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Research Article

CONVENTIONAL AND ULTRASONIC MEDIATED SYNTHESIS OF SOME NEW SUBSTITUTED THIADIAZOLE DERIVATIVES AND EVALUATION FOR THEIR ANTIMICROBIAL AND ANTITUBERCULAR ACTIVITIES

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ABSTRACT
A series of new substituted 1,3,4-thiadiazole derivatives were synthesized using Ultrasound irradiation technique. The structures of these compounds were established by means of FTIR, ^{1}H-NMR and elemental analysis. All the compounds were evaluated for antibacterial, antifungal and antitubercular activities. Most of the compounds have shown significant antibacterial, antifungal and antitubercular activity when compared with the standard drug.

Keywords: Thiadiazole, Azetidin-2-one, Antimicrobial Activity, Antitubercular Activity.

INTRODUCTION

Over the last two decades there has been rapid progress in synthetic organic chemistry associated with the search for new organic compound derivatives with desirable properties. Such compounds are widely used in the pharmaceutical industry. Ultrasound irradiation has been utilized to accelerate a number of synthetically useful reactions during the last few years. Cavitation is the formation, growth and collapse of bubbles in an irradiated liquid. This effect induces very high local pressure and temperatures inside the bubbles and enhances mass transfer and turbulent flow in the liquid[1]. Ultrasound has been utilized to accelerate a number of synthetically useful reactions, especially in heterocyclic chemistry[2-3].

Several five membered aromatic systems having three hetero atoms at symmetrical position have been studied because of their interesting physiological properties. The therapeutic effects of compounds containing 1,3,4-thiadiazole and 1,2,4-triazole rings have been well studied for a number of pathological conditions including inflammation[4-5], pain[6-8] or hypertension[9] . Moreover, synthesis of thiadiazoles and triazoles has attracted widespread attention due to their diverse applications as antibacterial[10], antimycobacterial[11-12], antimycotic[13-14], antifungal[15-16] and antidepressant agents[17].

Azetidinones are of great biological interest, especially as anti-tubercular[18], antibacterial[19-21] activities. Azetidin-2-one, a four membered β-lactum skeleton, has been recognized as useful building blocks for the synthesis of a large number of organic molecules by exploiting its ring stain[22]. The 2-azetidinone moiety is an essential part of the penicillin skeleton and substructure found in β-lactamase inhibitors such as clavulanic acid or sulbactam[23].

MATERIALS AND METHODS

MATERIALS AND INSTRUMENTATION

The compounds were synthesized using OSCAR Ultraclean Sonicator 103 according to the given synthetic scheme figure 1. Melting points were determined in open capillary method and are uncorrected. IR spectra were recorded on Shimadzu's IRAffinity1 FTIR spectrophotometer. Purity of the compounds was checked on silica Gel TLC plates. ^{1}H NMR spectra were recorded on 400-MHz and 500-MHz Bruker spectrometer in DMSO-d_6 or $CDCl_3$ using tetramethylsilane (TMS) as an internal standard.

General Synthesized Experiment Procedure

Preparation of 5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine (A_1)

By conventional method

A mixture of thiosemicarbazide (0.1mole), 4-chlorobenzoic acid (0.1 mole), & conc. Sulphuric acid (10 drops) was refluxed for 1 hr & poured onto crushed ice. The solid separated out was filtered, washed with water & recrystallized from ethanol.

By ultrasonic irradiation

The equimolar quantity of thiosemicarbazide (0.1 mol) and 4-chlorobenzoic acid (0.1 mol), were taken in 100ml of beaker with 15ml of ethanol and & conc. Sulphuric acid (10 drops) and the reaction mixture was subjected to Ultrasonic irradiation for 15-20 mins at temperature of 60°C. The product obtained was poured over ice water. The solid separated out was filtered, washed with water & recrystallized from ethanol.

Preparation of 5-(4-chlorophenyl)-N-(1-phenylethylidene)-1,3,4-thiadiazol-2-amine (B_1)

By conventional method

The equimolar amount of 5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine A_1 (0.01 mol) and benzaldehydes (0.188 mol) in ethanol was refluxed for 8 h in presence of few drops of glacial acetic acid. The progress and completion of the reaction were checked by TLC. After refluxing, excess of solvent was distilled off and mother liquor was dropped on crushed ice, filtered, dried and solids thus obtained were recrystallized from ethanol.

By ultrasonic irradiation

In a 100 mL beaker, 5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine A_1 (0.1 mol) and benzaldehyde (0.188 mol) were together suspended in 15ml ethanol. The mixture was subjected to Ultrasonic irradiation for 15-20 mins at temperature of 60°C. The product obtained was poured over ice water. The solid separated out was filtered, washed with water & recrystallized from ethanol to give B_1

Preparation of 3-chloro-1-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)-4-phenylazetidine-2-one (C_1)

By conventional method

To the mixture of 5-(4-chlorophenyl)-N-(1-phenylethylidene)-1,3,4-thiadiazol-2-amine B_1 (0.1 mol), chloroacetyl chloride (0.1 mol) and triethyl amine (1 ml) were added with stirring. These different reaction mixtures were refluxed for 4-6 h and excess of solvent then distilled off. The resultant mixtures were poured onto crushed ice, filtered, dried and solids thus obtained were recrystallized from ethanol.

By ultrasonic irradiation

A mixture of 5-(4-chlorophenyl)-N-(1-phenylethylidene)-1,3,4-thiadiazol-2-amine (0.1 mol) and Chloroacetyl chloride (0.1 mol) were taken in 100ml of beaker with 15ml of ethanol and & TEA (0.1ml) and the reaction mixture was subjected to Ultrasonic irradiation for 15-20 mins at temperature of 60°C. The product obtained was poured over ice water. The solid separated out was filtered, washed with water & recrystallized from ethanol to give C_1. The compounds C_2-C_{24} were synthesized following a similar procedure using and different aromatic aldehydes like benzaldehyde, 4-chlorobenzaldehyde and salicyaldehyde as shown in figure 1.

Antibacterial and Antifungal activity

The antimicrobial activity of the synthesized compounds was determined by cup-plate method[24]. The organisms selected for antibacterial activity were *S. aureus* ATCC 12598 and *E. coli* ATCC 25922. Similarly the antifungal activity was carried out by using *A. niger* ATCC 9029 and *C. albicans* ATCC 2091. The concentration of sample compounds was 100 mcg/mL. Ciprofloxacin and Fluconazole were used as standard drugs for antibacterial and antifungal activity respectively as shown in table 3.

Antitubercular activity

The antimycobacterial activity of compounds (**C_1-C_9**) was assessed against *M. tuberculosis*

H37Rv (ATCC 2729411) using the microplate Alamar blue assay (MABA). This methodology is nontoxic, uses thermally stable reagent and showed good correlation with proportional and BACTEC radiometric methods[25] and the activity is expressed as the minimum inhibitory concentration (MIC) in µg/mL. The final drug concentration tested was 0.01-100 µg/mL. A blue colour in the well was interpreted as no bacterial growth and pink colour was scored as growth. The MIC was defined as the lowest drug concentration, which prevented a colour change from blue to pink. The MICs of the compounds were given in Table-1. Streptomycin, pyrazinamide and Ciprofloxacin were used as standards. All the tested compounds showed satisfactory *in vitro* activity against streptomycin, pyrazinamide and Ciprofloxacin as standard drugs.

Spectral data

3-chloro-1-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)-4-phenylazetidin-2-one (C_1). FT-IR (KBr disc): 3094 (C-H aromatic), 2982 (C-H aliphatic), 1694 (C=O of β- lactum ring), 1591 (C=N), 1470 (C=C of aromatic ring), 1091 (N-N), 1176 (C-N), 760 (C-Cl),683 (C-S-C) ; H^1-NMR (δ ppm): 7.22- 7.82 (m, 9H, Ar-H),5.15 (s, 1H, -CH-Cl),4.11 (s, 1H, -N-CH).

3-chloro-1-(5-(2,4-dichlorophenyl)-1,3,4-thiadiazol-2-yl)-4-phenylazetidin-2-one (C_2). FT-IR (KBr disc): 3072 (C-H aromatic), 2945 (C-H aliphatic), 1692 (C=O of β- lactum ring), 1584 (C=N), 1473 (C=C of aromatic ring), 1093 (N-N), 1155 (C-N), 772 (C-Cl), 679 (C-S-C).

3-chloro-1-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)-4-phenylazetidin-2-one (C_3). FT-IR (KBr disc): 3063 (C-H aromatic), 2993 (C-H aliphatic), 1693 (C=O of β- lactum ring), 1606 (C=N), 1494 (C=C of aromatic ring), 1074 (N-N), 1178 (C-N), 789 (C-Cl), 685 (C-S-C) ; H^1-NMR (δ ppm): 7.22- 7.82 (m, 9H, Ar-H),5.15 (s, 1H, CH-Cl),4.11(s, 1H,-N-CH).

3-chloro-4-(4-chlorophenyl)-1-(5-(4-chlorophenyl)1,3,4-thiadiazol-2-yl)azetidin-2-one(C_4). FT-IR (KBr disc): 3093 (C-H aromatic), 2981 (C-H aliphatic), 1692 (C=O of β- lactum ring), 1591 (C=N), 1470 (C=C of aromatic ring), 1093 (N-N), 1176 (C-N), 760 (C-Cl), 682 (C-S-C).

3-chloro-4-(4-chlorophenyl)-1-(5-(2,4-dichlorophenyl)1,3,4-thiadiazol-2-yl)azetidin-2-one (C_5). FT-IR (KBr disc): 3068 (C-H aromatic), 2941 (C-H aliphatic), 1708 (C=O of β-lactum ring), 1587 (C=N), 1474 (C=C of aromatic ring), 1085 (N-N), 1155 (C-N), 772 (C-Cl), 678 (C-S-C) ; H^1-NMR (δ ppm): 7.29-7.89 (m, 7H, Ar-H),4.11 (s, 1H, -N-CH),5.25 (s, 1H, CH-Cl, J=6.5 HZ,4.77 (d, 1H, CH-Ar, J=11.0HZ).

3-chloro-4-(4-chlorophenyl-1-(5-*o*-tolyl-1,3,4-thiadiazol-2-yl)azetidin-2-one(C_6). FT-IR (KBr disc): 3061 (C-H aromatic), 2956 (C-H aliphatic), 1712 (C=O of β- lactum ring), 1597 (C=N), 1471 (C=C of aromatic ring), 1086 (N-N), 1167 (C-N), 790 (C-Cl), 707 (C-S-C).

3-chloro-4-(4-hydroxyphenyl)-1-(5-(4-methoxyphenyl)1,3,4-thiadiazol-2-yl)azetidin-2-one (C_7). FT-IR (KBr disc): 3068 (C-H aromatic), 2941 (C-H aliphatic), 1691 (C=O of β-lactum ring), 1574 (C=N), 1495 (C=C of aromatic ring),3180 (OH stretch), 1107 (N-N), 1168 (C-N), 773 (C-Cl),698 (C-S-C); H^1-NMR (δ ppm): (400 MHz, DMSO):5.80 (s, 1H, -C-OH), 7.87 (m, 8H, Ar-H), 5.25 (s, 1H, -CH-Cl, J=6.5 Hz),4.71 (d, 1H, CH-Ar, J=11.0 Hz), 3.81 (s, 3H, OCH_3),4.13 (s, 1H,-N-CH).

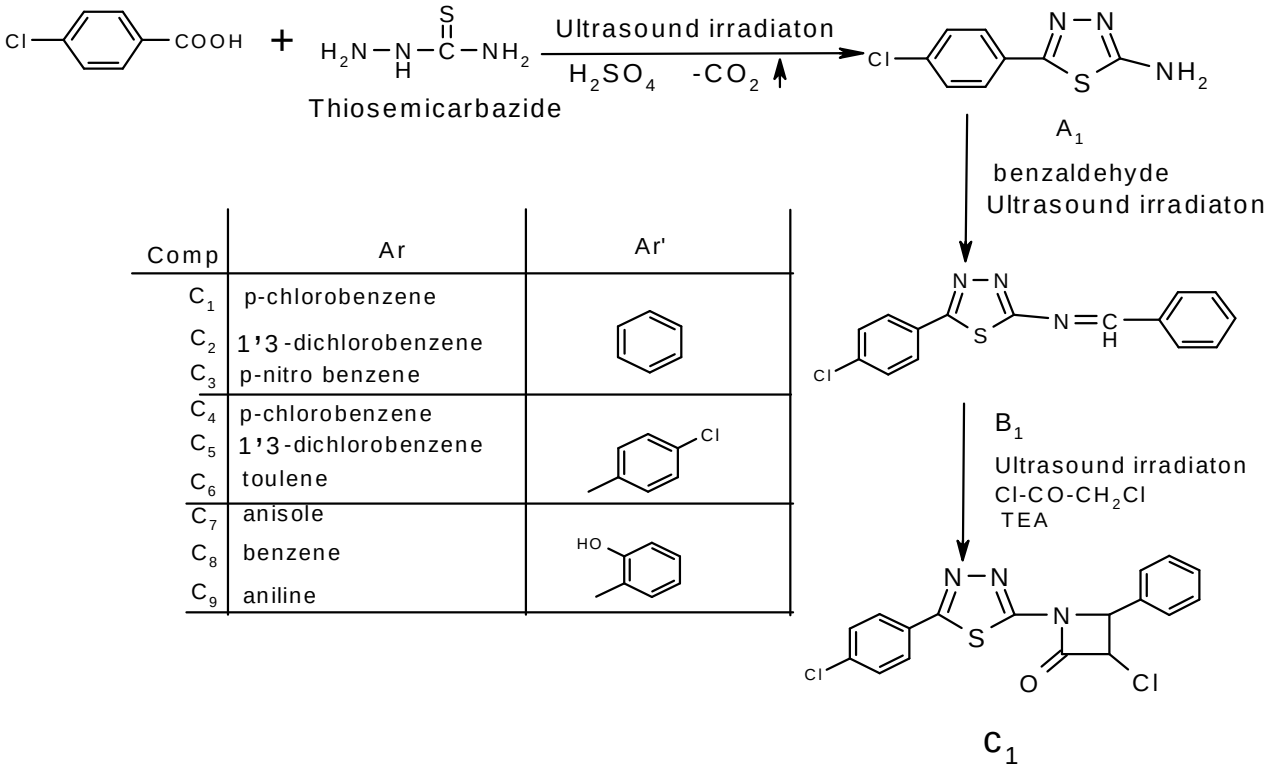
3-chloro-4-(4-hydroxyphenyl)-1-(5-phenyl-1,3,4-thiadiazol-2-yl)azetidin-2-one(C_8). FT-IR (KBr disc): 3068 (C-H aromatic), 2968 (C-H aliphatic), 1720 (C=O of β- lactum ring), 1568 (C=N), 1493 (C=C of aromatic ring), 1101 (N-N), 1149 (C-N), 783 (C-Cl), 707 (C-S-C).

1-(5-(4-aminophenyl)-1,3,4-thiadiazol-2-yl)-3-chloro-4-(4-hydroxyphenyl)azetidin-2-one(C_9). FT-IR (KBr disc): 3051 (C-H aromatic), 2983 (C-H aliphatic), 1720 (C=O of β- lactum ring), 1568 (C=N), 1470 (C=C of aromatic ring), 1084 (N-N), 1150 (C-N), 769 (C-Cl),707 (C-S-C).

RESULTS AND DISCUSSION

A series of Thiadiazole derivatives were synthesized using ultrasound irradiation by incorporating azetidinone moiety into 1,3,4 thiadiazole ring structure is given in figure 2.. The structures of these compounds were established by means of IR, ^{1}H NMR, and elemental analysis. The yield obtained from conventional synthesis and Ultrasonic methods as shown in table 1. The title compounds were screened for their antimicrobial and antitubercular activities.

Out of Nine compounds synthesized (**C_1-C_9**). Compounds **C_1, C_2, C_3, C_4, C_5, and C_7** have shown significant antibacterial activity. Remaining compounds have also shown moderate or weak antibacterial activity. Compounds **C_1, C_2, C_4, C_7, and C_8** have shown significant antifungal activity. Remaining compounds have also shown moderate or weak antifungal activity. The title compounds synthesized were screened for antitubercular activity against M. tuberculosis using microplate Almar Blue assay (MABA). All Compounds have shown satisfactory antitubercular activity and compounds C_3, C_4, C_8 and C_9 have got similar activity compared to standard drug streptomycin.



Comp	Ar	Ar'
C_1	p-chlorobenzene	
C_2	1'3-dichlorobenzene	
C_3	p-nitro benzene	
C_4	p-chlorobenzene	
C_5	1'3-dichlorobenzene	
C_6	toulene	
C_7	anisole	
C_8	benzene	
C_9	aniline	

Fig. 1: Synthetic scheme of Ultrasonic method for thiadiazole derivatives

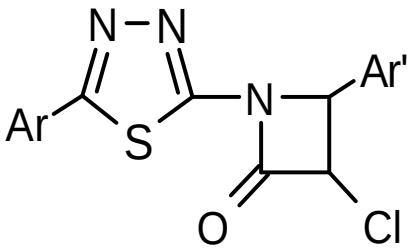


Fig. 2: Structure of thiadiazole derivatives

Table 1: Characterization data of compounds

Comp.	Mol. Formula	Ultrasound Method			Conventional Method		Elemental analyses Calculated (Found)		
		Time (min)	Yield (min)	m.p. (ºC)	Time (min)	Yield (%)	C	H	N
C_1	$C_{17}H_{11}Cl_2N_3OS$	22	94	241	360	62	54.27 (54.13)	2.95 (2.82)	11.17 (11.02)
C_2	$C_{17}H_{10}Cl_3N_3OS$	23	96	179	360	68	49.72 (49.63)	2.45 (2.53)	10.23 (10.14)
C_3	$C_{17}H_{11}ClN_4O_3S$	18	94	233	360	64	52.79 (52.59)	2.87 (2.73)	14.48 (14.38)
C_4	$C_{17}H_{10}Cl_3N_3OS$	22	91	243	360	58	49.72 (49.65)	2.45 (2.38)	10.23 (10.34)
C_5	$C_{17}H_9Cl_4N_3OS$	20	93	173	360	60	45.87 (45.73)	2.04 (2.12)	9.44 (9.5
C_6	$C_{17}H_{10}C_{l2}N_4O_3S$	23	90	237	360	62	48.47 (48.60)	2.39 (2.42)	13.30 (13.42)
C_7	$C_{17}H_{11}C_{l2}N_3O_2S$	22	93	243	360	61	52.05 (51.98)	2.83 (2.78)	10.71 (10.61)
C_8	$C_{17}H_{12}ClN_3O_2S$	23	92	139	360	62	57.06 (57.19)	3.38 (3.25)	11.74 (11.67)
C_9	$C_{17}H_{13}ClN_4O_2S$	20	94	227	360	59	54.77 (54.61)	3.51 (3.64)	15.03 (14.91)

Table 2: Antibacterial and Antifungal activities of the synthesized compounds

Comp.	Zone of Inhibition100mcg/mL (in mm)			
	E.coli	S.aureus	A.niger	C.albicans
C_1	**24**	**28**	**24**	**32**
C_2	**23**	**30**	**25**	**30**
C_3	**24**	**28**	20	23
C_4	**23**	**26**	**25**	**30**
C_5	**19**	**22**	22	18
C_6	18	20	23	18
C_7	**21**	**28**	**24**	**29**
C_8	20	25	**25**	**26**
C_9	18	25	17	20
Ciprofloxacin	**24**	**30**	--	--
Fluconazole	--	--	**26**	**32**

Table 3: Antitubercular activity of the synthesized compounds

Compound	MIC(µg/mL)
C_1	12.5
C_2	25
C_3	6.25
C_4	6.25
C_5	12.5
C_6	25
C_7	12.5
C_8	6.25
C_9	6.25
Streptomycin	6.25
Pyrazinamide	3.125
Ciprofloxacine	3.125

The MIC values were evaluated at concentration range, 0.8-100 µg/mL.
The figure in the table showed the value in µg/mL.

CONCLUSION

The satisfactory results obtained by the synthesized compounds, and the attracting significance of thiadiazole can be better explored in future as a potent candidate for antitubercular activity. It is also note worthy that the toxicity studies have been carried out for these compounds and least toxicity is being found in all these compounds.

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