Original Article

Synthesis, Characterization And Antimicrobial Activity Of Azetidinone Derivatives Based On 4-Amino-N-(4h-1,2,4-Triazol-4-Yl)Benzenesulfonamide

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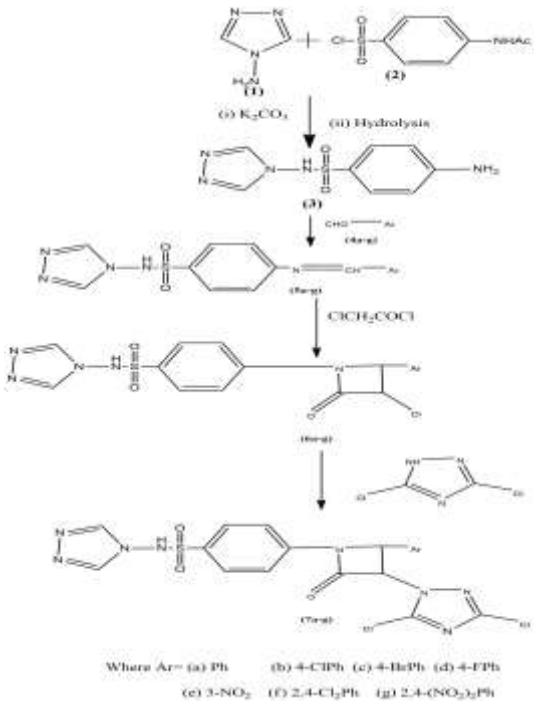
Abstract

The 4H-1,2,4-triazol-4-amine **(1)** reacted with 4-acetamidobenzene-1-sulfonyl chloride **(2)** followed by hydrolyzed to yield 4-amino-N-(4H-1,2,4-triazol-4-yl)benzene sulfonamide **(3).** The compound **(3)** was then condensed with various aromatic aldehydes **(4a-g)** afforded Schiff bases, 4-(arylideneamino)-N-(4H-1,2,4-triazol-4-yl) benzene sulfonamide **(5a-g)**. These **(5a-g)** further condensed with chloro acetyl chloride to novel azitidinone derivatives namely,4-(3-chloro-2-oxo -4-arylazetidin-1-yl)-N-(4H -1,2,4-triazol-4-yl) benzene sulfonamide **(6a-g).** On reaction of **(6a-g)** with 3,5-dichloro-1H-1,2,4-triazole yielded 4-(3(3,5-di chloro-1H-1,2,4-triazol-1-yl)-2-oxo-4-arylazetidin-1-yl)-N-(4H-1,2,4-triazol-4-yl) benzene sulfonamide **(7a-g)**. The elemental analysis and Spectroscopy techniques were used to determined the structure of all the novel synthesised compounds. The antibacterial and antifungal activities of novel synthesized compounds were tested.

Keywords: Schiff bases, Azetidinone, sulfonamide derivative, Triazole, Antibacterial activities and antifungal activities.

INTRODUCTION:

Sulpha drugs are well-known as ancient antibacterial drugs [1,2].4H-1,2,4-triazol-4amine (AT) is an important heterocyclic compound for many diversified for rug synthesis [3,4]. Many derivatives have been reported as antimicrobial, anticancer, anti-inflammatory, anti-T.B. drugs [5-7]. The various Schiff bases of AT have also been reported for medicinal purpose [815]. The heterocycylization azetidinone, thiazolidinone, etc of Schiff bases of AT has been well established by Patel et al [16]. Looking to the medicinal value of derivatives of AT and review of AT derivatives, it was found that AT an AT-sulfonamide derivatives has not been reported so far. The clubbing of AT and sulfonamide into on moiety may enhance the biological value of end product. Thus, the present paper comprises the heterocyclic derivatives like azetidinone based on sulfonamide-triazole derivatives shown in Scheme.



Scheme-1 Reaction Scheme

EXPERIMENTAL:

By Thermofinigun Flash EA (Italy) elemental analyzer the C,H,N, and other present atoms of all compounds determined. Known reported method applied for determination of sulfur and halogen [17]. Melting points were determined in open capillary tubes and were uncorrected. A Nicolet 400D spectrometer and Bruker spectrometer at 400 MHz used for IR spectra and ^{1}H NMR spectra. LC-MS of selected samples taken on LC-MSD-Trap-SL_01046. The 4-aminoN-(4H-1,2,4-triazol-4-yl)benzene sulfonamide (3) was prepared by reported method [18].

Synthesis of 4-(arylideneamino)-N-(4H-1,2,4-triazol-4-yl) benzene sulfonamide (5a-g): An equimolar mixture of 4-amino-N-(4H-1,2,4-triazol-4-yl)benzenesulfonamide **(3),** aromatic aldehyde **(4a-g),** glacial acetic acid in ethanol (30ml) was refluxed on a water bath for 4 hrs. The off white product was obtained after addition of 30ml ice water. The Schiff base was filtered, dried and recrystallized product from rectified spirit. The yield, melting points and other characterization data of these compounds designated as **(5a-g)** are given in Table -1.

Table: 1 Analytical Data and elemental analysis of compounds (5a-g)

Compd.	Molecular formula (Mol.wt.)	Yield (%)	M.P.* ^{0}C	Elemental Analysis									
				%C		% H		%N		%S		%X (X=Cl,Br,F)	
				Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
5a	C15H13N5O2S (327)	80	125	55.03	55.0	4.00	3.9	21.39	21.3	9.79	9.7	-	-
5b	C15H12N5O2SCl (361.5)	76	134	49.79	49.7	3.34	3.3	19.36	19.3	8.86	8.8	9.80	9.7
5c	C15H12N5O2SBr (406)	72	130	44.35	44.3	2.98	2.9	17.24	17.2	7.89	7.8	19.67	19.6
5d	C15H12N5O2SF (345)	73	147	52.17	52.1	3.50	3.4	20.28	20.2	9.28	9.2	5.50	5.4
5e	C15H12N6O4S (372)	67	129	48.38	48.3	3.25	3.2	22.57	22.5	8.61	8.6	-	-
5f	C15H11N5O2SCl2 (396)	74	140	45.47	45.4	2.80	2.7	17.67	17.6	8.09	8.0	17.89	17.8
5g	C15H11N7O6S (417)	71	135	43.17	43.1	2.66	2.6	23.49	23.4	7.68	7.6	-	-

***** *Uncorrected*

Synthesis of 4-(3-chloro-2-oxo-4-arylazetidin-1-yl)-N-(4H-1,2,4-triazol-4-yl) benzene sulfonamide (6a-g):

The schiff base, 4-(arylideneamino)-N-(4H-1,2,4-triazol-4-yl) benzene sulfonamide **(5a-g)** (0.003 mole) and TEA (triethyl amine) (0.006 mole) were dissolved in 1,4-dioxane (65 ml), cooled and stirred. To this well-stirred cooled solution, the chloro acetyl chloride (0.006 mole) was added dropwise. The reaction mixture was stirred for 4-5 hours at room temperature for more than 5 hrs. The resultant mixture was concentrated, cooled, poured into ice cold water. Then filtered, washed by ethanol and air-dried. Dissolved in THF solvent and reprecipitated by rectified spirit. All the compounds were characterized by elemental contents and data are shown in Table-2.

Table:2 Analytical data and elemental analysis of Compounds (6a-g)

Compd.	Molecular formula	Yield	M.P.*	Elemental Analysis

	(Mol.wt.)	(%)	^{0}C	%C		% H		%N		%S		%X(X=Cl,Br,F)	
				Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
6a	C17H14N5O3SCl (403)	64	178	50.56	50.5	3.49	3.4	17.34	17.3	7.94	7.9	8.78	8.7
6b	C17H13N5O3SCl2 (438)	61	170	46.59	46.5	2.99	2.9	15.98	15.9	7.32	7.3	16.18	16.1
6c	C17H13N5O3SClBr (482)	68	177	42.30	42.2	2.71	2.7	14.51	14.5	6.64	6.6	7.34 16.55	7.3 16.5
6d	C17H13N5O3SFCl (421)	60	180	48.40	48.3	3.11	3.1	16.60	16.5	7.60	7.5	8.40 4.50	8.3 4.4
6e	C17H13N6O5SCl (448)	58	184	45.49	45.4	2.92	2.9	18.72	18.7	7.14	7.1	7.90	7.8
6f	C17H12N5O3SCl3 (472)	55	175	43.19	43.1	2.56	2.5	14.81	14.8	6.78	6.7	22.50	22.4
6g	C17H12N7O7SCl (493)	61	172	41.35	41.3	2.45	2.4	19.85	19.8	6.49	6.4	7.18	7.1

***** *Uncorrected*

Preparation of 4-(3-(3,5-dichloro-1H-1,2,4-triazol-1-yl)-2-oxo-4-arylazetidin-1-yl)- N(4H-1,2,4-triazol-4-yl) benzene sulfonamide (7a-g):

In a round bottom flask, add 4-(3-chloro-2-oxo -4-arylazetidin-1-yl)-N-(4H -1,2,4triazol-4-yl)benzenesulfonamide **(6a-g)** (0.02 mol) and 3,5-dichloro-1H-1,2,4-triazole (0.03 mole) Triethyl amine (5ml) in ethyl alcohol (50ml). The mixture was heated at reflux with stirring for 4.5 hrs. The reaction mixture was cooled, filtered and washed with ethyl alcohol then cold water. Reprecipitation of **(7a-g)** was carried out using THF solvent. All the compounds were characterized by elemental contents and data are shown in Table-3.

The IR, NMR, LC-MS spectral features of compounds 5a-g, 6a-g and 7a-g are interpreted for their structural assignments. The details are given in results and discussion.

Table: 3 Analytical data and elemental analysis of Compounds (7a-g)

Compd.	Molecular formula (Mol.wt.)	Yield (%)	M.P.* ^{0}C	Elemental Analysis									
				%C		% H		%N		%S		%X (X=Cl,Br,F)	
				Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
7a	C19H14N8O3SCl2 (504)	60	222	45.16	45.1	2.79	2.7	22.17	22.1	6.35	6.3	14.03	14.0
7b	C19H13N8O3SCl3 (539.5)	57	229	42.28	42.2	2.43	2.4	20.76	20.7	5.94	5.9	19.70	19.6
7c	C19H13N8O3SCl2Br (584)	54	232	39.06	39.0	2.24	2.2	19.18	19.1	5.49	5.4	12.14 13.68	12.1 13.6
7d	C19H13N8O3SCl2F (523)	59	227	43.61	43.6	2.50	2.4	21.41	21.4	6.13	6.1	13.55 3.63	13.5 3.6
7e	C19H13N9O5SCl2 (550)	56	233	41.47	41.4	2.38	2.3	22.91	22.9	5.83	5.8	12.88	12.8
7f	C19H12N8O3SCl4 (574)	53	218	39.74	39.7	2.11	2.1	19.51	19.5	5.38	5.3	24.70	24.6
7g	C19H12N10O7SCl2 (595)	52	224	38.33	38.3	2.03	2.0	23.53	23.5	5.39	5.3	11.91	11.9

***** *Uncorrected*

RESULTS AND DISCUSSION:

It was observed that 4-amino-N-(4H-1,2,4-triazol-4-yl)benzenesulfonamide **(3)** on condensation with various aromatic aldehydes to yield Schiff bases,4-(arylideneamino)-N(4H-1,2,4-triazol-4-yl) benzene sulfonamide **(5a-g)**. The structures of **(5a-g)** were confirmed by elemental analysis and IR/NMR spectra showing absorption band at 3030-3050 cm^{-1} (C-H, of Ar.), 3130 cm^{-1} (NH), 1590-1585 cm^{-1} (C=N), 1160-1150,1330-1340cm^{-1} (SO_2NH), 1080cm^{-1} (C-Cl), 710 cm^{-1} (C-Br), 1260 cm^{-1} (C-F), 1550,1370 cm^{-1} (-NO_2) and 1342,1358m^{-1} (SO_2). ^{1}HNMR :7.65–8.50 (m,6H,Ar-H), 8.42(s,1H, NH),8.40(s,1H,CH), (a) 7.50- 7.80(m,5H,Ar-H), (b) 7.55-7.85(m,4H,Ar-H), (c) 7.60-7.80(m,4H,Ar-H), (d) 7.407.85 (m,4H,Ar-H), (e) 8.15 -8.40 (m,4H,Ar-H), (f) 7.40-8.10 (m,3H,Ar-H), (g) 8.45-9.20 (m,3H,Ar-H).The elemental contents of all compounds,**(5a-g)** are presented in Table -1.

The cyclocondensation of **(5a-g)** with chloro acetyl chloride resulted in formation of novel azitidinone derivatives namely,4-(3-chloro-2-oxo -4-arylazetidin-1-yl)-N(4H -1,2,4-triazol-4-yl)benzenesulfonamide **(6a-g)**. The structures assigned to **(6a-g)** were supported by the elemental analysis and IR/NMR spectra showing absorption bands at 1750- 1730 cm^{-1} (C=O of monocyclic β-lactam),3035-3050cm^{-1} (C-H, of Ar.), 3120 cm^{-1} (NH), 11601150,1335-1340cm^{-1} (SO_2NH), 1350(C-N),1080 cm^{-1} (C-Cl), 710 cm^{-1} (C-Br),1260 cm^{-1} (CF) and 1550.1370 cm^{-1} (C-NO_2). The ^{1}HNMR : 7.60–8.50 (m,6H,Ar-H), 8.45(s,1H,NH), 5.35(d,1H,C_2-H),5.65(d,1H,C_3-H), (a) 7.30-7.45 (m,5H,Ar-H), (b) 7.40-7.50(m,4H,Ar-H), (c) 7.00-8.00(m,4H,Ar-H), (d) 7.20-7.30(m,4H,Ar-H), (e) 7.58 -8.30 (m,4H,Ar-H), (f) 7.107.80(m, H, Ar-H), (g) 7.80-8.95 (m,3H,Ar-H). The elemental contents of all compounds,**(6ag)** are presented in Table -2.

The reaction of **(6a-g)** with 3,5-dichloro-1H-1,2,4-triazole resulted in formation of 4-(3-(3,5-di chloro-1H-1,2,4-triazol-1-yl)-2-oxo-4-arylazetidin-1-yl)-N-(4H-1,2,4-triazol4-yl) benzene sulfonamide **(7a-g)**. The post reaction of chlorine of azetidinone has been reported in literature [19]. The structures assigned to **(7a-g)** were supported by the elemental analysis and IR/NMR spectra showing absorption bands at 1750-1730 cm^{-1} (C=O of mono cyclic β-lactam), 3035-3050 cm^{-1}(C-H, of Ar.), 3115 cm^{-1} (NH), 1630-1635 cm^{-1} (C=N), 11601150,1332-1340cm^{-1} (SO_2NH), 1085cm^{-1} (C-Cl),710 cm^{-1} (C-Br),1260 cm^{-1} (C-F) and 1550,1370 cm^{-1} (C-NO_2). ^{1}HNMR : 7.60–8.50 (m,6H,Ar-H), 8.40(s,1H,NH), 5.32(d,1H,C_2-H),5.70(d,1H,C_3-H), (a) 7.30-7.40 (m,5H,Ar-H), (b) 7.40-7.48(m,4H,Ar-H), (c) 7.00- 7.90(m,4H,Ar-H), (d) 7.20-7.25 (m,4H,Ar-H), (e) 7.60 -8.10 (m,4H,Ar-H), (f) 7.107.60(m,3H,Ar-H), (g) 7.80-8.90 (m,3H,Ar-H). The elemental contents of all compounds,**(7ag)** are presented in Table -3.

The examination of data Table:1-3 reveals that the elemental contents are consistent with the predicted structures shown in scheme-1.The IR spectral and NMR signal of each compounds confirm the structure of all three series 5a-g,6a-g and 7a-g of compounds data also direct for assignment of the predicted structure.

The LC-MS of selected compounds 5e, 6e and 7e shows the peak of M+ ion peak at 373.1.8, 448.6 and 550.8, which is consistent of their molecular weight 372, 448 and 550, respectively. All these facts confirm the structures 5a-g, 6a-g and 7a-g.

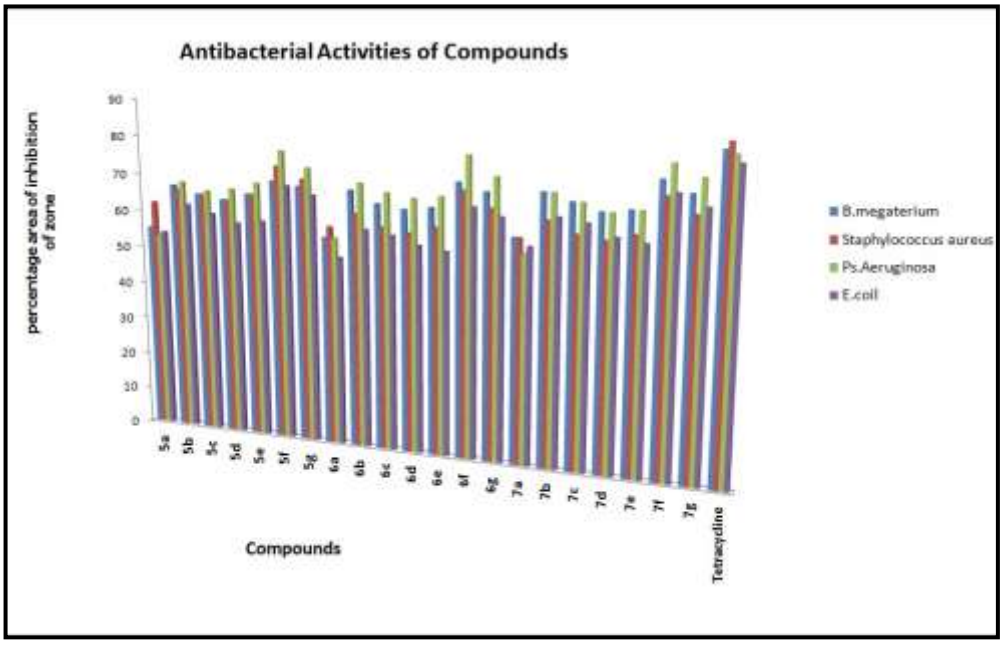
BIOLOGICAL SCREENING

Antibacterial Activities

Antibacterial activities of all the compounds were studied against gram-positive bacteria (*Staphylococcus aureus and B. megaterium*) and gram-negative bacteria (*E.coli, and Ps. Aeruginosa*) at a concentration of 50µg/ml by agar cup plate method [19-23]. Methanol system was used as control in this method. Under similar condition using tetracycline as a standard for comparison carried out control experiment. The percentage area of inhibition of zone measured. All the compounds show good toxicity against bacteria. However, compounds 7f and 7g were found more active against the above microbes. Other compounds found to be less or moderate active than standard tetracycline (Table –4). The compounds **(7a-g)** are more toxic than **(6a-g)** and **(5a-g).** The more toxicity of 7a-g compounds might be ue to presence of more chlorine atoms in their structures.

Table: 4 Antibacterial Activities of Compounds (5a-g), (6a-g) and (7a-g)

Compounds	percentage area of inhibition of zone			
	Gram +Ve		Gram -Ve	
	B. megaterium	*S. Aureus*	*E. Coli*	*Ps. Aeruginosa*
5a	55	62	53	54
5b	67	66	68	62
5c	65	65	66	60
5d	64	64	67	58
5e	66	66	69	59
5f	70	74	78	69
5g	69	71	74	67
6a	56	59	56	51
6b	69	63	71	59
6c	66	60	69	58
6d	65	59	68	56
6e	66	61	69	55
6f	73	71	80	67
6g	71	67	75	65
7a	60	60	56	58
7b	72	65	72	66
7c	70	62	70	65
7d	68	61	68	62
7e	69	63	69	61
7f	77	73	81	74
7g	74	69	78	71
Tetracycline	85	87	84	82



The results show that all the compounds display similar trend as studied for antibacterial activity.

Antifungal Activities

The fungicidal activity of all the compounds was studied by (agar cup method) at 1000 ppm concentration in vitro. Plant pathogenic organisms used were *Rhizopus nigricum***,** *Aspergillus niger, Fusarium oxyporium and Botrydepladia thiobromine*. The antifungal activity of all the compounds was measured on each of these plant pathogenic strains on a potato dextrose agar (PDA) medium. Such a PDA medium prepared from potato 200gm, dextrose 20gm, agar 20gm and water one liter. Five days old cultures were employed. The compounds to be tested were suspended (1000ppm) in a PDA medium and autoclaved at 120^{o} C for 15 min. at 15atm.pressure. These medium were poured into sterile Petri plates and the organisms were inoculated after cooling the Petri plates. The percentage inhibition for fungi was calculated after five days using the formula given below:

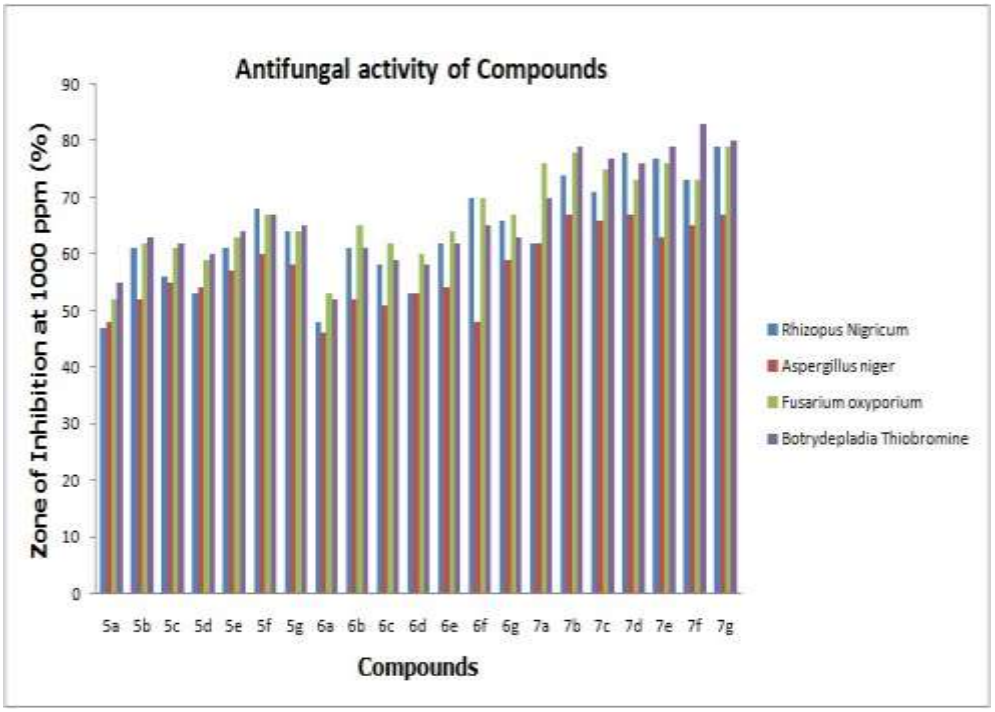
Percentage of inhibition = 100(X-Y) / X
Where, X = Area of colony in control plate
Y = Area of colony in test plate

The fungicidal activity displayed by all compounds **(5a-g), (6a-g)** and **(7a-g)** is shown in Table-5. The results show that all the compounds of three series are moderate to more toxic against fungi. More particularly the 7a-g compounds are more toxic than 5a-g an 6a-g compounds. 7f and 7g have been found as more toxic. This might be responsible due to chlorine atom.

Table: 5 Antifungal Activities of Compounds (5a-g), (6a-g) and (7a-g)

	Zone of Inhibition at 1000 ppm (%)			
Compounds	***Rhizopus Nigricum***	***Aspergillus niger***	***Fusarium oxyporium***	***Botrydepladia Thiobromine***
5a	47	48	52	55
5b	61	52	62	63
5c	56	55	61	62
5d	53	54	59	60
5e	61	57	63	64
5f	68	60	67	67
5g	64	58	64	65
6a	48	46	53	52
6b	61	52	65	61
6c	58	51	62	59
6d	53	53	60	58
6e	62	54	64	62
6f	70	48	70	65
6g	66	59	67	63
7a	62	62	76	70
7b	74	67	78	79
7c	71	66	75	77

7d	78	67	73	76
7e	77	63	76	79
7f	73	65	73	83
7g	79	67	79	80



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