Antimicrobial Evaluation and Comparative Study of Some 3,5 Di- Substituted Pyrazoles with Bromo Substituted Pyrazoles

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***Abstract*:** New substituted pyrazoles were prepared from 2 hydroxy-3,5-dichloro acetophenone in ethanol and aromatic aldehyde as starting materials through1,3-diketones as intermediates. These intermediates on reaction with hydrazines in alkaline media, finally converted into corresponding pyrazoles. The synthesized compounds were characterized by their physical properties, IR and NMR spectroscopic studies. The antimicrobial activity of synthesized pyrazoles was assessed by agar cup method and filter paper disc method. Purity of these heterocycles was checked by TLC. These compounds were tested for antimicrobial activity against pathogenic bacteria i.e; Staphylococeus aureus, Klebsiella pneumoniae, Salmonella typhi, Proteus vulgaris, Shigella flexueri, Escherichia coli & Pseudomonas aerugivosa. & MIC are found to have remarkable activity.
In the most cases pyrazole having bromo substitution on the styryl ring was found to be more efficient than the remaining against some microorganisms.
Keywords: *Pyrazoles, Phenyl hydrazine, 1,3-diketone, antibacterial activity, anti- fungal activity, some microorganisms.*

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I. Introduction:

Pyrazoles are five member ring heterocyclic compounds, have some structural features with two nitrogen atoms in adjacent position and are also called as Azoles [1]. Recently Pyrazole derivatives have been found in nature [1], β-[1-pyrazolyl]alanine was isolated from the seeds of water melons [Citurllus lanatus]. The best described property of almost every group of pyrazoles is in the treatment of inflammation and inflammation associated disorders, such as arthritis [2]. Pyrazole derivatives are the subject of many research studies due to their widespread potential biological activities such as antimicrobial[3], antiviral[4], antitumor[5,6], antihistaminic[7], antidepressant[8], insecticides[9] and fungicides[9].
Several pyrazole derivatives have been found to possess significant activities such as 5-α-red-uctase inhibitor[10], antiproliferative[11], antiparasitic[12], herbicides[13]. A good number of pyrazoles have also been reported to have interesting biological activities like anti-inflammatory[14] and antiprotozoal[15-16] which render them valuable active ingredients of medicine and plant protecting agents. Further, current literature indicates 1,2-pyrazole derivatives to possess various biological activities [17].

Substituted pyrazole and its analogs have been used as precursors for synthesis of various biologically active molecules. In the recent years, the efficiency of microwave chemistry in dramatically reducing reaction times has recently been prove in several different fields of organic chemistry [18], microwave assisted organic synthesis has shown significant improvement in the generation of combinatorial libraries of small molecules [19].
Taking into consideration the important biological activities of pyrazoles,

II. MATERIALS AND METHODS

2.1 Materials and physical measurements:

Melting points were measured by a Stuart Scientific melting point apparatus in open capillaries and are uncorrected. Infrared spectra (KBr discs) were recorded on a Bruker Alpha (FTIR) Spectrometer. 1H-NMR spectra were recorded on a Bruker spectrometer operating at 400 MHz using DMSO-d6 and $CDCl_3$ as a solvent with TMS as an internal standard. Elemental analysis was performed using a (EURO EA 3000 instrument). Acme silica was used for analytical TLC. All other analytical grade chemicals and solvents were obtained from commercial sources and used as received standard procedure.
Antimicrobial activity of the compounds was assayed by cup plate agar diffusion method [20]. The titled compounds were tested against pathogenic bacteria for their antibacterial activity by paper disk method [21]. The organisms tested were Staphylococeus aureus., Klebsiella pneumoniae , Salmonella typhi , Proteus vulgaris , Shigella flexueri , Escherichia coli & Pseudomonas aerugivosa . The solution of these compounds were prepared in DMSO as a solvent at a concentration of 50µ /ml. The culture medium used was nutrient agar. After 24 hours of inhibition at 37^0C, the zones of inhibition were measured in mm.

2.2 Preparation of new substituted pyrazoles:

A) General procedure for 1,3,5-trisubstituted pyrazoles:
A Mixture of 3-iodoflavanone, nucleophile such as Isonicotinic acid hydrazide, semicarbazide and thiosemicarbazide in pyridine (40ml) was refluxed for 5 hours . The reaction mixture was diluted by 1:1 HCl. The product obtained was crystallized from ethanol-acetic acid mixture to get pale yellow product.

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Physical data of 1,3,5-trisubstituted pyrazoles [3/4/5(a-e)]:

Compound	R_1	R_2	R_3	Yield (%)	m.p. (^{0}C)	Rf value	Molecular Formula
3a	H	OCH_3	$CONC_5H_4$	75	212	0.62	$C_{22}H_{15}O_3Cl_2N_3$
3b	H	H	$CONC_5H_4$	70	114	0.74	$C_{21}H_{13}O_2Cl_2N_3$
3c	Cl	H	$CONC_5H_4$	70	180	0.7	$C_{21}H_{12}O_2Cl_3N_3$
3d	NO_2	H	$CONC_5H_4$	72	218-222	0.81	$C_{21}H_{12}O_4Cl_2N_4$
3e	H	OH	$CONC_5H_4$	76	264	0.66	$C_{21}H_{13}O_3Cl_2N_3$
4a	H	OCH_3	$CONH_2$	75	210	0.55	$C_{17}H_{13}O_3Cl_2N_3$
4b	H	H	$CONH_2$	76	230	0.69	$C_{16}H_{11}O_2Cl_2N_3$
4c	Cl	H	$CONH_2$	70	236	0.51	$C_{16}H_{10}O_2Cl_3N_3$
4d	NO_2	H	$CONH_2$	75	234	0.90	$C_{16}H_{10}O_3Cl_2N_4$
4e	H	OH	$CONH_2$	75	250	0.67	$C_{16}H_{11}O_3Cl_2N_3$
5a	H	OCH_3	$CSNH_2$	70	220	0.52	$C_{16}H_{11}O_2Cl_2N_3S$
5b	H	H	$CSNH_2$	76	212	0.69	$C_{16}H_{11}O_2Cl_2N_3$ S
5c	Cl	H	$CSNH_2$	70	198	0.52	$C_{16}H_{10}OCl_3N_3$ S
5d	NO_2	H	$CSNH_2$	76	165	0.69	$C_{16}H_{10}O_3Cl_2N_4S$
5e	H	OH	$CSNH_2$	76	240	0.69	$C_{16}H_{11}O_2Cl_2N_3$

Spectral interpretation:

3a) IR (ν max, cm^{-1}):
3349(-OH Stretch), 2921(-C-H Stretch), 1602.1(C=N Stretch), 736(C-Cl Stretch)

NMR ($CDCl_3$+DMSO) (δ ppm):
3.7(S, 3H,-OCH_3), 3.47-3.62(dd,1Ha,-CH), 3.7-3.9(dd, 1Hb,-CH), 6.7-7.99(m, 6H,Ar-H)

4a) IR (ν max, cm^{-1}):
3386(-OH Stretch), 2924(-C-H Stretch), 1602.1(C=N Stretch), 727(C-Cl Stretch)

NMR ($CDCl_3$+DMSO) (δ ppm):
2.17(S, 3H, -OCH_3), 6.78(S, 2H, -NH_2), 6.83-8.03(m, 6H,Ar-H)

5a) IR (ν max, cm^{-1}):
3382(-OH Stretch), 2920.8(-C-H Stretch), 1602.4(C=N Stretch), 754.8(C-Cl Stretch), 1265.9(C-N Stretch)

NMR ($CDCl_3$+DMSO) (δ ppm):
2.60(S, 3H, -OCH_3), 6.7 (S, 2H, -NH_2), 6.9-7.9 (m, 6H,Ar-H)

2.2 B General procedure for 3, 5 – diaryl -1-substituted -4-bromo pyrazoles:

1-(2- hydroxy -3, 5-dichloro phenyl) -3-aryl-2-bromo propan -1, 3-diones was dissolved in ethanol & nucleophile such as isonicotinic acid hydrazide, semicarbazide, thiosemicarbazide was added to it. The reaction mixture was refluxed for about 2.5 hours in basic medium. It was cooled & poured in to water. The product was filtered, washed with water and crystallized from ethanol to obtained pale yellowish crystals of 3,5 - diaryl -1-substituted -4-bromo pyrazoles.

Physical data of 3, 5 – diaryl -1-substituted -4-bromo pyrazoles 6,7,8 (a-d):

Comp.	R_1	R_2	R_3	Yields %	M.P.^{0}C	Rf	Molecular formula
6a	H	H	C_5H_4NCO	75	208	0.95	$C_{21}H_{12}O_2Cl_2BrN_3$
6b	H	OCH_3	C5H4NCO	70	150	0.80	$C_{22}H_{14}O_3Cl_2BrN_3$
6c	Cl	H	C_5H_4NCO	70	280	0.89	$C_{21}H_{11}O_2Cl_3BrN_3$
6d	H	NO_2	C_5H_4NCO	60	210	0.92	$C_{21}H_{11}O_4Cl_2BrN_4$
7a	H	H	$CONH_2$	65	178	0.92	$C_{16}H_{10}O_2Cl_2BrN_3$
7b	H	OCH_3	$CONH_2$	70	172	0.88	$C_{17}H_{12}O_3Cl_2BrN_3$
7c	Cl	H	$CONH_2$	75	132	0.88	$C_{16}H_{10}O_2Cl_3BrN_3$
7d	H	NO_2	$CONH_2$	70	198	0.88	$C_{16}H_9O_4Cl_2BrN_4$
8a	H	H	$CSNH_2$	70	182	0.88	$C_{16}H_{10}OCl_2BrN_3S$
8b	H	OCH_3	$CSNH_2$	65	160	0.92	$C_{16}H_{10}O_2Cl_2BrN_3S$
8c	Cl	H	$CSNH_2$	70	228	0.86	$C_{16}H_9OCl_3BrN_3S$
8d	H	NO_2	$CSNH_2$	75	125	0.95	$C_{16}H_9O_3Cl_2BrN_4S$

Spectral Interpretation :

6a) IR (ν max cm^{-1}):

31264.7(-OH Stretch), 1657.2(C=O Stretch), 1613.4(C=N stretch), 682,716 (C-Cl Stretch).543.6(-C-Br Stretch)

NMR (CDCl $_3$+DMSO) (δ ppm)

1.25 (S, 1H,-OH), 6.87-8.77 (m, 11H, Ar –H)

7a) IR (ν max cm^{-1}):

3422.8(-OH Stretch) ,1664.5(C=O Stretch), 1594.9(C=N stretch),766,854 (C-Cl Stretch), 563.4(-C-Br Stretch), 1292(C-N Stretch)

NMR (CDCl $_3$+DMSO) (δ ppm)

8.39 (S, 1H,-OH), 8.02(S, 2H,-NH2), 6.87-8.00(m, 7 H, Ar –H)

III. ANTIMICROBIAL ACTIVITY:

Preparation of Inoculum:

The gram positive (Staphylococcus aureus and Klebsiella pneumoniae) and gram negative bacteria (Salmonella typhi, Proteus vulgaris, Proteus vulgaris, Shigella flexueri and Escherichia coli) were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A nm). The fungal inoculums (Aspergillus niger and A. flavus) was prepared from 5 to 10 day old culture grown on Potato dextrose agar medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A nm) to obtain a final concentration of approximately 105 spores/ml.

3.1. Anti-bacterial activity:

Determination by Agar cup method:

The antibacterial activity of pyrazole derivatives was studied by agar cup method [22, 23]. Glass Petri dishes used were sterilized and Nutrient broth was used as basal medium for testing bacteria. The Nutrient broth medium was prepared by taking Beet extract (1 gm/lit), Yeast extract (2 gm/lit), peptone (5.0 gm/it), NaCl (5 gm/lit) Agar (15gm/lit) and with pH (7.0), and plated into Petri dishes, allowed to solidification. The selected Bacterial culture, single colony was inoculated in to broth medium and kept for incubation for overnight at 25 0C. The overnight Bacterial culture was spread evenly over the entire surface and left undisturbed for few minutes to percolate the culture. Wells (4 mm) were created using a sterile borer into the solidified agar medium. The selected compounds were added to each well (100 & 50 µL) at peripheral and the reference compound (Chloramphenacol) was added at the centre. Thus the prepared plates were incubated at room temperature (at about 25^{0}C) for about 3-5 days. After incubation period the plates were collected and record the inhibition zone in mm

(from the margin of the well to surface of inhibition).Dimethyl sulphoxide (DMSO) was used as solvent to prepare the stock solutions (5 mg in 0.5ml) of the compounds initially and also to maintain proper control.

3.2. Antifungal Activity:

Determination by filter paper disc method:

The antifungal activity was tested by disc diffusion method [24, 25]. The potato dextrose agar was used as basal medium for testing fungi. The potato dextrose agar medium was prepared by taking yeast extract (3 gm/lit), peptone (10 gm/it), Dextrose (20 gm/lit) Agar (15 gm/lit) distilled water (1 lit) and with pH (6.0), and plated into Petri dishes, allowed to solidification. The potato dextrose agar plates were inoculated with each fungal culture (10 days in old) by point inoculation. The filter paper discs (5 mm in diameter) impregnated with 100 µl and 50 µl concentrations of the extracts were placed on test organism-seeded plates. DMSO was used to dissolve the tested compounds and was completely evaporated before application on test organism-seeded plates. Blank disc impregnated with solvent DMSO followed by drying off was used as negative control and Nystatin (10µg) used as positive control. The activity was determined after 72 hrs of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

Antimicrobial activity of 1,3,5-trisubstituted pyrazoles [3/4/5(a-e)]:

(Inhibition zone measured in mm)

Microorganisms	**3a**	**3b**	**3c**	**3d**	**3e**	**4a**	**4b**	**4c**	**4d**	**4e**	**5a**	**5b**	**5c**	**5d**	**5e**
S. aureus	17	13	16	20	20	16	12	13	12	12	12	18	15	13	12
K. pneumone	20	14	15	15	14	19	16	12	13	15	15	14	13	11	14
S. typhi	16	13	13	11	13	16	13	11	12	13	13	13	11	13	18
P. vulgaris	15	16	17	15	14	20	12	12	14	22	17	12	15	16	17
S. flexueri	16	17	15	11	16	12	15	11	15	18	14	18	11	15	12
E. colli	17	17	18	16	15	15	14	12	18	14	14	18	16	13	14
P. aerugivosa	21	16	17	18	17	15	15	11	12	15	17	22	16	12	16

Minimum inhibitory concentration of 1, 3, 5-trisubstituted pyrazoles [3/4/5(a-e)]:

(MIC value in µg/ml)

Comp	S. aureus	K. pneumone	S.typhi	P.vulgaris	S. flexueri	E. colli	P. aerugivosa
3a	150	150	200	200	150	150	200
3b	600	600	600	600	400	600	800
3c	300	450	600	450	300	450	450
3d	50	50	70.5	70.5	70.5	50	70.5
3e	200	400	200	400	300	300	300
4a	300	400	300	300	200	300	300
4b	300	300	400	400	300	300	200
4c	400	400	400	800	400	400	400
4d	100	100	100	200	100	100	150
4e	400	600	400	800	600	400	600
5a	300	300	400	400	200	300	300
5b	600	600	600	600	400	600	600
5c	600	400	400	800	400	400	600
5d	200	300	200	400	200	200	300
5e	400	800	600	600	600	600	800

Cl	25 (15)	28 (7.5)	25 (15)	32 (4)	25 (15.5)	25 (12.5)	28 (7.5)

Cl-Chloramphenacol

Antimicrobial activity of 3,5–diaryl-1-substituted-4-bromo pyrazoles 6, 7, 8(a-d)

Microorganisms	6a	6b	6c	6d	7a	7b	7c	7d	8a	8b	8c	8d
S.aureus	23	14	22	15	12	24	18	17	20	22	16	18
K. pneumoniae	23	12	25	22	21	14	14	23	21	14	22	15
S. typhi	14	17	16	24	14	18	20	21	17	18	15	16
P. vulgaris	19	18	17	20	15	23	14	15	16	15	16	19
S. flexueri	16	17	12	13	12	15	21	12	15	21	13	20
E. colli	21	13	18	15	17	14	21	13	17	16	21	12
P. aerugivosa	13	22	20	22	15	24	21	22	24	22	11	13

Highly active 20-30, moderately active 15-20, weakly active 11-15, less than 11 inactive

Minimum inhibitory concentration of 3, 5 – diaryl -1-substituted -4-bromo pyrazoles 6,7,8 (a-d) (MIC value in µ g/ml)

Comp	S. aureus	K.pneumone	S. typhi	P. vulgaris	S. flexueri	E. colli	P. aerugivosa
6a	150	150	200	100	150	100	150
6b	150	100	150	150	100	100	150
6c	600	600	600	600	400	400	600
6d	400	800	600	600	400	400	600
7a	150	150	100	150	100	100	200
7b	150	100	150	200	150	100	150
7c	400	800	800	600	400	400	600
7d	400	600	600	600	400	600	400
8a	150	150	150	150	200	150	200
8b	200	200	150	250	150	100	1500
8c	400	600	600	800	600	600	400
8d	400	800	600	600	400	600	600

Cl	25 (15)	28 (7.5)	25 (15)	32 (4)	25 (15.5)	25 (12.5)	28 (7.5)

Cl-Chloramphenacol

IV. RESULT & DISCUSSION:

S. aureus shows MIC value 50 µg/ml against 3d. 100 µg/ml against 4d. 150 µg/ml against 3a, 6a,6b,7a,7b,8a. 200 µg/ml against 3e,5d. 300 µg/ml against 4a,4b and 5a. 400 □ g/ml against 6d,7c,7d,8c,8d and 600 µg/ml against 3b,5b,5c and 6c.

K. pneumone shows MIC value 50 µg/ml against 3d. 100 µg/ml against 4d, 6b and 7b. 150 µg/ml against 3a, 6a,7a and 8a. 200 □ g/ml against 8b.300 µg/ml against 4b,5a,5d. 400 µg/ml against 3e,4a,4c,5c. 450 µg/ml against 3c. 600 µg/ml against 3b,4e,5b, 6c,7d,8c. and 800 µg/ml against 5e, 6d,7c,8d.

S. typhi shows MIC value 70.5 µg/ml against 3d. 100 µg/ml against 4d, 6b,7b,8a,8b. 200 µg/ml against 3a,3e,5d and 6a. 300 µg/ml against 4a. 400 µg/ml against 4b,4c,4e,5a,5c. 600 µg/ml against 3b,3c,5b,5e. us

P.vulgaris shows MIC value 70.5 µg/ml against 3d. 150 □ g/ml against 6b,7a,8a200 µg/ml against 3a,4d and 8b. 300 µg/ml against 4a. 400 µg/ml against 3e,4b, 5a,5d. 450 µg/ml against 3c. 600 µg/ml against 3b,5b,5e, 6c,6d,7c,7d and 8d. 800 µg/ml against 4c,4e,5c and 8c.

S. flexueri shows MIC value 70.5 µg/ml against 3d. 100 µg/ml against 4d and 6b. 150 µg/ml against 3a,6a,7b,8b. 200 µg/ml against 4a,5a,5d and 8a. 300 µg/ml against 3c,3e,4b. 400 µg/ml against 3b,4c,5b,5c, 6c,6d,7c,7d,8d. and 600 µg/ml against 4e,5e and 8c.

E. colli shows MIC value 50 µg/ml against 3d. 100 µg/ml against 4d, 6a,6b,7a.7b,8b. 150 µg/ml against 3a and 8a. 200 µg/ml against 5d. 300 µg/ml against 3e,4a,4b,5a. 400 µg/ml against 4b,4e,5c, 6d,7c. 450 µg/ml against 3c. and 600 µg/ml against 3b,5b,5e, 7d,8c,8d.

P. aerugivosa shows MIC value 70.5 µg/ml against 3d. 150 µg/ml against 4d, 6a,6b,7b,8b.. 200 µg/ml against 3a,4b, 5a,6a. 400 µg/ml against 4c, 5d,6c. 450 µg/ml against 3c. and 600 µg/ml against 5b,5c,4e, 4c,4d,5c,6d. 800 µg/ml against 3b,5e.

V. CONCLUSION:

The compounds were found to be active in MIC range 50-600 µg/ml for gram positive bacteria and 200-800 µg/ml for gram negative bacteria. Increasing MIC value have been observed with increasing zone of inhibition. The compound contains Hydroxy and Methoxy groups together was found to be fourfold more active than without hydroxy and methoxy groups compounds. In the most cases pyrazole having bromo substitution on the styryl ring was found to be more efficient than the remaining against some microorganisms.

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