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Synthesis and Biological Activity of some Novel 1, 3, 4-Thiadiazole derivatives

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Abstract : A novel synthesis of 3-chloro-4-(substituted)-1-(5-phenyl-1, 3, 4-thiadiazol-2-yl) azetidin-2-ones and their antimicrobial activity is described. The synthesis of the final compounds involves four steps. First step involves the synthesis of 2-amino-5-aryl-1,3,4-thiadiazole which is accomplished by cyclization of thiosemicarbazide in the presence of ferric chloride which is further converted into imines by the treatment with various substituted aldehydes. The final compounds were synthesized by Staudinger imine reaction of Schiff's bases.
Keywords: 1, 3, 4-thiadiazoles; 5-phenyl-1, 3, 4-thiadiazol-2-amine; azetidin-2-one; Anti-bacterial activity; Anti-fungal activity.

Introduction

Aromatic five membered heterocyclic systems having three hetero atoms at symmetrical positions have been studied because of their interesting physiological properties. It is also well established that various derivatives of 1,3,4 - thiadiazoles exhibit broad spectrum of pharmacological properties such as anti-inflammatory [1], antiviral [2], anticonvulsant [3,4], antifungal [5], anthelmintic,[6] CNS depressant[7] and diuretic[8] activities, when properly substituted in the 2- and 5-positions.[9] In the medical field one of the best known drugs having a 1,3,4-thiadiazole moiety is acetazolamide, which is a carbonic anhydrase inhibitor launched in 1954. Its indications and usage are many including the treatment of glaucoma, epilepsy and congestive heart failure.[10] Natural and synthetic azetidinone derivatives occupy a central place among medicinally important compounds due to their diverse and interesting antibiotic activities.
Hence in this article we wish to report the synthesis of derivatives having molecular entity of both 1, 3, 4-thiadiazole and azetidinone moiety. We have also reported the effect of this molecular frame work on their antimicrobial activity.

Experimental

The chemicals and solvents were of reagent grade. Melting points were determined by open capillary method and are uncorrected. The IR spectra were recorded on a Fourier Transform IR spectrometer (8400S, Shimadzu) at M.S. Ramaiah College of pharmacy, Bangalore. 1H NMR spectra were recorded on NMR spectrometer (AMX-400, Bruker) at Indian Institute of Science Bangalore using $CDCl_3$ and chemical shifts (δ) are reported in parts per million downfield from internal reference Tetramethylsilane (TMS). Mass spectra were provided at by Uwin Global Services, Bangalore, which were recorded on Mass spectrometer (LCMS-2010 A, Shimadzu).

(a) General procedure for synthesis of 2-amino 5-aryl-1, 3, 4-thiadiazole [01][07]

Aromatic aldehyde (0.04 mole) was dissolved in warm alcohol and thiosemicarbazide (0.004 mole) dissolved in hot water. The solution of aromatic aldehyde was added slowly to a solution of thiosemicarbazide with continous stirring, white coloured solid (thiosemicarbazone) was formed which was filtered off and recrystallised from 50% aq.alcohol. Thiosemicarbazone (0.005mole) was suspended in 150ml of distilled water in a 500ml beaker and ferric chloride (0.015mole) was dissolved in 150ml of distilled water. The solution of thiosemicarbazone and ferric chloride were mixed in a round bottom flask and the contents were heated at 80-90^{0}C for 3 hours and was filtered hot. A mixture of citric acid and sodium citrate was added to the solution and stirred.

After cooling the whole solution to room temperature, it was taken in a bigger vessel (to account for the increase in volume) and neutralized with 10% aqueous ammonia. The precipitate obtained was filtered and re-crystallized from 25% aqueous ethanol. The yields of the purified product are 77.7%.

2-amino 5-aryl-1, 3, 4-thiadiazole (01) Brown powder; bp. 218-220^{0}C; % yield 77.7 ; Rf 0.34 (Benzene : Acetone::1:1); IR (KBr)ν 3280.69, 3272.05 (NH); 3085.32 (Ar.C-H); 690.47(C-S stretching); 1633(C=N); 1H NMR (400 MHz, $CDCl_3$) δ 7.2-8.7 (5H Ar CH); δ 5.4 (2H,NH_2) ; MS (APCI +) *m/z* 178$(M+H)^+$.

(b)General procedure for synthesis of 2-[substituted benzylimino-5-phenyl]-1,3,4-thia diazole 2, [2a-g] [11]

Benzaldehyde (0.019mole) and 2-amino 5-phenyl 1, 3, 4-thiadiazole (0.0096mole) were mixed in a porcelain dish and heated on water bath for 1.5 hrs. The mixture was frequently stirred with a glass rod , globules of water soon appeared on the surface of the oil .The mixture was cooled and stirred well, the yellow coloured solid mass obtained was recrystallised from methylated spirit. The yield of purified products is between 70-79%.

2-[benzylimino-5-phenyl]-1, 3, 4-thiadiazole (2) Yellow powder; mp 82-85^{0}C; % yield 75.4; Rf 0.87 (Benzene:acetone::1:1); IR (KBr) ν 3070.46 (Ar.CH) ; 1583.45 (C=N); 707.83 (C-S); 1H NMR (400 MHz, $CDCl_3$) δ 7.2-8.2 (10H Ar H) δ 8.4 (1H CH) ; MS (APCI +) *m/z* 264 $(M-H)^+$.

(c) General procedure for synthesis of 3-chloro-4-substituted aryl-1-(5-phenyl-1, 3, 4-thia diazol-2-yl) azetidin-2-ones 3,[3a -g] [12]

A solution of 1ml of chloro-acetyl chloride in 1, 4-Dioxan (0.012M) was added dropwise to a well stirred solution of 2-[benzylimino-5-phenyl]-1, 3, 4-thiadiazole [Schiff's base] (0.01M) and Triethylamine (0.02M) in 1, 4-Dioxan. After the addition has been completed, the solution was stirred for 24 hrs. The reaction mixture was poured into

ice cold water. The separated solid was filtered and purified from Dioxan: water (80:20). The yield of purified products is between 55-65 %.

3-chloro-4- substituted aryl -1-(5-phenyl-1, 3, 4-thiadiazol-2-yl) azetidin-2-one (3)

White powder; mp 208-210^{0}C; % yield 58.6; Rf 0.576 (Chloroform: Methanol::9: 1); IR (KBr) ν 3028.03 (Ar.CH); 1704.96(C=O of CONH); 1299.93 (C-N); 829.33 (C-Cl); 686.61(C-S stretching); 1H NMR (400 MHz, $CDCl_3$) δ 7.3-8 (10H Ar H);δ 4.5 (1H, N-CH);δ 5.4 (1H, CH-Cl); MS (APCI +) *m/z* 343 $(M+H)^+$.

3-chloro-4-(4-hydroxyphenyl)-1-(5-phenyl-1,3,4-thiadiazol-2-yl)azetidin-2-one (3a). White powder ; mp 218-220^{0}C ; % yield 64.4; Rf 0.52 (Chloroform : Methanol::9 : 1); IR (KBr) ν 3174.61(O-H); 3024.18 (Ar.CH); 1706.88(C=O of CONH);1348.15(C-O stretching); 1299.3 (C-N); 1H NMR (400 MHz, $CDCl_3$) δ 7.2-8 (9H, Ar H);δ 4.4 (1H, N-CH); δ 5.5 (1H, CH-Cl); δ 9.4 (1H, OH); MS (APCI +) *m/z* 358 $(M+H)^+$.

3-chloro-4-(2-hydroxy phenyl)-1-(5-phenyl -1,3,4-thiadiazol-2-yl)azetidin-2-one (3b). Brown powder ; mp. 198-200^{0}C ; % yield 58.8 ; Rf 0.55 (Chloroform: Methanol::9:1); IR (KBr) ν 3174.61 (O-H);3028..03 (Ar.CH);1706.88 (C=O of CONH); 1346.22 (C-O stretching);1H NMR (400 MHz, $CDCl_3$) δ 7.3-8 (9H, Ar H);δ 4.4 (1H, N-CH);δ 5.2 (1H, CH-Cl);δ 8.8 (1H, OH); MS (APCI +) *m/z* 358 (M^+).

3-chloro-4-(3,4,5-trimethoxyphenyl)-1-(5-phenyl-1,3,4-thiadiazol-2-yl)azetidin-2-one (3c). Cream coloured powder; mp. 200-205^{0}C; % yield 63.6; Rf 0.60 (Chloroform : Methanol::9 :1); IR (KBr) ν 3058.89(Ar C-H) 1697.24 (C=O CONH);1296.08(C-N); 1172.64 (C-O str); 1H NMR (400 MHz, $CDCl_3$) δ 7.5- 8 (7H, Ar H);δ 5.1 (1H, N-CH);δ 5.6 (1H, CH-Cl);δ 3.8 (4H, OCH_3) MS (APCI +) *m/z* 434 (M^+).

3-chloro-4-(2,4-dichlorophenyl)-1-(5-phenyl-1,3,4-thiadiazol-2-yl)azetidin-2-one (3d). White powder ; mp. 203-205 ^{0}C; % yield 60.9; Rf 0.52 (Chloroform : Methanol::9:1); IR (KBr) ν 3087.82 (Ar CH);1706.17 (C=O CONH);823.55(C-Cl); 1299.3 (C-N), 1H NMR (400 MHz, $CDCl_3$) δ= 7.5-8.3 (8H, Ar H) δ 5.0 (1H,

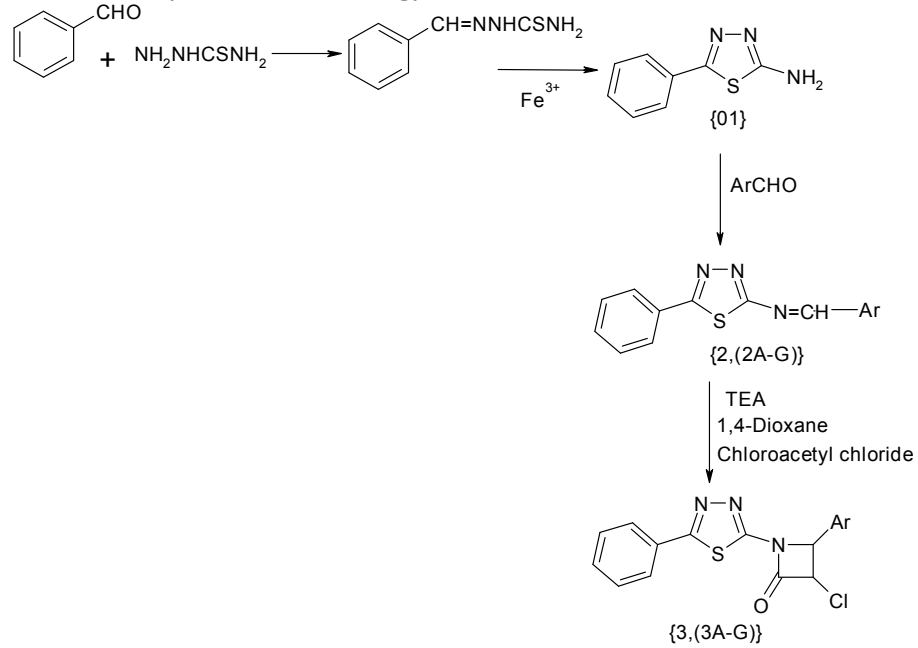
N-CH); δ 5.5 (1H, CH-Cl); MS (APCI +) *m/z* 411 (M^+).

3-chloro-4-(3-ethoxy-4-hydroxyphenyl)-1-(5-phenyl-1,3,4-thiadiazol-2-yl) azetidin-2-one (3e). Brown powder; mp. 178-180^{0}C; % yield 57.5 ; Rf 0.50 (Chloroform:Methanol::9:1); IR (KBr) ν 3176.54 (O-H);3058.89 (Ar C-H);2989.46 (Alkyl C-H); 1705.08(C=O CONH); 1H NMR (400 MHz, $CDCl_3$) δ 7.0-8.0 (8H, Ar H);δ 5.2 (1H, N-CH); δ 5.6 (1H, CH-Cl);δ 4.2 (2H, CH_2);δ 1.5 (3H, CH_3);δ 9.9(1H, OH) ; MS (APCI +) *m/z* 402 (M^+).

3-chloro-4-(4-dimethylphenyl)-1-(5-phenyl-1,3,4-thiadiazol-2-yl)-azetidin-2-one (3f). Yellow powder; mp. 205-208^{0}C; % yield 57.3; Rf 0.59 (Chloroform : Methanol::9:1); IR (KBr) ν 3024.18 (Ar C-H);2947.03 (Alkyl C-H);1706.88 (C=O of CONH);1299.93 (C-N); 1H NMR (400 MHz, $CDCl_3$) δ= 7.5-8.0 (9H, Ar H);δ 5.0 (1H, N-CH);δ 5.4 (1H, CH-Cl); δ 3.3 (3H, CH_3);MS (APCI -) *m/z* 385 $(M-H)^-$.

3-chloro-4-(4-nitrophenyl)-1-(5-phenyl-1,3,4-thiadiazol-2-yl)azetidin-2-one (3g). White powder; mp.198-200^{0}C; %yield 64.9; Rf 0.63 (Chloroform: Methanol::9:1); IR (KBr) ν 3056.96 (Ar C-H);1708 (C=O of CONH); 1348.15 (Ar NO_2); 1299.93 (C-N)833.19 (C-Cl); 1H NMR (400 MHz, $CDCl_3$) δ= 7.5-8.3 (9H, Ar H);δ 4.5 (1H, N-CH);δ 5.3 (1H, CH-Cl);MS (APCI +) *m/z* 385 (M^+).

Scheme for Synthetic Methodlogy:



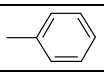
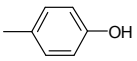
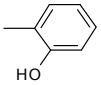
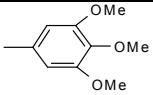
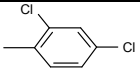
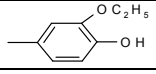
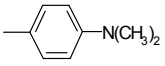
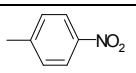
S.No.	Compound	Ar
1	3	
2	3a	
3	3b	
4	3c	
5	3d	
6	3e	
7	3f	
8	3g	

Table-I (A): Results of Antimicrobial Activity

Compounds	Ar	ANTIBACTERIAL ACTIVITY Zone of Inhibition (mm)				ANTIFUNGAL ACTIVITY Zone of Inhibition (mm)	
		S.aureus	***P.aeru***	***E.coli***	***E.cocci***	***A. niger***	***A. flavus***
[3]	Phenyl	10.3	07	9.6	8.6	8.3	8.3
[3A]	P-hydroxyphenyl	09	09	8.6	9.3	8.0	9.3
[3B]	2-hydroxy phenyl	11	8.6	09	10	6.3	5.6
[3C]	3,4,5-trimethoxyphenyl	11.3	7.6	10.3	7.6	9.3	8.6
[3D]	2,4-dichlorophenyl	15.3	11.6	11.6	12.3	10.6	11.3
[3E]	3-ethoxy-4-hydroxyphenyl	11.3	8.6	10	10	8.3	8.3
[3F]	4-dimethyl amino phenyl	11	6.3	09	8.6	8.6	8.6
[3G]	4-nitro phenyl	17.6	10.6	12.6	11.6	12	10.6
Norfloxacin	---------	18.3	14.6	23	15.6	--------	---------
Gatifloxacin	---------	23	15	23.6	18.6	---------	---------
Amphotericin B	---------	----------	-------	-------	---------	16.3	13.6
Clotrimazole	----------	----------	--------	--------	--------	18	15.3
Control(DMF)		NI	NI	NI	NI	NI	NI

NOTE: - Average zone diameter in mm of triplicates
NI : - No inhibition

Results and Discussion

i) Antibacterial activity

The antibacterial activity of newly synthesized 1, 3, 4-thiadiazole derivatives was carried out by agar diffusion method against *Staphylococcus aureus and Enterococci (*gram-positive) and *Pseudomonas aerugenosa and Escherichia coli (gram-negative)* using Norfloxacin and Gatifloxacin as standard reference drugs. The results are presented in Table-IA.

All compounds have shown antibacterial activity against the gram-positive and gram-negative bacteria tested.

The order of the antibacterial activity for the synthesized compounds is as follows.

a) Against *Staphylococcus aureus*

3G (17.6mm) > 3D (15.3mm) > 3E, 3C (11.3mm) > 3B,3F (11mm) >3(10.3) >3A (09mm)

b) Against *Enterococci*

3D (12.3mm) > 3G (11.6mm) > 3B, 3E (10mm) > 3A (9.3mm) > 3, 3F (8.6mm) >3C (7.6mm)

c) Against *Pseudomonas aeruginosa*

3D (11.6mm) > 3G (10.6mm) > 3A (09mm) > 3B,3E (8.6mm),3C (7.6mm) >3(7mm) >3F(6.3mm)

d) Against *Escherichia coli*

3G (12.6mm) > 3D(11.6mm) > 3C (10.3mm) > 3E (10mm) > 3 (9.6mm) > 3B,3F (9mm) >3A (12mm)

ii) Antifungal activity

The antifungal activity was evaluated against *Aspergillus niger* and *Aspergillus flavus* by agar diffusion method. The standards used are Clotrimazole and Amphotericin B. The results are presented in Table-IA.

All compounds have shown antifungal activity and the order of activity is as follows.

a)* Against *Aspergillus niger

3G(12mm) > 3D (10.6mm) > 3C (9.3mm) > 3F (8.6mm) > 3,3E (8.3mm) > 3A (8mm) > 3B (6.3mm)

b)* Against *Aspergillus flavus

3D (11.3mm) > 3G (10.6mm) > 3A (9.3mm) > 3F, 3C (8.6mm) > 3,3F (8.3mm) > 3B (5.6mm)

Conclusion

The objective of the present work was to synthesize, purify, characterize and evaluate the antimicrobial activity of the newly synthesized derivatives containing the molecular entity 1, 3, 4-Thiadiazole

tethered with 2-azetidinone. The yield of the products ranged from 55-65%. The purity was checked by TLC. The structures of the newly synthesized compounds **3,[3a-g]** are characterized and confirmed by spectral data viz. IR, 1H NMR and Mass spectra and all the synthesized compounds **3,[3a-g]** were screened for antimicrobial activity. From this study it may be concluded that 1, 3, 4-Thiadiazole tethered with 2-azetidinone and its derivatives show moderate to good antimicrobial activity.

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