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SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF NOVEL PYRAZOLONE DERIVATIVES

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ABSTRACT

*Mannich reaction of various ethyl-2-substituted phenyl hydrazono-3-oxobutyrates **(2a-h)** with furan-2-carbohydrazide **(3)** afforded 1-(furan-2-carbonyl)-3-methyl-4-(2-phenyl hydrazono)-1H-pyrazol-5(4H)-one **(4a-h)**. The structures of all these compounds **(4a-h)** were recognized on the basis of analytical and spectral studies. The newly synthesized compounds were evaluated for their antimicrobial activity against various bacteria and fungi.*

Keywords: pyrazole, furan-2-carbohydrazide, antimicrobial activity, spectral studies.

INTRODUCTION

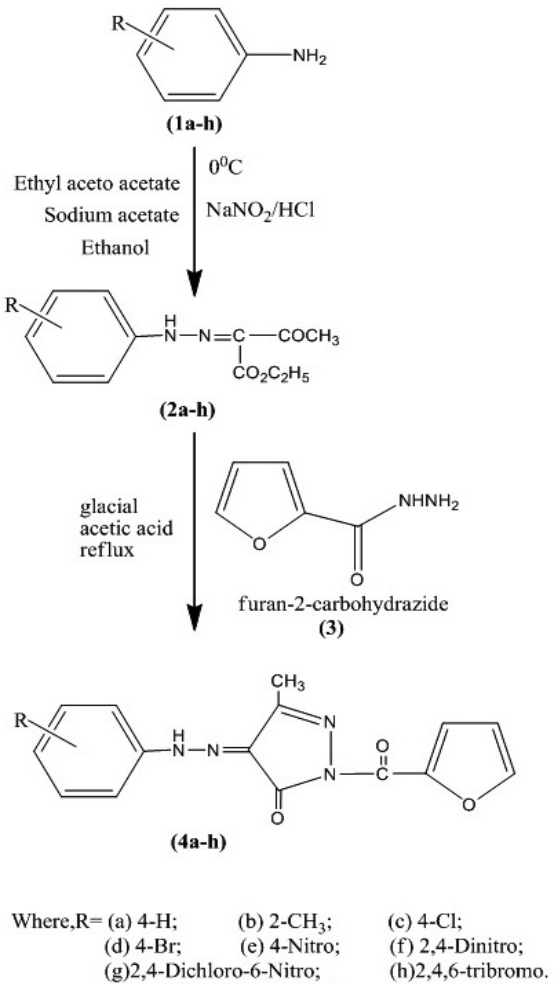
The arylazopyrazoles are generally prepared by combination of aryl-azo-ethyl actoacetate derivatives and hydrazine derivatives [1-6]. Hydrazide and their heterocyclic products exhibit miscellaneous biological activities including antibacterial, antifungicidal, analgesic, antituberculosis, anticancer, antiinflammatory properties [7-21]. The hydrazide containing heterocyclic compounds are widely used in medicine, agriculture and industry. Also pyrazole and its derivatives are particularly interesting because of their potential application in medicinal chemistry as antiperdiferative [22], antiinflammatory [23, 24], antipyretic [24], anticancer [25], antioxidant [26], antifungal [27] and enzyme inhibitory agents [28]. Hence, it was thought of interest to merge both of arylazopyrazole and hydrazide moieties which may enhance the drug activity of compounds to some extent or they might possess some of the above mentioned biological activities. From this point of view, the objective of the present work is to prepare new derivatives of furan carbonylhydrazide containing arylazopyrazole moiety. Hence the present communication comprises the synthesis of 1-(furan-2-carbonyl)-3-methyl-4-(2-phenyl hydrazono)-1H-pyrazol-5(4H)-one **(4a-h)**. The synthetic approach is shown in Scheme 1.

EXPERIMENTAL

Melting points were determined in open capillary tubes and were uncorrected. The IR spectra were recorded in KBr pellets on a Nicolet 400D spectro-meter. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO with TMS as internal standard on a Bruker spectrometer at 400 MHz and 100 MHz, respectively. LC-MS of selected samples were taken on LC-MSD-Trap-SL_01046. Purity of compounds was checked by TLC on silica gel plates and the spots were visualized by UV lamp.

Synthesis of ethyl-2-substituted phenyl hydrazono-3-oxobutyrates (2a-h)

Substituted aniline **(1a-h)** (0.01mole) was dissolved in a mixture of HCl (8 ml) and water (6 ml) and cooled to 0°C in ice bath. A cold aqueous solution of sodium nitrate (0.03 mole) was added to it. The diazonium salt solution was filtered into a cooled solution of



Scheme 1.

ethyl acetoacetate (0.01 mole) and sodium acetate (0.12 mole) in ethanol (50 ml).The resulting solid was washed with water and recrystallized from EtOH/MeOH. IR (KBr, cm^{-1}): 3355-3360 (N-H), 3030-3080 (C-H of Ar.), 2815-2850 (-OCH_2), 2950, 1370 (-CH_3, CH_2), 1620-1640 (C=N), 1695 (C=O ketone),1725 (C=O ester). ^{1}H NMR (400MHz, DMSO-d_6, δ/*ppm*): 1.25 (t, 3H, CH_3), 2.35 (s, 3H, $COCH_3$), 4.29 (q, 2H, $COCH_2$),11.62 (s, 1H, NH); **(2a)**: 6.89-7.37 (s, 5H, ArH); **(2b)**: 2.36 (s, 3H, CH_3), 6.74-7.19 (s, 4H, ArH); **(2c)**:7.11-7.26 (s, 4H, ArH); **(2d)**: 6.56-7.38 (s, 4H, ArH); **(2e)**: 7.24-8.08 (s, 4H, ArH); **(2f)**: 8.01-8.92 (s, 3H, ArH); **(2g)**: 8.04-8.20 (s, 2H, ArH); **(2h)**: 7.01-7.08 (s, 2H, ArH). ^{13}C –NMR (100MHz, DMSO, δ/*ppm*):14.2 (CH_3), 62.6 (OCH_2), 27.1 (CH_3), 163.5 (C=O ester),196.4 (C=O), 126.9 (C=N); **(2a)**: 114.6-143.7 (Ar-C); **(2b)**: 17.9 (CH_3), 113.4-142.1 (Ar-C); **(2c)**: 118.2 -130.4 (Ar-C); **(2d)**: 116.9-142.5 (Ar-C); **(2e)**: 113.7-149.5 (Ar-C); **(2f)**: 116.9-139.1 (Ar-C); **(2g):** 125.1-140.8 (Ar-C); **(2h)**: 109.8-152.7 (Ar-C). The yields, melting points and other characterization data of these compounds are given in Table 1.

Synthesis of 1-(furan-2-carbonyl)-3-methyl-4-(2-phenylhydrazono)-1H-pyrazol-5(4H)-ones (4a-h)

To ethyl-2-substituted phenyl hydrazono-3-oxobutyrate **(2a-h)** (0.002 mole) dissolved in glacial acetic acid (20 ml), a solution of furan-2-carbohydrazide **(3)** (0.002 mole) in 25 ml of glacial acetic acid was added and the mixture was refluxed 10-12 h. It was

Table 1. Physical and Analytical Data of the Compounds Synthesized (**2a-h**).

Compound No.	R	Molecular Formula (Mol.mass.)	LC-MS Data	M.P.* °C	Yield %	Elemental Analysis		
						C%	H%	N%
						Calcd. (Found)	Calcd. (Found)	Calcd. (Found)
2a	H	$C_{12}H_{14}N_2O_3$ (228)	245	94-96	72	63.15 (63.1)	6.14 (6.1)	12.28 (12.2)
2b	2-Me	$C_{13}H_{16}N_2O_3$ (242)	260	98-99	80	64.46 (64.4)	6.61 (6.6)	11.57 (11.4)
2c	4-Cl	$C_{12}H_{13}N_2O_3Cl$ (268.5)	281	112-115	76	53.63 (53.6)	4.84 (4.8)	10.42 (10.3)
2d	4-Br	$C_{12}H_{13}N_2O_3Br$ (313)	324	114-117	82	46.00 (45.9)	4.15 (4.1)	8.94 (8.8)
2e	4-NO_2	$C_{12}H_{13}N_3O_5$ (279)	297	82-84	65	51.61 (51.5)	4.65 (4.6)	15.05 (14.9)
2f	2,4-Dinitro	$C_{12}H_{12}N_4O_7$ (324)	339	118-120	66	44.44 (44.4)	3.70 (3.6)	17.28 (17.2)
2g	2,4-Dichloro-6-Nitro	$C_{12}H_{11}N_3O_5Cl_2$ (348)	364	126-128	77	41.37 (41.3)	3.16 (3.1)	12.06 (11.9)
2h	2,4,6-Tribromo	$C_{12}H_{11}N_2O_3Br_3$ (471)	492	122-125	84	30.57 (30.5)	2.33 (2.3)	5.94 (5.8)

***** *Uncorrected*

Table 2. Physical and Analytical Data of the Compounds Synthesized (**4a-h**).

Compound No.	R	Molecular Formula	LC-MS Data	M.P.* °C	Yield %	Elemental Analysis		
						C%	H%	N%
						Calcd. (Found)	Calcd. (Found)	Calcd. (Found)
4a	H	$C_{15}H_{13}N_4O_2$ (281)	290.5	152-154	62	64.05 (64.0)	4.62 (4.6)	19.92 (19.8)
4b	2-Me	$C_{16}H_{15}N_4O_2$ (295)	302.5	157-159	64	65.08 (64.9)	5.08 (4.9)	18.98 (18.9)
4c	4-Cl	$C_{15}H_{12}N_4O_2Cl$ (315.5)	336	168-172	57	57.05 (57.0)	3.80 (3.8)	17.74 (17.7)
4d	4-Br	$C_{15}H_{12}N_4O_2Br$ (360)	382	171-173	54	50.00 (49.9)	3.33 (3.2)	15.55 (15.5)
4e	4-NO_2	$C_{15}H_{12}N_5O_4$ (326)	345	165-168	53	55.21 (55.2)	3.68 (3.6)	21.47 (21.4)
4f	2,4-Dinitro	$C_{15}H_{11}N_6O_6$ (371)	386	156-158	59	48.51 (48.4)	2.96 (2.9)	22.64 (22.6)
4g	2,4-Dichloro-6-Nitro	$C_{15}H_{10}N_5O_4Cl_2$ (395)	418	173-175	60	45.56 (45.5)	2.53 (2.5)	17.72 (17.6)
4h	2,4,6-Tribromo	$C_{15}H_{10}N_4O_2Br_3$ (518)	537	169-171	59	34.74 (34.7)	1.93 (1.9)	10.81 (10.7)

then cooled and allowed to stand overnight. The resulting solid was filtered off dried and crystallized from methanol. IR (KBr, cm^{-1}): 3330 and 3155 (NH), 1624-1640 (C=N), 3030-3088 cm^{-1} (C-H of Ar.), 2960, 1380 cm^{-1} (-CH_3), 1692 (C=O ketone), 1640 (C=O amide) and 1165 cm^{-1} (C-O). ^{1}H NMR (400MHz, DMSO-d_6, δ/*ppm*): 2.42 (s, 3H, CH_3), 11.62 (s, 1H, NH); **(4a)**: 6.90-8.92 (s, 9H, ArH); **(4b)**: 2.24 (s, 3H, CH_3), 6.84-8.92 (s, 8H, ArH); **(4c)**: 7.20-8.94 (s, 8H, ArH); **(4d)**: 6.85-8.93 (s, 8H, ArH); **(4e)**: 7.24-8.94 (s, 8H, ArH); **(4f)**: 7.90-8.92 (s, 7H, ArH); **(4g)**: 7.94-8.95 (s, 6H, ArH); **(4h)**: 7.71-8.95 (s, 6H, ArH). ^{13}C-NMR (100 MHz, DMSO, δ/*ppm*): 12.1 (CH_3), 163.5 (-CO amide), 172.4 (-CO ketone), 129.4 (C=N); **(4a)**: 114.2-156.7 (Ar-C); **(4b)**: 17.8 (CH_3), 113.5-156.6 (Ar-C); **(4c)**: 118.4-156.6 (Ar-C); **(4d)**: 117.9-156.5 (Ar-C), **(4e)**: 116.8-159.9 (Ar-C); **(4f)**: 118.1-156.6 (Ar-C); **(4g)** 125.3-156.8 (Ar-C); **(4h)**: 110.7-156.4 (Ar-C). The yields, melting points and other characterization data of these compounds are given in Table 2.

BIOLOGICAL SCREENING

Antibacterial activities

Antibacterial activities of all the compounds were studied against gram-positive Bacteria (*Bacillus subtilis and Staphylococcus aurous*) and gram-negative Bacteria (*E. coli, Salmonella typhi and Klebsiella promioe*) at a concentration of 50 μg/ml by agar cup plate method [29]. Methanol system was used as control in this method. Under similar conditions, using tetracycline as a standard for comparison, we carried out a control experiment. The zone of inhibition was measure in mm (Table 3).

Antifungal activity

The fungicidal activity of all the compounds **(4a-h)** was studied at 1000 ppm concentration in vitro plant pathogenic organisms listed in Table 4. The antifungal activities of all the samples were measured on each of these plant pathogenic strains on potato dextrose agar (PDA) medium. Such a PDA medium contained potato 200 g, dextrose 20g, agar 20 g and water 1 litre and five days old cultures were employed. The compounds to be tested were suspended (1000 ppm) in a PDA medium and autoclaved at 120°C for 15 min at 15 atm pressure. These mediums were poured into sterile Petri plate and the organisms were inoculated after cooling the Petri plate. The percentage inhabitation for fungi was calculated after 5 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.

Table 3. Antibacterial Activity of Compounds (**4a-h**).

Compounds No.	Zone of Inhibition(mm) (Activity Index)^{std}				
	Gram +ve		Gram -ve		
	Bacillus subtilis	*Staphylococcus aurous*	*Klebsiella promioe*	*Salmonella typhi*	*E. coli*
4a	56 (0.70)	42 (0.76)	50 (0.57)	44 (0.57)	55 (0.76)
4b	51 (0.64)	47 (0.85)	61 (0.70)	55 (0.72)	58 (0.80)
4c	71 (0.89)	48 (0.87)	79 (0.90)	67 (0.88)	63 (0.87)
4d	68 (0.86)	43 (0.78)	78 (0.89)	63 (0.82)	59 (0.81)
4e	55 (0.69)	46 (0.83)	71 (0.81)	43 (0.56)	57 (0.79)
4f	66 (0.83)	44 (0.80)	58 (0.66)	64 (0.84)	56 (0.77)
4g	67 (0.84)	47 (0.85)	75 (0.86)	62 (0.81)	52 (0.72)
4h	70 (0.88)	50 (0.90)	80 (0.91)	66 (0.86)	61 (0.84)
Tetracycline	79	55	87	76	72

***(Activity Index) std** = Zone of Inhibition of the sample/ Zone of Inhibition of the standard.*

The fungicidal activity of all compounds **(4a-h)** are shown in Table 4.

RESULTS AND DISCUSSION

The synthetic approach assumed for the synthesis of intermediate and target compounds is illustrated in the Scheme 1. Condensation of various ethyl-2-substituted phenyl hydrazono-3-oxobutyrate **(2a-h)** with furan-2-carbohydrazide **(3)** afforded 1-(furan-2-carbonyl)-3-methyl-4-(2-substituted phenyl hydrazono)-1H-pyrazol-5(4H)-one **(4a-h)**.

The IR spectra of compounds **2a-h** displayed C=N stretching at 1620-1640 cm^{-1}, N-H stretching at 3355-3360 cm^{-1}, C-H of aromatic stretching at 3030-3080 cm^{-1}, C=O of ketone stretching at 1695 and ester C=O stretching at 1725 cm^{-1}. The ^{1}H NMR shows triplate at δ 1.25 indicating the presence of CH_3 protons. In the aromatic region complex multiplate at δ 6.56-8.92 was observed. The singlet at δ 2.35 indicating the presence of $COCH_3$ and quartate at δ 4.29 indicating the presence of $COCH_2$. A broad singlet at δ 11.62 was obsered for one NH proton. The ^{13}C-NMR shows peak at δ 14.2, 27.1 for CH_3, δ 62.6 for OCH_2, δ 163.5 for ester carbonyl carbon,

Table 4. Antifungal Activity of Compounds (**4a-h**).

Zone of Inhibition at 1000 ppm (%)					
Compounds No.	*Botrydepladia Thiobromine*	*Nigrosspora Sp.*	*Penicillium Expansum*	*Trichothesium Sp.*	*Rhizopus Nigricuns*
4a	60	71	73	55	52
4b	71	65	61	57	69
4c	72	78	71	79	74
4d	65	66	66	68	65
4e	54	63	53	65	70
4f	63	58	50	76	58
4g	71	62	61	59	55
4h	76	75	72	77	69

196.4 for ketone carbonyl carbon, 126.9 for C=N carbon and 113.4-152.7 for aromatic carbon.The C, H, N analysis data of all compounds are presented in Table 1.

The IR spectra of compounds **4a-h** displayed C=N stretching at 1624-1640 cm^{-1}, N-H stretching at 3330 and 3155 cm^{-1}, C-H of aromatic stretching at 3030-3088 cm^{-1}, C-O of furan at 1165cm^{-1}, C=O of ketone stretching at 1692 cm^{-1} and amide C=O stretching at 1640 cm^{-1}. The ^{1}H NMR shows triplate at δ 2.42 indicating the presence of CH_3 protons. In the aromatic region complex multiplate at δ 6.84-8.95 was observed. A broad singlet at δ 11.62 was obsered for one NH proton. The ^{13}C NMR shows peak at δ 12.1 for CH_3, δ 163.5 for amide carbonyl, δ 172.4 for ketone carbonyl carbon, δ 129.4 for C=N carbon and δ 110.7-159.9 for aromatic carbon.The C, H, N analysis data of all compounds are presented in Table 2.

The examination of spectra and elemental data reveals that they are consistent with the predicted structure as shown in Scheme 1.The LC-MS data of **4a** shows the peak of M^+ ion at 290.5 which is consistent of molecular mass of **4a** i.e. 281. All these facts confirm the structures **4a-h**.

The examination of antibacterial activity data reveals that the compounds **4c** and **4h** are more active against the gram-positive and gram-negative bacteria, but less active compared to tetracycline. Other compounds were less or moderate active than **4c** and **4h**.

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