Synthesis and Antimicrobial Studies of Triazole Derivatives from Substituted Benzoic acid

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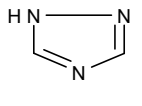
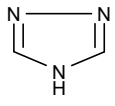
Abstracts- A large number of 1,2,4 triazole derivatives containing ring system have been of various variety of drug candidates including Anti-inflammatory ,Antifungal , Anti microbial ,Antibacterial ,Anti conversant Agents. The Rapid Developments of drug resistant, new agent should preferably have chemical characterization that clearly differs from those of existing agent. **2-(Dimethyl amino)-N-(3-mercapto-5-phenyl-4*H*-1,2,4-triazol-4-yl)-acetamide and its derivatives was perared by reacting aromatic esters (from synthesized aromatic acid)with hydrazine hydarate followed by reaction with alcoholic potassium hydroxide and carbon disulphide,the obtain potassium dithiocarbazinates were cyclized with hdrazine to 1.2.4 triazole and it furher reactd wuth chloroacetyl chlorids and the with amine.** The newly synthesized compounds were characterized by IR, NMR and Mass specra. The synthesized compounds were evaluzted for antibacterial nad anti fungal activity by ager diffusion method. All the compounds ***NJ-01,NJ-02*** and **NJ-03** at a concentration of *1000,500,250,125,,62.5 and 31.5 µg/ml*.and compound were screened for their antibactrail activity againest ***Staphylococus aureis , Bacillus sublilis*** (Gram postive bacteria) ***Escherichia coli, Psedomonas aeruginosa*** (Gram negative bacteria) by disk diffusin method. compounds show good antibacterial activity againest ***staphylococus aureus , Bacillus sublilis***. all compounds exhibited good antifunfgal activity againest ***candida albicans*** fungus.

Key words:-Triazole,Benzoic Acid, Antibacterial activity, Antifungal Activity and staphylococus aureus.

Introduction :- Five member aromatic ring with three atom are called 1,2,4 Triazole is also pyrodiazole.



1H -1,2,4-triazole



4H -1,2,4-triazole

S-Triazole or 1, 2, 4-Triazole

A large number of 1,2,4 Triazo a hetrocyclic derivative exhibit therapeutic activities such as antifungal[1] , anticonvulsant[2],antitubercular[3], antiinflamatory[4] and antimicribial[5] actiovity.

1, 2, 4 triazole ring has been incorporated is a wide variety of therapeutic interesting drug like ribavirin (antiviral agent) and fluconazole, itracanazole (antifungal agent) [6,] Thus there need to these compound for the developments of novel molecules with activity.

Various 1, 2, 4-triazole derivatives were synthesized by reacting aromatic acid esters with hydrazine hydrate followed by reaction with alcoholic potassium hydroxide and carbon disulphide. The obtained potassium dithiocarbazinates were cyclized with hydrazine to yield 1, 2, 4-triazole. And further reacted with chloro acetyl chloride and then with amines to give title compound.

Fungal and bacterial infection has been an important complication and major cause of mortality in immune compromised individual suffering from tuberculosis and cancer[07].

Other antifungal like azoles derivatives (fluconazole, an orally active triazole agent, and itraconazole), thiocarbamates are some agents actually working in patients with impaired resistance. While these new compounds are often used in treatment of fungal infections, resistance to these drugs is increasing; moreover many of currently available drugs have an undesirable side effect, which clearly indicates an urgent need for development of new antimicrobial agents [08, 09].

All the synthesized compounds were recrystallized and their purity was checked by performing thin layer chromatography. The structure of the compounds was confirmed on the basis of IR, NMR & ^{1}H NMR spectral data.

All newly synthesized triazole derivatives were screened for their activity against fungi ***Candila albicans*** and compound were evaluated for antibacterial activity against ***Staphylococus auraus , Bacillus sublilis*** (Gram postive bacteria) ***Escherichia coli, Psedomonas aeruginosa*** (Gram negative bacteria).

Experimeimental:-

In the present study some triazole derivatives have been synthesized and screened for their biological activity. The progress of reaction was monitored by thin layer chromatography

using plate coated silica gel G of 0.25 mm thickness. Eluents used were hexane and ethyl acetate (6:4) as a solvent system and iodine vapors was used as a detecting agent. Spot were visualized through iodine chamber. Solubility of newly synthesized triazole derivative was determined in various organic solvents at room temperature. Melting points of the synthesized compounds was determined by open capillary method and will be uncorrected.

Syntheses of compounds were carried out as per following scheme

Step-I: Synthesis of esters of aromatic acid

A mixture of substituted benzoic acid (0.3 mol), 130 mL of absolute alcohol and 3.3 mL of conc. H_2SO_4 was refluxed for 2 h on water bath. After completion of reaction, excess of ethanol was distilled off and content was transferred into separating funnel containing 310 mL distilled water. Carbon-tetrachloride (20 mL) was added, aqueous layer and ester layer were separated. Ester layer (lower layer) was taken in another separating funnel and shaked it with a strong solution of sodium bicarbonate until all free acid was removed and no further evolution of carbon dioxide occur. Washed once with water and dried by pouring into a small conical flask containing 7.5g magnesium sulphate. Cork the flask, shaked for 2 minutes then carbon tetrachloride was distilled off under reduced pressure. The resulting colourless liquid was collected and the completion of reaction was checked by TLC using hexane and ethyl acetate (6:4) and iodine vapour as a detecting reagent.

Step-II: Synthesis of hydrazide of synthesized ester

Produced aromatic esters (0.1 mol) and 80 % hydrazine hydrate (0.1 mol) was refluxed on a water bath for 15 min. Enough absolute ethanol was added to obtain a clear solution. Again contents were refluxed for 2 h. Excess alcohol was evaporated and solution was cool down**.** The solid obtained was separated and recrystallised from ethanol to get the needle shaped crystals.

Step-III: Synthesis of potassium dithiocarbazinate

Substituted aromatic hydrazides (0.02 mol), KOH (0.012 mol) and CS_2 (0.015 mol) in absolute ethanol (350 mL) were stirred for 10 h. After the completion of reaction

ether (200 mL) was added. The obtained precipitate was filtered, washed and dried. The synthesized dithiocarbazinate was used for the next step without further purification.

Step-IV: Synthesis of 5-aryl-4-amino-3-mercapto-1,2,4-triazole

Substituted produced dithocarbazinate (0.1 mol), hydrazine hydrate (0.3 mol) and water (30 mL) was refluxed for 3 h, H_2S was evolved during the reaction and clear solution resulted, enough cold water was added and cooled to 5^0c. Acidified the cooled solution with dil. HCl. Obtained precipitate was filtered, washed and recrystallized from 95% ethanol.

Step-V: Synthesis of 5-aryl-4-(chloroacetylamino)-3-mercapto-1,2,4- triazole:

In a two necked flask fitted with reflux condenser containing 100 mL benzene and obtained compound and separating funnel contained chloro acetyl chloride in 30 mL benzene. The mixture was refluxed and chloro acetyl chloride was added in small portions. After addition of chloro acetyl chloride, solution was again refluxed for 5-6 h, cooled and contents were poured on crushed ice. The obtained precipitate was filtered, washed and recrystallized from absolute ethanol.

Step-VI: Synthesis of amino derivative of 5-aryl-4-(chloroacetylamino) -3-mercapto-1,2,4-triazole:

Synthesized substituted 5-aryl-4-(chloroacetylamino)-3-mercapto-1, 2, 4- triazole (0.03 mol), respective amines (0.03 mol) and 75 mL benzene was taken in round bottom flask. The contents were refluxed for 5-6 h and cooled. Filtered the precipitate and washed with distilled water several times to remove traces of hydrochloride. Product obtained was recrystallized from appropriate solvent.

Result and Discussion :-

01. 2-(Dimethylamino)-N-(3-mercapto-5-phenyl-4*H*-1,2,4-triazol-4-yl)-acetamide (NJ-01)

IR (KBr,cm^{-1}):3310 (N-H stretching), 3116.0 (aromatic C-H stereching), 2962.6 (C-H streching of methyl group), 2928.8 & 2858.7 (C-H streching of methylene group),2584.5 (S-H streching),1660.6 (C=O streching), 1614.8(C=N streching), 1580.0 (C=C streching), 760.8 (C-H out of plane bending), 670.0 (C-S streching)

MS(m/s): M^+ calculated – 277

^{1}H-NMR (400 MHz,DMSO-d_6) δ 8.0(s, 1H, -NH), 7.2-7.6(m, 5H, -C_6H_5), 3.25(s, 2H, -CH_2), 3.1(s, 1H, -SH), 2.3(s, 6H, -CH_3)

02. 2-(Dimethyl amino)-N-(3-mercapto-5-p-tolyl-4*H*-1, 2, 4-triazol-4-yl)-acetamide

IR (KBr,cm^{-1}):3226.6(N-H stretching),3106.0 (aromatic C-H stereching),2958.0 (C-H streching of methyl group), 2928.8 & 2848.9 (C-H streching of methylene group),2584.5 (S-H streching),1662.6 (C=O streching),1611.4 (C=N streching), 787.8 (C-H out of plane bending)

MS(m/s): M^+ calculated – 291

^{1}H-NMR (400 MHz,DMSO-d_6) δ 8.0(s, 1H, -NH), 7.0-7.2(m, 4H, -C_6H_4), 3.25(s, 2H, -CH_2), 3.1(s, 1H, -SH), 2.3(s, 3H, -CH_3), 2.1 (s,3H,-CH_3)

03. 2-(Dimethylamino)-N-(3-mercapto-5-(4-methoxyphenyl)-4*H*-1,2,4-triazol-4-yl)-acetamide

IR (KBr,cm^{-1}):3310 (N-H stretching), 2952.6 (C-H streching of methyl group), 2928.8 & 2858.7 (C-H streching of methylene group),2584.5 (S-H streching),1660.6 (C=O streching),1614.8(C=N streching),1585.0 (C=C streching), 773.4 (C-H out of plane bending), 614.5 (C-S streching)

MS(m/s): M^+ calculated – 277

^{1}H-NMR (400 MHz,DMSO-d_6) δ 8.0(s, 1H, -NH), 6.7-6.9(m, 5H, -C_6H_4), 3.7(s, 3H, -OCH_3), 3.25(s, 2H, -CH_2), 3.1(s, 1H, -SH), 2.3 (s,3H,-CH_3)

In vitro antimicrobial studies:-

The synthesized compounds were subjected to anti microbial screening by cup plate method for zone of inhibition. The antibacterial activity was tested against various gram positive and gram negative bacteria and antifungal activity against various fungal stains compared with (Ampicillin and Griseofulvin) using solvent control. The results were described in Table No. 02 and 03.

The synthesized compounds were subjected to biological evaluations. The compounds were evaluated for antibacterial and antifungal activities. The activity studies suggest that

novel 1, 2, 4 Triazole derivatives compound had showed moderate antibacterial and antifungal activity.

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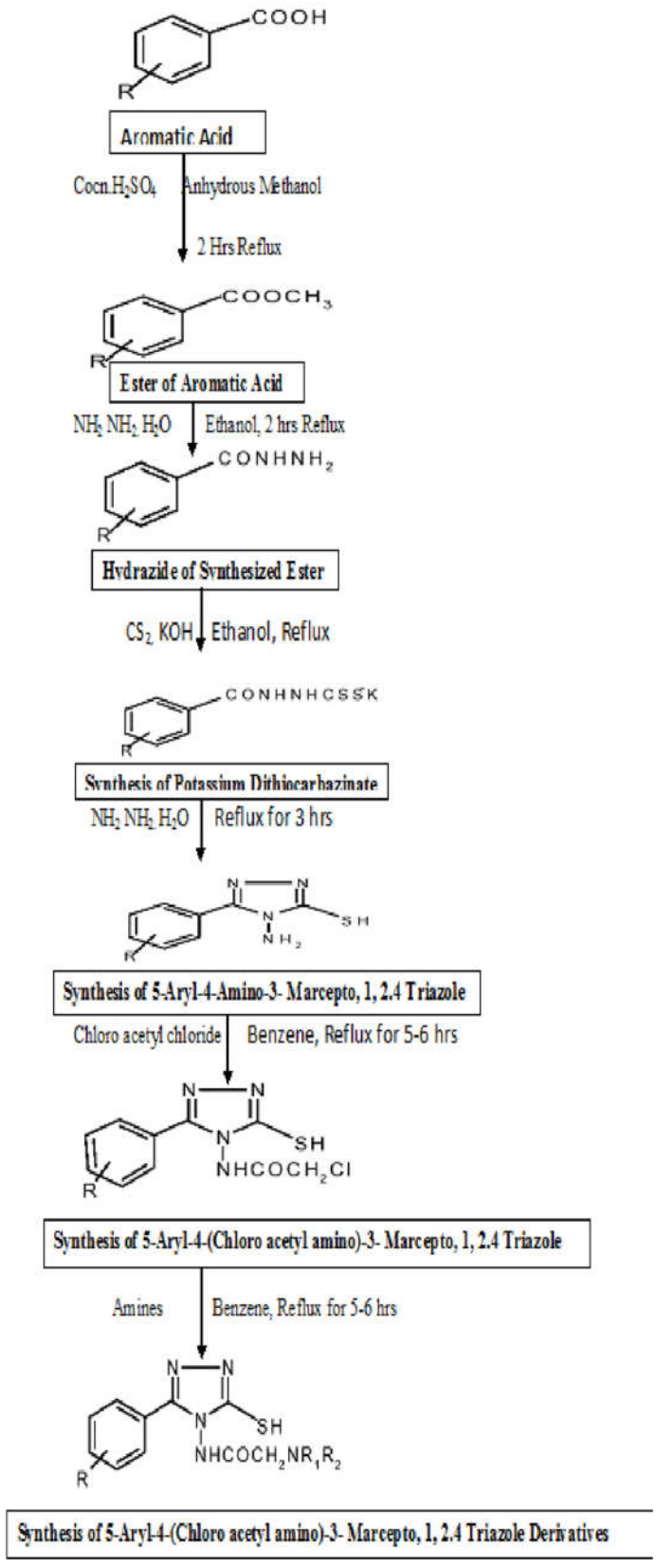

S.No.	Compounds	R	R_1 & R_2
01.	NJ-01	-H	Methyl Amine
02.	NJ-02	$-CH_3$	Methyl Amine
03.	NJ-03	$-OCH_3$	Methyl Amine

Table 1: Physical parameters of triazole derivatives synthesized compounds

S.No.	S. No.	R	Mol. Form.	Rf value	Molecular weight	% Yield	M.P. (°C)
01.	NJ-01	H	$C_{12}H_{15}O_1N_5S_1$	0.56	277	56%	170-172
02.	NJ-02	CH_3	$C_{13}H_{18}O_1N_5S_1$	0.48	291	48%	158-160
03.	NJ-03	OCH_3	$C_{13}H_{18}O_2N_5S_1$	0.59	307	55%	212-214

Table 2: Data of antimicrobial activity of synthesized 1,2,4-Triazole Derivatives

S. No.	Compound	Diameter of zone of inhibition (mm)				
		B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***	***C. albicans***
01.	NJ-01	14	16	15	14	13
02.	NJ-02	15	18	16	15	14
03.	NJ-03	16	19	17	15	15
Ampicillin		16	20	18	15	-
Griseofulvin		-		-	-	16

Table 3: Data of antibacterial and antifungal activity of synthesized 1, 2, 4-Triazole Derivatives

S. No.	Comp.	*B. subtilis*				*S. aureus*				*E. coli*				*P. aeruginosa*				*C. albicans*			
Dilution		I	II	III	IV-V	I	II	III	IV-V	I	II	III	IV-V	I	II	III	IV-V	I	II	III	IV-V
1	NJ-01	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+
5	NJ-02	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+
9	NJ-03	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+
Ampicillin		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
Griseofulvin																		-	-	-	-

I-1000μg/ml, II-500μg/ml, III-250μg/ml, IV-125μg/ml, V-62.5μg/ml

(-) indicates absence of growth; (+) indicates presence of growth

Fig I- The bacterial screening indicated that among the compound no. the compounds NJ-02 and NJ-03 moderately activity against all tested bacterial stain *staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa.*

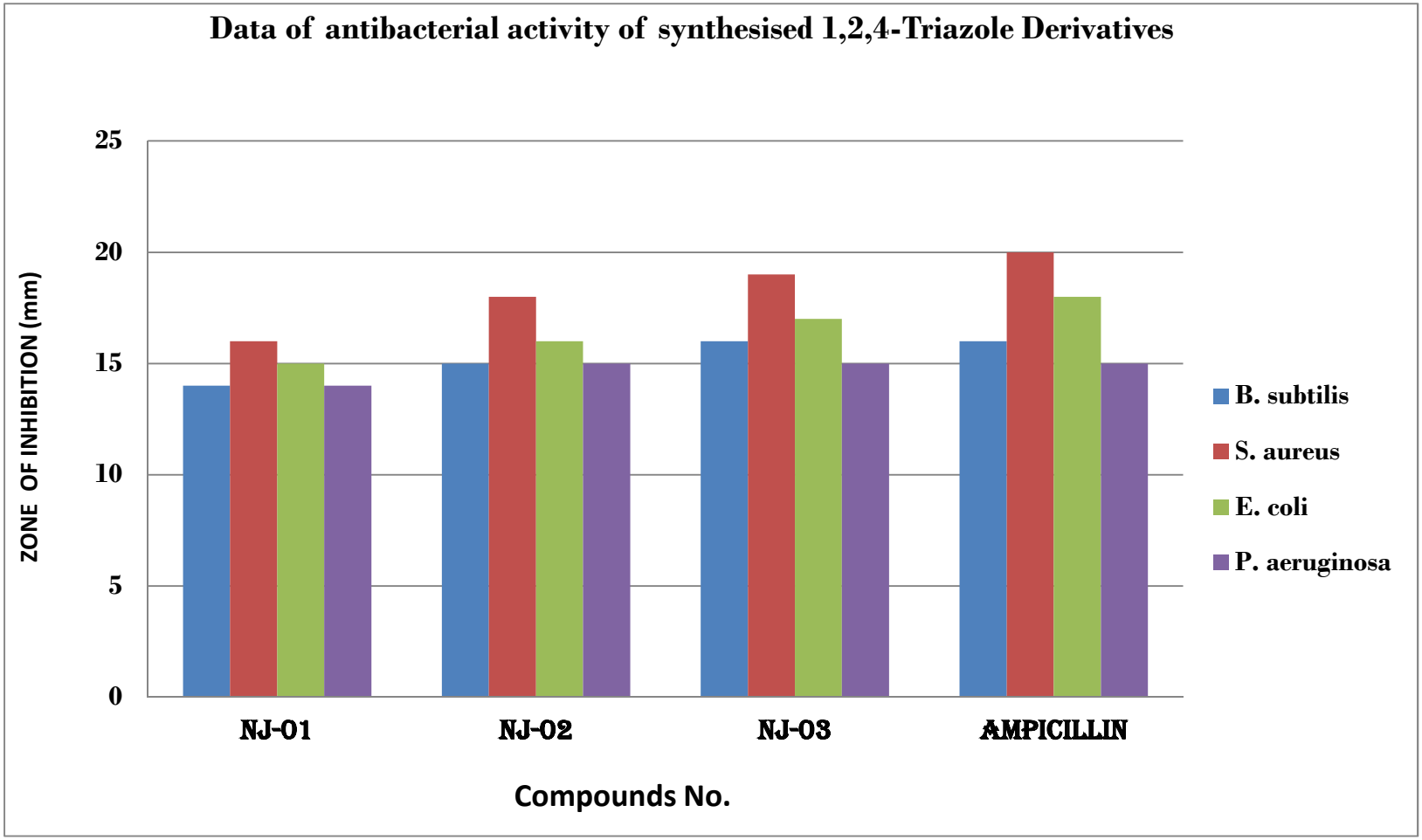

Fig.II -Antifungal screening indicated that among the compound NJ-03 reveled that the test compounds showed moderate activity against *Candida albicans*.