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SYNTHESIS AND CHARACTERIZATION OF 4-ARYL TRIAZOLE RING SYSTEM AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

Synthesis of some new 4-Aryl triazoles and its derivatives were synthesized. 4-aryl triazoles were obtained by cyclization reaction of the potassium salt of substituted dithiocarbazinic acids with aromatic amines. These compounds have been characterized on the basis of elemental analyses, IR, ^{1}H NMR and MASS Spectrometry. Compounds have been screened for their antibacterial activity.

KEYWORDS: **Anti Microbial screening, 4-aryl triazole.**

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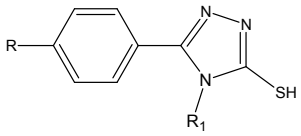
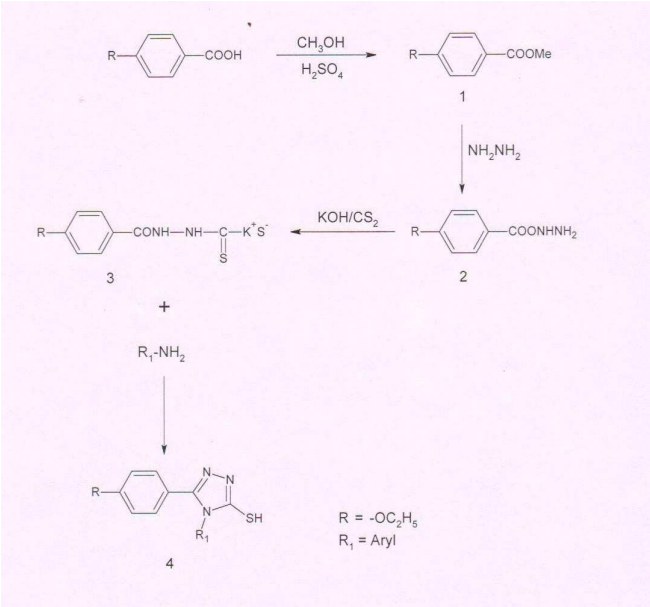
INTRODUCTION

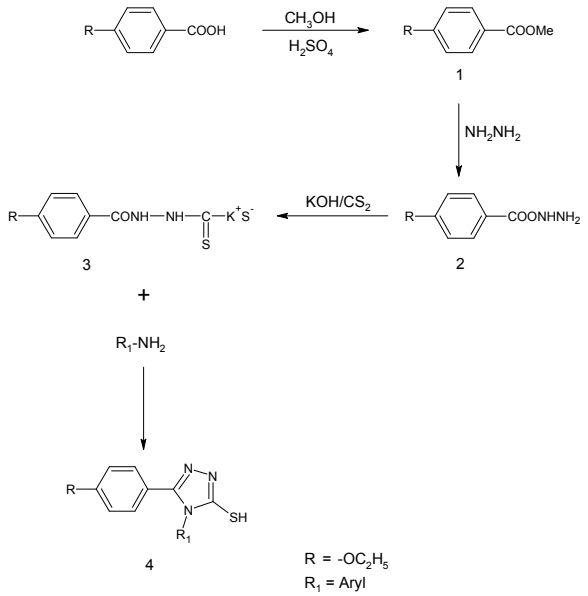
Amongst the five member nitrogen containing heterocycles, the position of nitrogen atom at 1,2 and 4 activates the ring. 1,2,4-triazole and its derivatives represent one of the most biologically active classes of compounds, possessing a wide spectrum of activities[1-4]. Many workers have reported the different activities of 4-aryl triazoles.Chambers *et al.* [5] have investigated 4-aryl triazoles to be useful in the treatment of neurogenerative disease. Pier *et al*.[6] reported as irreversible antagonist at the A_3, A_2A Adenosine receptor[7].Further many workers have reported 4-aryl triazoles as aromatic –steroid sulfatage inhibitors[8], GSKK-3 inhibitors[9], anticancer[10], fungicidal[11], antibacterial[12], anti-inflammatory[13], PKB (protein kinas B) inhibitors[14]. The triazole derivatives have also been reported as better therapeutic agents[15]. The scientific literature also stated that the antiviral [16] and antibacterial [17,18] activities of thiourea their derivatives are due to the presence of the –NH-C(S)-NH- function in the molecule and the changes in this activity depend on the nature of its sub stutuents. With an aim to synthesize better therapeutic agents, we have investigated some new 4-aryl triazole derivatives which have been described as under. All the synthesized compounds have been characterized by using Infrared and ^1H nuclear magnetic resonance spectroscopy. The compounds have been screened for their in vitro biological assay like antibacterial activity towards Gram positive and Gram negative bacterial strains. The above literature survey of recent years demonstrates that the synthesis of triazole derivatives of type A RRR been undertaken by heating potassium salt with different aromatic amines.



R= $-OC_2H_5$ R1= Aryl

Scheme 1





The synthetic route for the present work is depicted in scheme 1. Synthesis of 3-mercapto-4-N-Aryl-5-(4-Ethoxy phenyl)-1,2,4-Trizoles. Requiredasthe starting material was prepared according to the method in the literature [19]. Esterification of 1 with methanol in the presence of sulphuric acid gave ester derivative 2. whichon hydrozinolysis with hydrazine furnished hydrazide derivative 3. The hydrazide react with KOH and CS_2 in methanol gave 4. Condensation of 4 with various aromatic amines in DMF gave the corresponding 4-aryl triazole derivatives. The cyclization reaction is fairly general, facile, clean and efficient and is devoid of any side products. The products are obtained in good yields.(65%) and in a state of high purity. The structural assignments to compounds 2-5 were based on their elemental analyses and Spectral (IR,^{1}H NMR and MASS) data.

EXPERIMENTAL

Melting Points were determined on Gallen-Kamp melting point apparatus and are uncorrected. All the compounds were routinely checked for their homogeneity by TLC on silica gel-G plates. IR spectra were recorded in KBr on a Perkin-Elmer BX series FT-IR spectrophotometer, ^{1}H NMR spectra on a 400 MH_z spectrometer using TMS as internal slandered and satisfactory C, H and N analyses were obtained for all the compounds. The bacterial strains studied were identified strains and were obtained from the National Chemical Laboratory (NCL), Pune, India.

(1) Synthesis of 4-ethoxy benzoate

A mixture of 4- ethoxy benzoic acid 1,(1.66gm,0.01mole), 25ml methanol and 1ml of conc. H_2SO_4 was refluxed for 6 hours. The reaction mixture was poured in to ice. The product was isolated and treated waith saturated sodium bicarbonate solution.
Yield 87%, M.P. - 219^{0}C

(2) Synthesis of 4-ethoxy benzoic acid hydrazide

A equimoler mixture of ester 2 and hydrazine hydrate was heated for 10 hours and poured into ice. The separated soild was filtered, washed with water and recrystallized from ethyl alcohol to afford 3.
Yield 83%, M.P. - 156^{0}C.

(3) Synthesis of Potassium 4-ethoxy benzoic acid hydrazide dithiocarbamate

A mixture of hydrazine 3, (1.80g 0.01mole) KOH (0.5g,0.01mole) and CS_2 in methanol (20 ml) was stirred for 12 hours and poured in to ice.The product 4 was filtered, washed with water and recrystallized from diethyl ether.
Yield 78 %, M.P. - 196^{0}C

(4) Synthesis of 5-(4-ethoxyphenyl)-4-(4-methylphenyl)-4H-1,2,4-triazole-3-thiol

A mixture of potassium salt 4 (2.94gm, 0.01mole)and p-toludine(1.07gm,0.01mol) was heated up to evolution of H_2S gas, DMF was added to this mixture and contents were poured into ice. The crude product was filtered and crystallized from ethyl alcohol.
Yield 65%, M.P. - 190^oC

RESULTS AND DISCUSSION

Synthesis

The following compounds have been synthesized

4a. 5-(4-ethoxyphenyl)-4-(4-methylphenyl)-4*H*-1,2,4-triazole-3-thiol
4b. 4,5-bis(4-ethoxyphenyl)-4*H*-1,2,4-triazole-3-thiol
4c. 4-(4-chlorophenyl)-5-(4-ethoxyphenyl)-4*H*-1,2,4-triazole-3-thiol
4d. 5-(4-ethoxyphenyl)-4-(2-nitrophenyl)-4*H*-1,2,4-triazole-3-thiol
4e. 5-(4-ethoxyphenyl)-4-(3-nitrophenyl)-4*H*-1,2,4-triazole-3-thiol
4f. 4-(2,5-dimethylphenyl)-5-(4-ethoxyphenyl)-4*H*-1,2,4-triazole-3-thiol
4g. 4-(2,3-dichlorophenyl)-5-(4-ethoxyphenyl)-4*H*-1,2,4-triazole-3-thiol
4h. 5-(4-ethoxyphenyl)-4-(2-methylphenyl)-4*H*-1,2,4-triazole-3-thiol
4i. 4-(3-chloro-4-fluorophenyl)-5-(4-ethoxyphenyl)-4*H*-1,2,4-triazole-3-thiol
4j. 4-(2-methoxyphenyl)-5-(4-ethoxyphenyl)-4*H*-1,2,4-triazole-3-thiol

(4a) IR (KBr) : 2932 (C-H Str. Asym Aliphatic), 1426 (C-H Str. Sym Aliphatic), 3075 (C-H Str. Aro.), 1513 (C=C Str. Ring. Ske), 1611 (C=N Str.), 1255 (C-N Str.), 669 (S-H).
(4a) ^{1}H NMR : δ 1.36 (3H, t, -OCH_2CH_3), 2.43 (3H, s, Ar-CH_3), 4.19 (2H, d, -OCH_2CH_3), 6.77 (2H, d, Ar-H), 7.16 (2H, d, Ar-H), 7.22 (4H, d, Ar-H), 11.6 (1H, s, SH)
(4a) MS : m/z 107, 136, 154, 297, 311.
Physical constant of 5-(4-ethoxyphenyl)-4-(4-methylphenyl)-4*H*-1,2,4-triazole-3-thiol are given in **table 1**.

TABLE 1
CHARACTERIZATION DATA OF COMPOUND 4

Comp.	-Ar	Molecular Formula	Molecular Weight (gm)	M.P. ^{0}C	Yield %	R_f* Value	% of C, H and N		
							C	H	N
4a	4-CH_3-C_6H_4	$C_{17}H_{17}ON_3S$	311.38	190	66	0.63	65.62	5.45	13.52
							65.67	5.50	13.49
4b	4-OCH_3-C_6H_4	$C_{17}H_{17}O_2N_3S$	327.38	196	79	0.59	62.39	5.27	12.87
							62.36	5.23	12.83
4c	4-Cl-C_6H_4	$C_{17}H_{14}OClN_3S$	331.80	160	81	0.78	57.95	4.27	12.67
							57.91	4.25	12.60
4d	2-NO_2-C_6H_4	$C_{16}H_{14}O_2N_4S$	342.35	240	59	0.65	56.17	4.05	16.42
							56.13	4.12	16.36
4e	3-NO_2-C_6H_4	$C_{16}H_{14}O_3N_4S$	342.35	260	77	0.45	56.15	4.18	16.31
							56.13	4.12	16.36
4f	2,4-CH_3-C_6H_3	$C_{18}H_{19}ON_3S$	325.40	162	74	0.51	66.45	5.89	12.88
							66.43	5.88	12.91
4g	2,3- Cl-C_6H_3	$C_{16}H_{13}OCL_2N_3S$	366.24	169	83	0.70	52.51	3.63	11.42
							52.47	3.58	11.47
4h	2-CH_3-C_6H_4	$C_{17}H_{17}ON_3S$	311.38	166	78	0.66	63.61	5.55	13.53
							65.57	5.50	13.49
4i	3-Cl-4-F-C_6H_3	$C_{16}H_{13}OClFN_3S$	349.79	135	64	0.61	57.93	4.27	12.69
							57.91	4.25	12.66
4j	2-OCH_3-C_6H_4	$C_{17}H_{17}O_2N_3S$	327.38	110	68	0.55	62.38	5.27	12.87
							62.36	5.23	12.83

ANTI BACTERIAL ACTIVITY

The antibacterial activity of the title compounds 4 was examined against the bacteria *Bacillus cereus, Pseudomonas aeriginosa, Klebsiella pneumoniae, Micrococcus flavus* and *Citrobactor freundii* by ager well diffusion method. After 24 hours incubation at 37^0C the zone of inhibition was measured in mm.

TABLE 2
ANTIBACTERIAL SCREENING RESULTS OF COMPOUND 4

Compound	Inhibition Zone in mm				
	Gram positive (+)		Gram negative (-)		
	B. cereus	*M. flavus*	*K. pneumoniae*	*P. aeriginosa*	*C. freundii*
4a	0.4	0.0	0.0	0.0	0.0
4b	0.4	0.0	0.0	0.0	0.0
4c	0.4	0.0	0.4	0.	0.0
4d	0.1	0.0	0.0	0.0	0.4
4e	0.0	0.0	0.0	0.0	0.0
4f	1.2	0.0	0.45	1.4	0.7
4g	0.45	0.0	0.4	0.0	0.0
4h	0.45	0.0	0.4	0.0	0.0
4i	0.38	0.0	0.45	0.0	0.0
4j	0.45	0.0	0.25	0.6	0.0
Citrobactor	4.5	2.0	4.5	1.9	0.85

ANTI FUNGAL ACTIVITY

The antifungal activity of the title compounds 4 was examined against the bacteria *Candida tropicalis, Candida albicans, Cryptococcus neofomans, Trichospor onbeigelii*, and *Aspergillius flavus* by agar well diffusion method. After 24 hours incubation at 37^0C the zone of inhibition were measured in mm.

TABLE 3
ANTIFUNGAL SCREENING RESULTS OF COMPOUND 4

Compound	Inhibition Zone in mm				
	C. tropicalis	*C. albicans*	*C. neoformans*	*T. onbeigelii*	*A. flavus*
4a	1.25	1.5	3	1.5	1.5
4b	1	0.0	1.5	1	1
4c	1.15	1.5	1	1.15	0.90
4d	1.15	1	0.0	1.15	0.80
4e	1.15	0.0	2.5	0.0	0.0
4f	1.25	1.5	1.15	1.15	1.15
4g	1.15	0.0	1.5	1.25	1.25
4h	1	0.0	1.5	1.2	1.15
4i	0.0	1.25	0.0	1	1
4j	0.0	0.0	2.0	0.0	0.0
Fluconazole	4.5	3.5	2.2	1.8	2.1

Preparation of the plates and microbiological assays

A loopful of the given test strain was inoculated in 20ml of N-broth (Nutrient Broth). To activate the given bacterial strain, it was incubated for 24 hours in an incubator at 37^0C. The Agar well diffusion method is used for antibacterial assay. 28-30 ml of molten agar (Mueller Hinton Agar No. 2) was added into the 100 mm diameter Petri plate. Care should be taken to avoid air bubbles during inoculation and pouring. To maintain sterile

condition, all these procedures were done in the laminar air flow. The media was allowed to solidify. After solidification of the media, well was made in the plates with the help of cup-borer (0.85 cm) and then it was filled with the synthesized 4-aryl triazole derivatives solution (dissolved in DMSO). The antibacterial activities of these synthesized compounds were determined by the inhibition zone formed by these compounds against the particular test bacterial strain. Synthesized compound are screened for their antibacterial activity. However all the synthesized compound show less antibacterial activity than standard drug citrobactor. Among all the synthesized compound, compound 4f show equal or more activity against bacterial. Compound 4a show more activity against *C. neoformans* than standard drug fluconazole. Compound 4f give moderate activity against all fungicidal bacterial. 4e show more activity against *C. neoformans* Compound 4j give good activity against *C. neoformans*.

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