cytotoxic, cancer cell line.

INTRODUCTION

Mankind has been invaded by infectious diseases since ages. To counteract these infections, a large number of antimicrobial drugs have been invented by scientists. However, the change in the lifecycle of microbes and the resistance developed thereafter has become a serious issue[1]. There is always a necessity to search better antimicrobial agents and the inventions of such drugs have an important role in medicinal chemistry[2]. Sulfur containing heterocyclic compounds 1,3,4-thiadiazoles are the valuable structural part of bioactive compounds[3]. 5-Phenyl-1,3,4-thiadiazole is familiar group of bioactive compounds with numerous pharmacological activities[4].

Various pharmacological activities reported for 1,3,4-thiadiazole analogues are anticancer[5-7] antimicrobial[8-10], antitubercular[11, 12], anti-inflammatory[13], analgesic[14], anticonvulsant[15], antihypertensive[16], diuretic[17], local anesthetic[18] and antioxidant activities[19].Hence, it is envisaged to synthesize 1,3,4-thiadiazole analogs and screened them for antimicrobial and anticancer activities.

EXPERIMENTAL

All chemicals and solvents were procured from Merck Chemicals, Spectrochem (P) Ltd. and Qualigens Chemicals, Mumbai, India. Purity of these compounds was checked by thin layer chromatography (TLC) on silica gel G glass plates for which iodine vapors was the detecting agent. R_f values of synthesized compounds were found by taking different proportions of benzene and acetone (Table-1). The synthesized compounds were recrystallized from ethanol and water mixtures. Melting points of all the synthesized compounds were determined in open capillaries and are uncorrected. Structures of intermediates were established on the basis of melting point and various functional groups test. Infra-red spectra (in KBr)

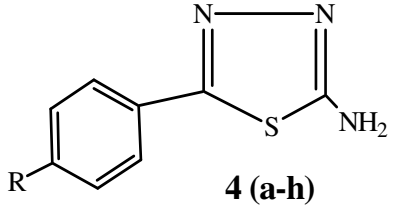
were determined on a Shimadzu IR Affinity-1 FTIR spectrophotometer. ^{13}C NMR spectra in dimethyl sulphoxide (DMSO) were recorded at strength of 400 MHz on Jeol-JMS spectrometer using TMS as an reference standard ((chemical shift, δ ppm). Mass spectra were scanned on Shimadzu LC-MS 2010A spectrometer. The percentage estimation of carbon, hydrogen, nitrogen and sulphur were determined using Euro-Vector EA-3000 analyzer. The elemental analyses for C, H, N and S established for these compounds were established to be within ±0.5% limits of theoretical values.

General method for the synthesis of methyl 4-substituted benzoate, 2(a-h)
The 4-substituted benzoates **2(a-h)** were prepared according to reported procedure[20]. These esters were used for the synthesis of the intermediate, benzoyl thiosemicarbazides.

General method for the synthesis of 4-substituted benzoyl thiosemicarbazide 3 (a-h)
The 4-substituted benzoyl thiosemicarbazides were synthesized according to reported methods[21, 22]. The thiosemicarbazide (0.015 mol) and benzoic acid esters (0.01 mol) were dissolved in 50 mL of methanol by heating. Then, reaction mixture was refluxed for 8-10 h. The ice-water mixture was added to resulting solution. The solid which got separated was dried and recrystallized using rectified spirit.

General method for synthesis of 2- amino 5-(4-substituted phenyl)-1, 3, 4-thiadiazole[23, 24] 4(a-h)
The 4-substituted benzoyl thiosemicarbazide (0.02 mol) **(3a-h)** was added portion wise to concentrated sulphuric acid (20 mL) with shaking. The mixture was heated between 60-70^{0}C for 5 h. The reaction mixture was kept aside at room temperature overnight. This solution was poured over crushed ice. The compound was precipitated by addition of NH_4OH solution. The precipitate, so obtained was washed thoroughly with distilled water to free from the sulphates. The dried product was recrystallized from chloroform.
The physical constants and data of the title compounds are provided in Table-1.

Table-1: Physical Properties of 5-(4-substituted phenyl)-1, 3, 4-thiadiazole-2-amines, 4(a-h):



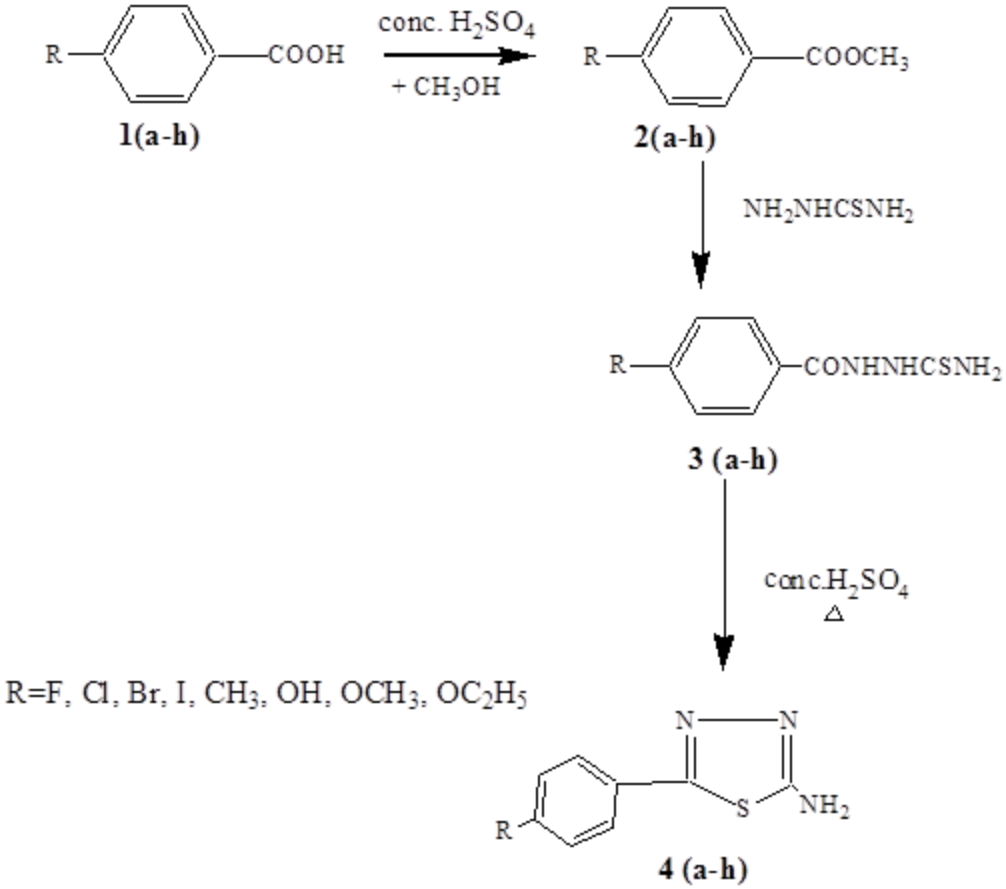
4 (a-h)

Compounds	R	Molecular Formula	Molecular Weight	Yield (%)	M.P. (°C)	R_f Value	Benzene:Acetone
4 (a)	F	$C_8H_6FN_3S$	195.22	63.6	233-35	0.77	8:2
4 (b)	Cl	$C_8H_6ClN_3S$	211.67	66.4	227-29	0.83	9:1
4 (c)	Br	$C_8H_6BrN_3S$	256.12	72.4	224-26	0.80	9:1
4 (d)	I	$C_8H_6IN_3S$	303.12	78.2	282-84	0.86	9:1
4 (e)	CH_3	$C_9H_9N_3S$	191.25	70.5	218-20	0.78	8:2
4 (f)	OH	$C_8H_7N_3OS$	193.23	65.8	214-16	0.66	7:3
4 (g)	OCH_3	$C_9H_9N_3OS$	207.25	73.4	225-27	0.72	7:3
4 (h)	OC_2H_5	$C_{10}H_{11}N_3OS$	221.06	75.2	238-40	0.68	8:2

Spectral data and elemental analysis data of title compounds **(4 a-h)** are presented as follows:

5-(4-Fluoro-phenyl)-[1, 3, 4]thiadiazole-2-amine (4a)
IR (KBr ν, cm^{-1}): 3372 (N-H stretching), 3064 (C-H str, aromatic), 1632 (C=N str, ring) 1562-1448 (C=C str, aromatic), 1056 (C-F str), 862 (C-S-C str, thiadiazole); ^{13}C NMR 400 MHz, (DMSO-d_6, δ, ppm):

175.2 (C-5), 163.1 (C-4'), 162.2 (C-2), 130.3 (C-2',C-6'), 129.8 (C-1'), 117.1 (C-3',C-5'); LCMS (m/z): 195 (M^+), 196 (M^+ +1); Elemental Analysis ($C_8H_6FN_3S$), Found % (Calculated %): C, 49.04 (49.22); H, 3.06 (3.10); N, 21.42 (21.52); S, 16.36 (16.43).



Scheme-1: Synthesis of 5-Phenyl-1, 3, 4-thiadiazole-2-amines 4 (a-h)

5-(4-Chloro-phenyl)-[1, 3, 4] thiadiazole-2-amine (4b)

IR (KBr ν, cm^{-1}): 3378 (N-H stretching), 3064 (C-H str, aromatic), 1634 (C=N str, ring) 1562-1448 (C=C str, aromatic), 880 (C-S-C str, thiadiazole), 761 (C-Cl str); ^{13}C NMR 400 MHz, (DMSO-d_6, δ, ppm): 174.2 (C-5), 162.3 (C-2), 135.1 (C-4'), 132.3 (C-1'), 129.8 (C-3',C-5'), 128.2 (C-2',C-6'); LCMS (m/z): 211 (M^+), 213 (M^+ +2); Elemental Analysis ($C_8H_6ClN_3S$), Found % (Calculated %): C, 45.22 (45.39); H, 2.82 (2.86); N, 19.78 (19.85); S, 15.10 (15.15).

5-(4-Bromo-phenyl)-[1, 3, 4] thiadiazole-2-amine (4c)

IR (KBr ν, cm^{-1}): 3381 (N-H stretching), 3076 (C-H str, aromatic), 1642 (C=N str, ring) 1570-1454 (C=C str, aromatic), 890 (C-S-C str, thiadiazole), 644 (C-Br str); ^{13}C NMR 400 MHz, (DMSO-d_6, δ, ppm): 173.8 (C-5), 162.8 (C-2), 133.2 (C-1'), 131.4 (C-3',C-5'), 129.3 (C-2',C-6'), 122.8 (C-4'); LCMS (m/z): 256 (M^+), 258 (M^+ +2); Elemental Analysis ($C_8H_6BrN_3S$), Found % (Calculated %): C, 37.38 (37.52); H, 2.33 (2.36); N, 16.35 (16.41); S, 12.46 (12.52).

5-(4-Iodo-phenyl)-[1, 3, 4] thiadiazole-2-amine (4d)

IR (KBr ν, cm^{-1}): 3384 (N-H stretching), 3078 (C-H str, aromatic), 1643 (C=N str, ring) 1572-1455 (C=C str, aromatic), 892 (C-S-C str, thiadiazole), 534 (C-I str); ^{13}C NMR 400 MHz, (DMSO-d_6, δ, ppm): 174.2

(C-5), 162.3 (C-2), 138.2 (C-3',C-5'), 132.8 (C-1'), 128.7 (C-2',C-6'), 95.1 (C-4'); LCMS (m/z): 303 (M^+), 304 (M^+ +1); Elemental Analysis ($C_8H_6IN_3S$), Found % (Calculated %): C, 31.55 (31.70); H, 1.94 (2.00); N, 13.80 (13.86); S, 10.52 (10.58).

5-(4-Methyl-phenyl)-[1, 3, 4] thiadiazole-2-amine (4e)
IR (KBr ν, cm^{-1}): 3387 (N-H stretching), 3080 (C-H str, aromatic), 2932 (C-H str, aliphatic), 1645 (C=N str, ring), 1574-1460 (C=C str, aromatic), 1379 (C-H bend, aliphatic), 896 (C-S-C str, thiadiazole); ^{13}C NMR 400 MHz, (DMSO-d_6, δ, ppm): 173.7 (C-5), 162.6 (C-2), 131.5 (C-4'), 130.2 (C-1'), 128.6 (C-3',C-5'), 126.4 (C-2',C-6'), 21.8 (Ar-CH_3); LCMS (m/z): 191 (M^+), 192 (M^+ +1); Elemental Analysis ($C_9H_9N_3S$), Found % (Calculated %): C, 56.38 (56.52); H, 4.70 (4.74); N, 21.92 (21.97); S, 16.72 (16.77).

5-(4-Hydroxy-phenyl)-[1, 3, 4] thiadiazole-2-amine (4f)
IR (KBr ν, cm^{-1}): 3462 (O-H, stretching), 3383 (N-H str), 3077 (C-H str, aromatic), 1642 (C=N str, ring), 1570-1550 (C=C str, aromatic), 1081 (C-O str), 894 (C-S-C str, thiadiazole); ^{13}C NMR 400 MHz, (DMSO-d_6, δ, ppm): 173.2 (C-5), 161.2 (C-2), 158.1 (C-4'), 128.3 (C-2',C-6'), 125.7 (C-1'), 115.8 (C-3',C-5'); LCMS (m/z): 193 (M^+), 194 (M^+ +1); Elemental Analysis ($C_8H_7N_3OS$), Found % (Calculated %): C, 49.62 (49.73); H, 3.61 (3.65); N, 21.68 (21.75); S, 16.55 (16.60).

5-(4-Methoxy-phenyl)-[1, 3, 4] thiadiazole-2-amine (4g)
IR (KBr ν, cm^{-1}): 3390 (N-H stretching), 3081 (C-H str, aromatic), 2945 (C-H str, aliphatic), 1648 (C=N str, ring), 1576-1464 (C=C str, aromatic), 1375 (C-H bend, aliphatic), 1064 (C-O, str), 898 (C-S-C str, thiadiazole); ^{13}C NMR 400 MHz, (DMSO-d_6, δ, ppm): 174.2 (C-5), 162.5 (C-2), 160.4 (C-4'), 127.9 (C-2',C-6'), 125.2 (C-1'), 114.3 (C-3', C-5'), 56.5 (Ar-O-CH_3); LCMS (m/z): 207 (M^+), 208 (M^+ +1); Elemental Analysis ($C_9H_9N_3OS$), Found % (Calculated %): C, 52.07 (52.16); H, 2.32 (4.38); N, 20.70 (20.75); S, 15.41 (15.47).

5-(4-Ethoxy-phenyl)-[1, 3, 4] thiadiazole-2-amine (4h)
IR (KBr ν, cm^{-1}): 3394 (N-H stretching), 3084 (C-H str, aromatic), 2948 (C-H str, aliphatic), 1650 (C=N str, ring), 1579-1465 (C=C str, aromatic), 1383 (C-H bend, aliphatic), 1067 (C-O, str), 890 (C-S-C str, thiadiazole); ^{13}C NMR 400 MHz, (DMSO-d_6, δ, ppm): 174.3 (C-5), 162.4 (C-2), 158.7 (C-4'), 128.4 (C-2',C-6'), 125.7 (C-1'), 115.4 (C-3',C-5'), 65.2 (Ar-O-CH_2-CH_3), 15.2 (Ar-O-CH_2-CH_3); LCMS (m/z): 221 (M^+), 222 (M^+ +1); Elemental Analysis ($C_{10}H_{11}N_3OS$), Found % (Calculated %): C, 54.19 (54.28); H, 4.97 (5.01); N, 18.93 (18.99); S, 14.45 (14.49).

Antimicrobial Activity
The synthesized compounds **4(a-h)** were evaluated for antibacterial activity against *Staphylococcus aureus* (MTCC7443; Gram+ve), *Bacillus subtilis* (MTCC121; Gram+ve), *Escherichia coli* (MTCC118; Gram-ve) and *Pseudomonas aeruginosa* (MTCC424; Gram-ve) bacterial strains[25, 26]. While, the antifungal activity was tested against two fungi, *Aspergillus niger* and *Candida albicans* by disc diffusion technique[26, 27]. Concentrations of standard drugs, Fluconazole and Ciprofloxacin (20 µg/mL) were used for antifungal activity and antibacterial activity, respectively.
Accurately weighed 10 mg test compound was dissolved in dimethyl sulphoxide (DMSO) and volume made up to 10 mL in volumetric flask (1.0 mg/mL). This solution was further diluted with DMSO to a concentration of 100 µg/mL. Similarly, solutions of 20 µg/mLof concentration of each Ciprofloxacin and Fluconazole were prepared from their stock solutions.
For Antibacterial activity, nutrient broth having composition 2.0 g yeast extract, 1.0 g beef extract 5.0 g peptone and 5.0 g of sodium chloride was added to distilled water and volume made up to 1000 mL. Nutrient Agar medium was prepared by adding 2.0% of agar to nutrient broth and adjusting the pH to 7.4. For antifungal studies, Sabouraud's dextrose medium having composition: peptone (10.0 g), dextrose

(40.0 g) was dissolved in 1000 mL of distilled water and pH adjusted to 5.7. Finally, agar medium was prepared by adding 1.5% of agar to it.

Whatman no. 1 filter paper was employed to prepare the paper discs of about 6 mm in diameter. The paper discs and media were sterilized by autoclaving at 121°C and 15 lb/inch pressure for 20 minutes. The agar medium was transferred to each petriplate in the aseptic conditions. A standard inoculum (5 $x10^5$ c.f.u./mL) was properly distributed onto the surface of both sterile nutrient agar and dextrose medium plates. The sterile discs of 6 mm in diameter previously moistened with the standard drug or the test solutions were kept on the agar culture plates aseptically. The bacterial and fungal plates were incubated at 37±1°C for 24h and 25±1°C for 72 h respectively. The zone of inhibition was measured and the percentage of inhibition was calculated by comparing them with standard drugs (Table-2).

Minimum inhibitory concentrations (MIC) were determined for only compounds with remarkable effects. The various concentrations of test compounds in DMSO like 90, 80, 70, 60, 50, 40, 30 and 20 µg/mL were first prepared and checked for inhibition. Further dilutions were made and tested till the concentration inhibiting growth was achieved (Table-3).

Anticancer Activity

Anticancer study of synthesized compounds was carried out against human breast cell line (MCF-7) using SRB (Sulfo Rhodamine-B dye) assay[28, 29]. The cell suspensions were prepared in the appropriate growth medium to produce about 100 µL volume with the cell density of $1x10^4$ cells/well. The 100 µL aliquots of cell suspensions were transferred into 96-well micro-titer plates which were incubated for 120 h. The cell lines were kept at 37°C in a 5% v/v CO_2 with 95% humidity. Cultures were developed within a period of 7 days and culture medium changed at least one time in the 5 days. The 100 µL of culture with optimal cell density was taken in each well of 96-well titer plates. The test compounds were properly dissolved in dimethyl sulphoxide (DMSO) which was diluted to obtain various concentrations of 10, 20, 40 and 80 µg/mL. 100 µL of each concentration was added to the wells containing the cell suspension and 100 µLof DMSO solvent to control cells. The cells with the test compound were incubated for 48 h and fixed using 100 µl of cold 40% w/v of trichloroacetic acid at 4°C for 1 h. Thereafter, the sufficient volume of cold distilled water was added to wash the plates for five times. Aliquots of 50 µL of 0.4% w/v of SRB dye in 1% v/v acetic acid solution was added to each well of dried 96 well-plates and thencells were kept with dye stain for about 30 minutes. The dye was removed readily by washing the plates with 50 mL of 1% v/v acetic acid and rinsed 4-5 times till dye bonded with cells only, was retained. The 100 µL of 10mM Tris base maintaining pH 10.5 were added in each well of the dried plates to solubilise the contents of dye. A shaker was generally used to tremble the treated plates smoothly for 20 minutes and therefore, in each well the absorbance was read on a plate reader at 492 nm. The same operation was done for all the samples and got the observations in triplicate as well as LC_{50}, (drug concentration that kill the cell growth), TGI, (drug concentration that inhibit total cell growth) and GI_{50} values in µg/mL (drug concentration that inhibit 50% of cell growth) were determined by comparing with doxorubicin used as standard drug (Table-4).

Table- 2: Data for Antimicrobial Activityof Title compounds (4a-h)

Compounds (100 µg/mL)	Diameter of zone of inhibition ±SD (mm)[@]					
	S. aureus	*B. substilis*	*E. coli*	*P. aureginosa*	*A. niger*	*C. albicans*
4 (a)	18.33±0.29 (90.9%)	18.50±0.87 (85.4%)	17.67±0.29 (79.1%)	14.33±0.29 (70.5%)	11.33±0.29 (51.1%)	10.50±0.50 (51.2%)
4 (b)	17.17±0.29 (85.1%)	17.67±0.29 (81.5%)	16.83±0.58 (75.4%)	13.67±0.29 (67.2%)	9.67±0.58 (43.6%)	10.17±0.58 (49.6%)
4 (c)	14.17±0.58 (70.3%)	15.50±0.50 (71.5%)	14.83±0.58 (66.4%)	12.17±0.29 (59.9%)	11.50±0.50 (51.9%)	10.17±0.29 (49.6%)
4 (d)	12.67±0.29 (62.8%)	13.83±0.58 (63.8%)	11.67±0.29 (52.3%)	11.00±0.50 (54.1%)	9.83±0.29 (44.3%)	9.50±0.50 (46.3%)

4 (e)	9.17±0.29 (45.5%)	9.67±0.58 (44.6%)	9.33±0.58 (41.8%)	9.33±0.58 (45.9%)	11.67±0.29 (52.6%)	11.50±0.50 (56.1%)
4 (f)	11.50±0.50 (57.0%)	11.83±0.29 (54.6%)	13.50±0.50 (60.5%)	11.83±0.76 (58.2%)	13.00±0.50 (58.6%)	13.50±0.87 (65.9%)
4 (g)	11.33±0.29 (56.2%)	11.50±0.50 (53.1%)	10.50±0.50 (47.0%)	10.83±0.29 (53.3%)	12.83±0.76 (57.9%)	13.33±0.58 (65.0%)
4 (h)	13.00±0.50 (64.5%)	13.67±0.29 (63.1%)	12.00±0.50 (53.7%)	9.50±0.50 (46.7%)	12.67±0.29 (57.1%)	12.67±0.29 (61.8%)
Ciprofloxacin (20 µg/mL)	20.17±0.29 (100%)	21.67±0.58 (100%)	22.33±0.76 (100%)	20.33±0.58 (100%)	--	--
Flucazole (20 µg/mL)	--	--	--	--	22.17±029 (100%)	20.50±0.50 (100%)
DMSO	--	--	--	--	--	--

[@] The percentage zone of inhibition of test compounds was calculated against various microbialstrains with reference to standard and solvent control and given as % inhibition.
'--' indicates the zone of inhibition of control (DMSO) was considered as negligible.

Table-3: Data for Minimum Inhibitory Concentrations of Title compounds (4a-h)

Compounds	[#]Minimum Inhibitory Concentrations (MICs), µg/mL					
	S. aureus	*B. substilis*	*E. coli*	*P. aeruginosa*	*A. niger*	*C. albicans*
4 (a)	20	22	24	32	44	48
4 (b)	28	26	30	36	ND	ND
4 (c)	34	36	38	38	ND	ND
4 (f)	36	38	40	40	34	32
4 (g)	ND	ND	ND	ND	42	36
Ciprofoxacin	18	20	20	24	ND	ND
Fluconazole	ND	ND	ND	ND	26	24

[#] ND indicates the MIC of those compounds were not determined

Table-4: Data of Anticancer activity on Human Breast cancer cell line (MCF-7)

S. No.	Compound Code	ClogP	[a]LC_{50}	TGI	GI_{50}	[b]Activity
1	4 (a)	1.63	>80	>80	46.8	Ma
2	4 (b)	2.20	>80	>80	39.3	Ma
3	4 (c)	2.35	>80	>80	35.4	Ma
4	4 (f)	1.11	>80	>80	24.0	Ga
5	4 (g)	1.55	>80	>80	28.1	Ga
Standard	Doxorubicin	2.82	>80	<10	<10	Ga
Control	DMSO	--	--	--	--	--

[a]LC_{50}= drug concentration that kill the cell growth in µg/mL; TGI= drug concentration that inhibit total cell growth in µg/mL; GI_{50} = drug concentration that inhibit 50% cell growth in µg/mL
[b]Ga and Ma indicates the good and moderate anticancer activity of potent antimicrobial agents respectively; '-'indicates the activity of control was considered as negligible

RESULTS AND DISCUSSION

The 5-(4-substituted phenyl)-1,3,4-thiadiazole-2-amines were prepared by dehydrocyclization of the 4-substituted benzoyl thiosemicarbazides using concentrated sulphuric acid.
Infrared, NMR, Mass spectral methods as well as elemental analysis used to elucidate the structures of all synthesized compounds. The dehydrocyclization of benzoyl thiosemicarbazides to 1,3,4-thiadiazoles was confirmed by IR absorption bands at 860 to 896 cm^{-1} due to the presence of C-S-C linkage of

thiadiazoles, absorption bands from 1632 to 1650 cm^{-1} which are appeared because of stretching vibration of C=N bond. Infra-red absorption bands appeared from 3372 to 3394 cm^{-1}due to stretching vibration of N-H of NH_2 group in the synthesized compounds. ^{13}C NMR spectra confirmed the positions of carbons (at δ, ppm about 162 of C-2 and about 174 of C-5 in the thiadiazole nucleus) of the title compounds. The consistent molecular ion peaks of the synthesized compounds confirm their molecular weights and also chemical structures.

Compound **4(a)** and **4(b)** were found to be most active possessing bacterial inhibition between 80% to 91% as compared to Ciprofloxacin at the concentrations of 20 µg/mL against *Staphylococcus aureus* and *Bacillus substilis*. Compound **4(c)** was moderately active showing average percentage inhibition between 70% to 75% whereas compounds **4(d)** and **4(h)** showed mild inhibitory effect ranging from 62% to 65%. Rest of the compounds exhibited the inhibition from 44% to 57% considered to be inactive.

The compound **4(a), 4(b), 4(c)** and **4(f)** showed moderate antibacterial activity ranging between 59% to 80% inhibition as compared to Ciprofloxacin against *Escherichia coli* and *Pseudomonas aeruginosa*. Weak activity (47% to 55% of inhibition) was shown by compound **4(d)** and **4(h).** Compounds **4(e)** and **4(g)** exhibited mild inhibitory action (Table-2).

Some of the compounds of the series were prominent in their action against the fungal strains i.e. *Aspergillus niger*and *Candida albicans*. The maximum inhibitory action (57% to 66% inhibition) was shown by the compounds **4(f), 4(g)** and **4(h)**. The remaining compounds of said series showed mild antifungal activities.

The MIC values were determined for the most potent compounds and found to be 20 to 36 µg/mL for *Staphylococcus aureus*, 22 to 38 µg/mL for *Bacillus subtilis*, 24 to 40 µg/mL for *Escherichia coli* and 32 to 40 µg/mL for *Pseudomonas aeruginosa*. Compounds **4(a), 4(b)** and **4 (c)** were most active antibacterial agents within the synthesized series which are less active against the Gram –ve than Gram +ve bacteria. The range of MIC was 34 to 44 µg/mL for *Aspergillus niger* and 32 to 48 µg/mL for *Candida albicans* (Table-3).

In this study, the anticancer activity of the synthesized compounds was evaluated by determining LC_{50}, TGI, and GI_{50} values in µg/mL using sulforhodamine-B dye method[30]. The compounds **4(f)** possessing LC_{50} value >80; TGI value >80 and GI_{50} = 24.0 µg/mL and 4(g) with LC_{50} value >80; TGI value >80 and GI_{50} = 28.1 µg/mL showed good anticancer activity while **4(a), 4(b)** and **4(c)** exhibited the moderate activity with same values of LC_{50} >80; TGI values >80 but the GI_{50} values 35.4, 39.3, 46.8 µg/mL respectively.

In accordance, with the data obtained from *in vitro* anticancer studies, all the synthesized compounds showed moderate to good activity when compared with Doxorubicin as a standard drug. The compound **4(f)** and **4(g)** have shown comparatively good anticancer activity.

The value of clogP (determined using ChemDraw ultra 6.0) in the cytotoxic inhibition determines the permeability of drug to cross the cell membrane which should be sufficient to produce inhibitory action. From the results, it was found that the potent compounds possessing lowest clogP values showed good activity. Therefore, the cell permeability and anticancer activity of the synthesized compounds have been correlated to develop the variety of new anticancer agents. The results of anticancer activity of substituted 1, 3, 4-thiadiazoles against human breast cancer cell lines were summarized in Table-4.

In the present study, it was observed that the synthesized compound **4(a)** not only (R=4-fluorophenyl on C-5 position of thiadiazole nucleus) produced highest antimicrobial activity against all bacterial strains but also **4(b)** and **4(c)** possess significant antibacterial activity.

In contrast to this, the compounds **4(f)** (R=4-hydroxy phenyl) and **4(g)** (R=4-methoxy phenyl) showed slightly lower antibacterial but higher antifungal activities. The antibacterial activities of compounds **4(e)** (R=methylphenyl) and **4(g)** (R=methoxyphenyl) were found to be lowest one. The antifungal activity of **4(b) and 4(d)** was exceptionally lower than that of **4(f).**

Analysis of structure activity relationships suggests that 4-substitution on aromatic ring at C-5 position of 1, 3, 4-thiadiazole nucleus determines antibacterial and antifungal activities. The electron withdrawing group (F, Cl and Br) at *para* position of aromatic ring on C-5 position of thiadiazoles nucleus is necessary for potency of such antibacterial agents.

In this study, a series of 5-(4-substituted phenyl)-1, 3, 4-thiadiazole-2-amines were synthesized. The structure-anticancer activity relationship was discovered from data that the ability of substituent at *para* position to withdraw or donate electrons to the phenyl ring of thiadiazoles is an essential part in such anticancer heterocyclic compounds[30, 31]. When an electron releasing group was substituted at the *para* position such as hydroxyl group in the phenyl ring (compound **4f**) which showed prominent anticancer activity on MCF-7 cell line. There was a replacement of the hydroxyl group (compound **4f**) by more electron-releasing methoxy group (compound **4g**) and responsible for decrease in the activity. In this relation, it may be noticed that the bulkiness of the substituent gives similar results for anticancer property. When the bulky methoxy group (compound **4g**) was introduced at *para* position by replacing the hydroxyl group (compound **4f**) on the aromatic ring reduces potency significantly. The compound **4(c)** having bromine atom at 4-position of aromatic ring, showed improved anticancer activity when comparing with the chlorine analogs (compound **4b**). Therefore, 5-phenyl 1, 3, 4-thiadiazole would be suggested as a pharmacophore for prominent anticancer activity of these bioactive compounds.

From the SAR study, it can be suggested that a most favorable electron density, the bulkiness of of 4-substituent at phenyl ring on C-5 position of 1,3,4-thiadiazole may be responsible to a great extent for showing maximum anticancer property against the cell line used in this study. The presence of the hydrogen atoms attached to the amino nitrogen as a hydrogen bonding region and phenyl moiety as hydrophobic region of thiadiazoles are essential for activity. From the above discussion, it has been determined that structural alterations in 1, 3, 4-thiadizoles led to more prominent antimicrobial and anticancer agents.

CONCLUSION

In summary, a simple synthetic scheme was produced for 4-substitued 5-phenyl 1,3,4-thiadiazole-2-amines 4(a-h) which were elucidated by Infrared, NMR and Mass spectral techniques. All these compounds were evaluated for antimicrobial activity against different bacterial and fungal strains by disc diffusion method and anticancer activity on breast cancer cell line using SRB assay.

The biological data indicates that compounds **4(a), 4(b)** and **4(c)** exhibited significant bacterial inhibition whereas compounds **4(f)** and **4(g)** showed reasonable inhibition against both fungal strains at a given concentration when compared to standard drug. The compounds **4(f)** and **4(g)** exhibited good to moderate anticancer activities.

However, these compounds could be further screened for the antibacterial activities against a variety of Gram+ve and Gram-ve bacteria to achieve their broad spectrum profile. It is possible that structural modifications of synthesized compounds will increase the antifungal activity while bioactive compounds may be further used to screen cytotoxic effect against other cell lines such as colon and lung cancer cell lines.

It is rational that further molecular modifications in these compounds will be of much interest for finding the valuable antimicrobial and anticancer agents.

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