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Innovative synthesis and anti-microbial activity study of innovative Mannich bases containing 2-phenoxy-1,3,2-dioxa phospholanes and indole systems

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ABSTRACT

In the present study, synthesis, characterization, antimicrobial activity on some novel pyrazolone derivatives has been taken up. Reaction of (4Z)-2-{4-[(15Z)-4-(2-phenylhydrazono)-4,5-dihydro-3-methyl-5-oxopyrazol-1-yl] phenylamino}-N'-(2-oxoindolin-3-ylidene) acetohydrazide and acetophenones were refluxed in methanol containing a catalytic amount of glacial acetic acid for 4 hours furnished the corresponding (19Z)-2-{4-[(4E)-4-(2-substituted aryl hydrazono) -4,5 -dihydro-3 –methyl -5-oxopyrazol -1-yl] phenylamino}-N'-(1-phenylethylidene) acetohydrazide step-1 product in excellent yields. Step-I products and excessive acetic anhydride was refluxed to afforded (4E)-4-(2-substituted aryl hydrazono)-1-{4-[(4-acetyl-4,5-dihydro-5-methyl-5-phenyl-1,3,4-oxadiazol-2-yl) aminomethyl] phenyl}-3-methyl-1H-pyrazol-5(4H)-one step-II product. The structural assignments to compounds Step-I product and Step-II product were based on their elemental analysis and spectral (IR, ^{1}HNMR and MS) data. The anti-bacterial activity of synthesized compound was studied by the disc diffusion method against pathogenic bacteria and fungi.

Keywords: anti-microbial activity, Mannich bases, phenoxy-1, 3, 2-dioxa phospholanes, indole systems

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INTRODUCTION

Synthesis of 1,3,4-oxadiazole derivatives containing pyrazole-3-one moiety
1,3,4-oxadiazole is a thermally stable and neutral hetero aromatic molecule.1,3,4-oxadiazoles have a wide variety of users, particularly as biologically active compounds in medicine, agriculture, as dye stuffs, UV absorbing and fluorescent materials, heat resistant polymers and scintillators

Literature Review:
A review of literature concerning the synthesis and biological activity of 1,3,4-oxadiazoles is given below.

Substituted 1,3,4-oxadiazoles are of considerable pharmaceutical material interest, which is documented by a steadily increasing number of research publications and patents. For instance 2-aminio-1,3,4-oxadiazole acts as muscle relaxant[2] and shows antimitotic activity[3]. Analgesic, anti inflammatory, anticonvulsive, diuretic and antiemetic properties are exhibited by 5-aryl-2-hydroxymethyl-1,3,4-oxadiazole derivaties[4]. 2-hydroxyphenyl-1,3,4-0xdiazole acts as a hypnotic and sedative drug[5]. Some material applications of 1,3,4-oxadiazoles are in the area of photosensitizers[6], liquid crystals[7] and organic light emitting diodes[8].

i. Reported synthetic routes-1:
Several derivatives of 5-substituted-1,3,4-oxadiazoles [1] [X=SH, R=2/3-$CH_3C_6H_4$] Synthesized by Kuharia and co workers[9] were reported to posses hypoglycemic activity and found to be less toxic than the corresponding hydrazides

Srivastava et al[10] reported that 2-substituted-1,3,4-oxadiazoles and their derivatives [2] are active as antifungal and antiviral agents. All the tested compounds showed virucidal activity against virus SRV to the extent of 4.48% in *vivo* at 1µg/mL.

Symmetrical 2,5-disubstituted-1,3,4-oxadiazoles [3] synthesized by Sharma and co-workers were for CNS depressant and anticovulsant activities[11].

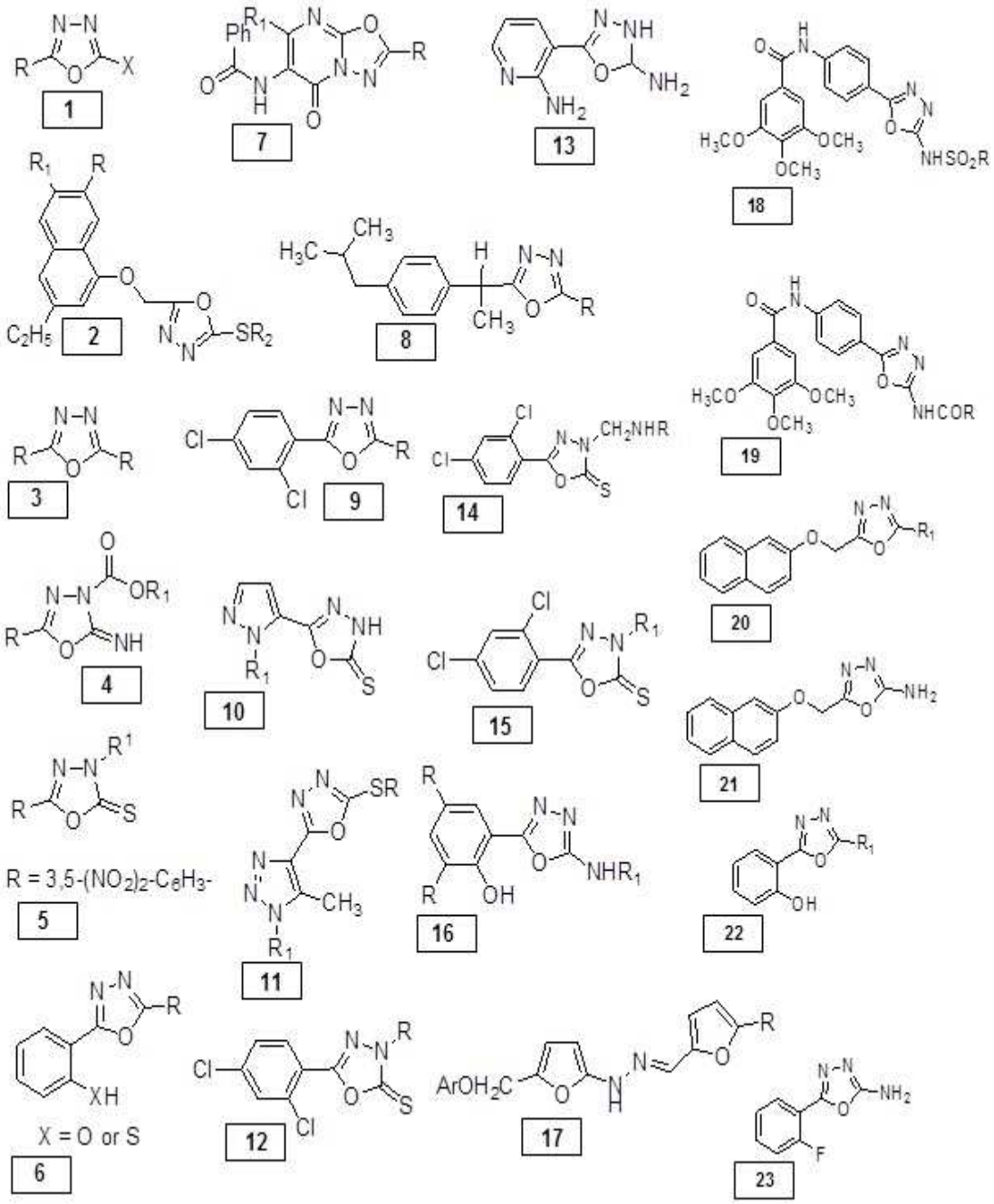

Figure-1: Chemical structures for reported methods

A German patent[12] described the synthesis of imino oxadiazole carboxylate [4] [R = C_6H_5, R_1 = C_2H_5] which acts a drug and agrochemical.

Nigam et al.,[13] reported the synthesis of some 3-(substituted aminomethyl)-5-(3,5-dinitrophenyl)-1,3,4-oxadiazol-2-thiones [5] [R^1 = $CH_2NR_1R_2$; NR_1R_2 = N-methylanilino, N-ethylanilino, morpholino, piperidino, N-phenylpiperazino]. These compounds were screened for their cardiovascular and anti-inflammatory activity. The compounds were found to be non-toxic and psychotropic in nature.

Lee et al.,[14] reported the synthesis of 1,3,4-oxadiazole derivatives [6] having phenol or thiophenol group. Treatment of a suspension of salicylic acid hydrizide in toluene with acetic anhydride or an acid chloride in the presence of an equimolar amount of methanesulphonic acid at room temperature, and then heating to reflux gave 1,3,4-oxadiazoles in 43-68% yield.

Yadava et al.,[15] synthesized a series of 2,7-diaryl-6-benzamido-6,7-dihydro-5H-1,3,4-oxadiazolo[3,2-a]-pyrimidin-5-ones 7 in an one pot reaction and compared their fungi toxicity with a commercial sample.Dithane M-45 against H. *oryzae* and *C. Saccharil* by agar plate method. Few of the tested compounds showed fungi toxic action almost equivalency to that of Dithane M-45 at 1000ppm concentration.

Yadava et al.,[16] reported the synthesis and activity of 2-aryl-5-(α-methyl-4-isobutylbenzyl)-1,3,4-oxadiazoles [8]. These compounds exhibited significant antimicrobial and anti-inflammatory activities.

2,5-disubstituted-1,3,4-oxadiazoles [9] prepared by Zhang and Qian[17] were tested for their fungicidal and insecticidal activities.

Synthesis, antimicrobial activity and heterocyclic ring transformation of 5-(pyrazol-5-yl)-1,3,4-oxadiazol-2(3H)-thiones [10] were reported by Shawali and co-workers[18].

Synthesis and antibacterial of 2-(1-aryl-5-mrthyl-1,2,3-triazol-4-yl)-1,3,4-oxadiazole derivatives [11] were reported by Zhang et al.,[19]. Most of the compounds were active against *E.coil, p.aeruginosa, B.subtilis and S.aureus.*

Dutta and Kataky[20] synthesized benzyl derivatives and diethylthiophosphonates of alkyl/aryl/aminomethyl-5-(2,4-dichlophenyl)-1,3,4-oxadiazol-2-thiones [12] [R = CH_2NHR^1,$CH_2N(R^1)COPh$, CH_2N (R^1) (PS) $(OEt)_2$] with a view to get more potent insecticidal and bacterial compounds.

Synthesis and pharmacological properties of some new derivatives of 2-amino-5-(2-amino-3-pyridyl)-1,3,4-oxadiazole [13] were reported by Liszkiewicz and co-workers[21].

3-arylaminomethyl-5-(2,4–dichlorophenyl)-1,3,4-oxadiazole-2-thiones [14] [R = H,2-ClC_6H_4, 4-ClC_6H_4, 2-$COOHC_6H_4$, 2-$NO_2C_6H_4$, 3-$NO_2C_6H_4$, 4-$NO_2C_6H_4$, 2-$CH_3C_6H_4$, 4-$CH_3C_6H_4$, 4-$COOHC_6H_4$,2-$OCH_3C_6H_4$,4-$OCH_3C_6H_4$, -$CH_2C_6H_5$, N-$(CH_2)_2CH_3$] and3-alkyl/aryl/alkyl-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole-2-thiones 15 [R^1 = -CH_3, -CH_2CH_3, n-$(CH_2)_3CH_3$, -$CH_2C_6H_5$, -$CH_2C_6H_5OCH_3$(4), -CH_2COOEt] were synthesized by Goswami et al.,[22]. These substituted oxadiazolethiones were screened for their fungitoxic properties. Most of the compounds showed toxicity against two test organisms, *Curvularia* verruciformis and *alternaria tenuis.* The degree of inhibition ranged from 38-100% for a few compounds.

A novel series of 2-substituted amino-5-aryl-1,3,4-oxadiazole [16] [R = H, Br, R_1 = C_6H_5, m-$CH_3C_6H_4$, *p*-ClC_6H_4, *p*-BrC_6H_4, $CH_2C_6H_5$, CH_2CH_2-BrC_6H_4, $CH_2C_6H_5$, $CH_2CH_2CH_2CH_3$, C_6H_{11}] derivatives were synthesized for their potential anticonvulsant activity by Omar and Aboul Wafa[23].

Synthesis of 5-substituted-1,3,4-oxadiazole-2-(5-substituted-2-furfuraldehyde) hydrazones [17] [R = 2,4-dichlorophenyl, 4-chlorophenyl, nitro; Ar = *p*-chlorophenyoxymethyl, o-chlorophenoxymethyl, β-napthyloxymethyl, *p*-cresyloxymethyl] was reported from our laboratory[24]. These compounds were screened for their biological activity against both Gram-positive and Gram-negative bacteria.

Synthesis of 2-arylsulfonamido-5-p-(3',4',5'-trimethoxy benzamido phenyl)-1,3,4-oxadiazoles [18] and 2-substituted benzamido-5-(3',4',5'-trimethoxy benzamido phenyl)-1,3,4-oxadiazoles [19] was reported by Joshi et al.,[25]. The compounds have been tested for their antimicrobial and antifungal activities.

Synthesis, anticonvulsant and antimicrobial activity of 2,5,-disubstituted-1,3,4-oxadiazoles [20] and 2-amino-5-substituted-1,3,4-oxadiazoles [21-23] was studied by Khan and co-workers[26].

Figure-1 represents the all reported molecules chemical structures for above discussion.

ii. Reported synthetic routes-2:

Khanna and co-workers[40] have reported the synthesis and anticatatonic activity of 4-(5-aryl-1,3,4-oxadiazol-2-methyl)-1-phenyl piperazines [24].

Reddy and reddy[41] reported the synthesis of quinazoloinyl-1,3,4-oxadiazoles [25] by heating a mixture of 2-hydrazinocabonyl quinalzolin-4(3H)-one with aromatic acids at 280ºC for 6 hours.

Reaction of hydrazides with various 4-substituted aromatic acids in the presence of $POCl_3$ afforded 2-(4-substituted anilinomethyl/ethyl)-1,3,4-oxadiazoles [26].

Dubey and Sangwan[43] have reported the synthesis and antifungal activity of 2-aryl-5-(3,5-diphenylpyrazol-4-yioxymethyl)-1,3,4-oxadiazoles [27].

Shah and co-workers have reported the synthesis and antimicrobial activity of 2-aryl-5-p-(nicotinamidophenyl)-1,3,4-oxadiazoles [28].

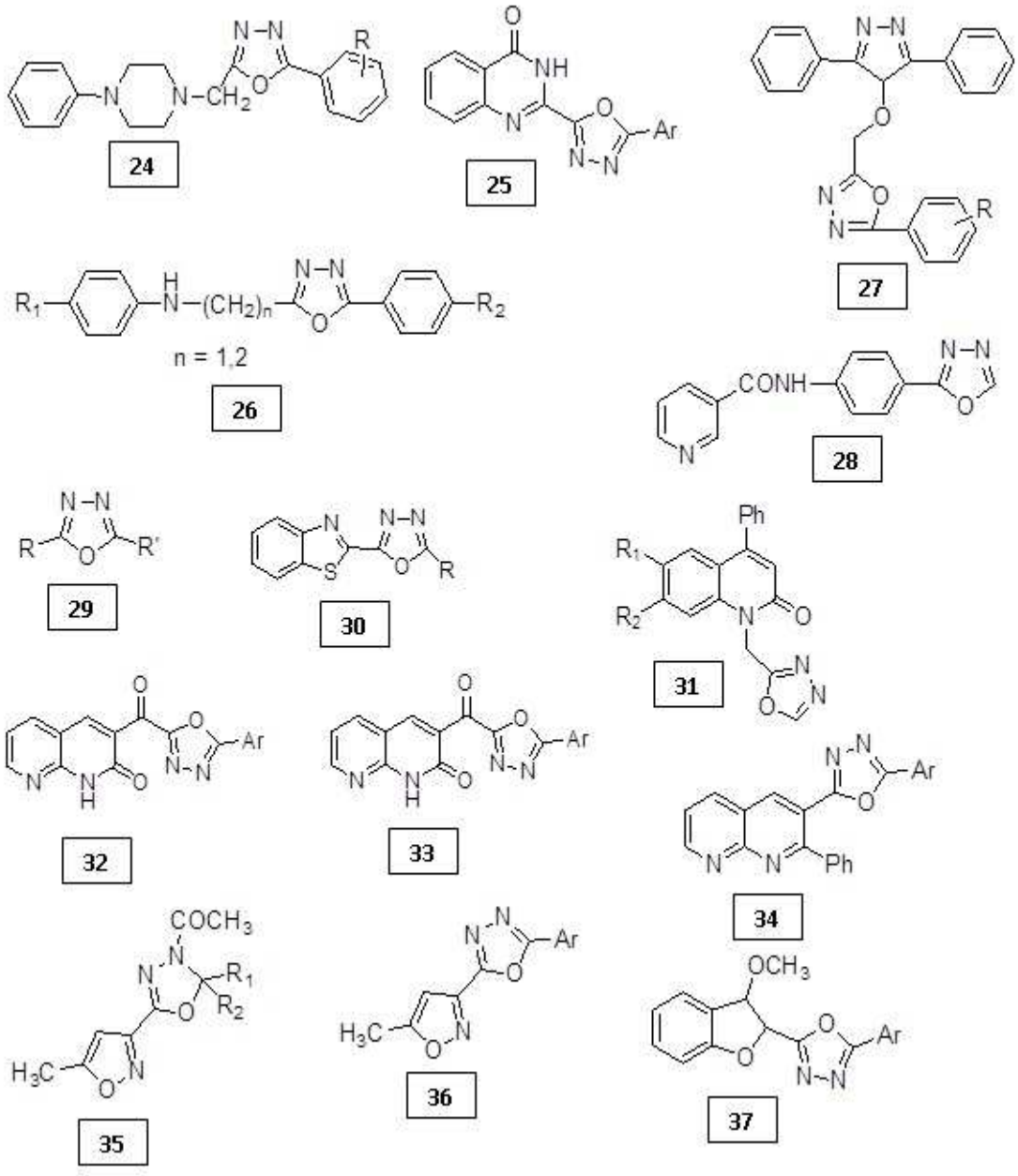

Figure-2: Chemical structures for reported methods

Lokanatha Rai and Lingann [45] 4synthesized 2,5-diaryl-1,3,4-oxadiazoles [29] by the oxidation of acylhydrazones with chloramines-T trihydrate.

Singh et al.,[46] reported chloramines-T mediated synthesis and antibacterial and antifungal activities of 2-benzothiozolyl-5-aryl-1,3,4-oxadiazoles [30].

Deshmukh and Shelar[47] have reported the synthesis of quinolinyl-1,3,4-oxadiazoles [31].

Mogilaiah et al.,[48] have reported the synthesis and antimicrobial activity of some new 1,3,and 4-oxadiazolyl-1, 8-naphthyridines [32 and 33].

Mogilaiah et al.,[49] have described the synthesis and antibacterial activity of 3-(5-aryl-1,3,4-oxadiazol-2-yl)-2phenyl-1-napthyridines[34].

Hui et al.,[50] have described the synthesis and antibacterial activities of 1,3,4-oxadiazole derivatives [35 and 36] containing 5-methyisoxazole moiety.

Basavaraja et al.,[51] have described the synthesis and antibacterial activity of 1,3,4-oxadiazofurans [37].

Synthesis of1,3,4-oxadiazole derivatives for varied applications such as biologically active molecules and/or in materials science is a actives area of research even today as evident by the growing number of research publications added to chemical literature each year[27-39]. Prompted by the above observations, a project was under taken to synthesize pyrazolone containing 1,3,4-oxadiazole derivatives and to study their biological activity.

Such of our studies form the subject matter of this chapter. In chapter III, the synthesis of a series of 2-(4-acetyl-5-methyl-5-phenyl-4,5-dihydro-[1,3,4]oxadiazol-2-methyl)-5methyl)-4-(4'-substituted arylhydrazono)-2,4-duhydro pyrazol-3-one is described and the newly formed compounds were screened for their antimicrobial activities.

MATERIALS AND METHODS

(4E)-4-(2-substituted aryl hydrazono)-1-{4-[(4-acetyl-4,5-dihydro-5-methyl-5-phenyl-1,3,4-oxadiazol-2-yl) aminomethyl] phenyl}-3-methyl-1H-pyrazol-5(4H)-one synthesis involved two steps and figure-3 represents the complete synthetic route.

STEP-1 SYNTHESIS:
2-{4-[(4Z)-4-(2-substituted aryl hydrazono)-4, 5-dihydro-3-methyl-5-oxopyrazol-1-yl] phenyl amino} acetohydrazide (starting material-1) employed in the present investigation was prepared as per procedure described in Chapter II of this thesis, (19Z)-2-{4-[(4E)-4-(2-substituted aryl hydrazono)-4,5-dihydro-3-methyl-5-oxopyrazol-1- yl] phenylamino}-N'-(1-phenylethylidene) acetohydrazide step-1 product (a-j) were obtained in very good yields by the condensation of starting material-1 with appropriate ketone

In a typical example a mixture of 2-{4-[(4Z)-4-(2-substituted aryl hydrazono)-4, 5-dihydro-3-methyl-5-oxopyrazol-1-yl] phenyl amino} acetohydrazide and acetophenone was refluxed in methanol containing a catalytic amount of gL acetic acid for 4 hours. After usual work the hydrazone step-1 product (a) was obtained in 84% yield, m.p.236ºC.

The above reaction of step-1 product with acetophenone has been extended to *p*-methylacetophenone, *p*-chloroacetophenone, and *p*-nitroacetophenone. The physical characteristics of step-1 product (a-j) are reported in Table-5. The compounds synthesized step-1 product has characterized by means of their elemental analysis IR, ^{1}HNMR, and MS data.

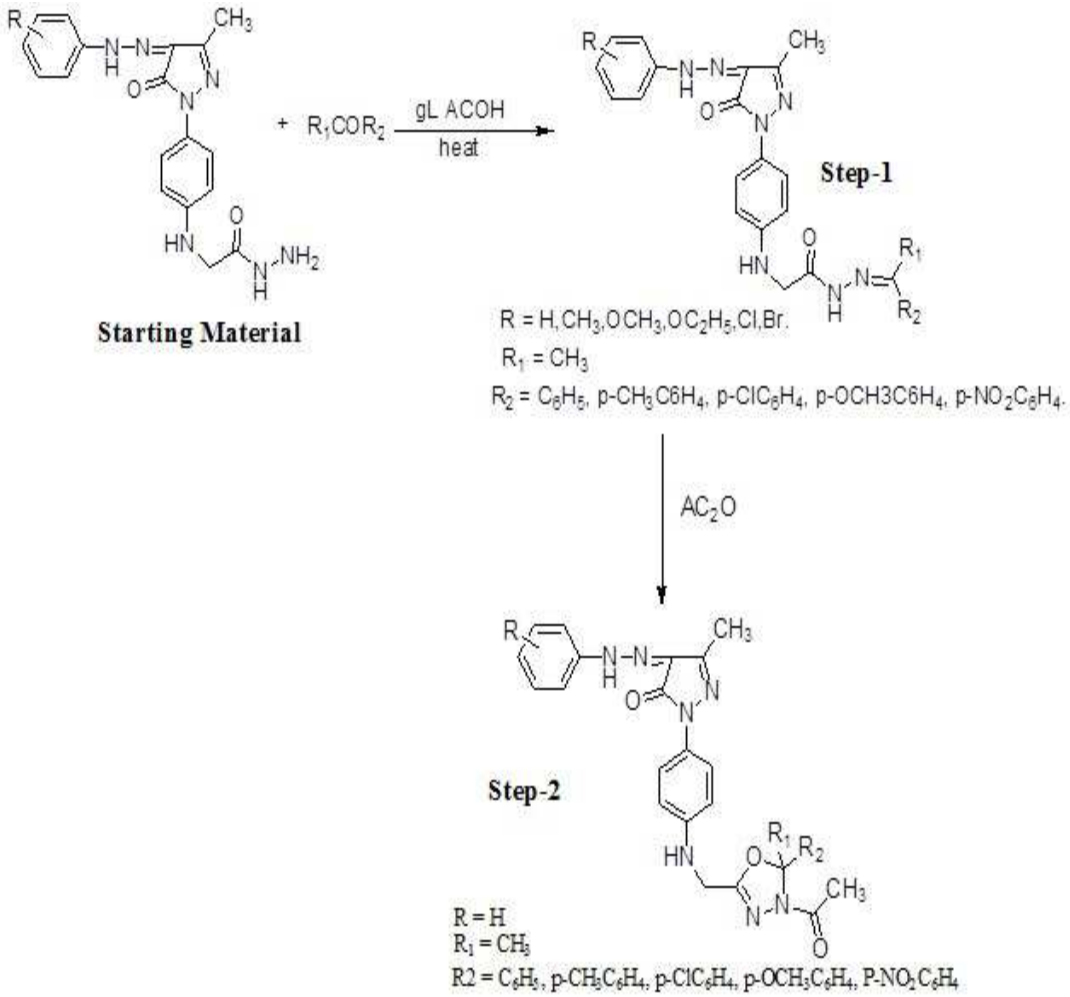

Figure-3: Synthesis of (4E)-4-(2-substituted aryl hydrazono)-1-{4-[(4-acetyl-4,5-dihydro-5-methyl-5-phenyl-1,3,4-oxadiazol-2-yl) aminomethyl] phenyl}-3-methyl-1H-pyrazol-5(4H)-one

I. IR Spectra

The IR (KBr) spectra of step-I product (a) shows absorption bands around 3150, 1665 and 1600 cm^{-1} due to NH, C = O and C = N functional groups respectively. The data are presented in Table-1.

Table-1: IR spectral data of step-I product

Step-I product	R	R_1	R_2	ν_{max} in cm^{-1}		
				NH	C=O	C=N
a	H	CH_3	C_6H_5	3185	1665	1600
b	CH_3	CH_3	C_6H_5	3175	1670	1602
c	OCH_3	CH_3	C_6H_5	3200	1665	1605
d	OC_2H_5	CH_3	C_6H_5	3190	1670	1604
e	Cl	CH_3	C_6H_5	3210	1650	1605
f	Br	CH_3	C_6H_5	3215	1660	1602
g	H	CH_3	$CH_3C_6H_4$	3195	1670	1605
h	H	CH_3	ClC_6H_4	3190	1675	1604
i	H	CH_3	$OCH_3C_6H_4$	3205	1660	1605
j	H	CH_3	$NO_2C_6H_4$	3180	1660	1604

II. ^{1}HNMR Spectra

The ^{1}HNMR (200MHz) spectra of (19Z)-2-{4-[(4E)-4-(2-substituted aryl hydrazono)-4,5-dihydro-3-methyl-5-oxopyrazol-1-yl] phenylamino}-N'-(1-phenylethylidene) acetohydrazide step-1 product (a-j) were recorded in DMSO-d_6 and data furnished in Table-2.

Table-2: ^{1}HNMR spectra data of step-1 product				
Step-1 product	R	R_1	R_2	^{1}HNMR (200MHz) (DMSO-d_6) (δppm)
a	H	CH_3	C_6H_5	1.52(s, 3H, CH_3), 2.35(s, 3H, N=CH_3), 4.1 (s, 1H, Ar-NH), 6.8(s, 1H, Ar-NH), 7.0-7.1(m, 10H, C_6H_4), 7.20(s, 2H, NCH2CO), 7.4(d, 2H, C_6H_4), 7.7(d, 2H, C_6H_4), 10.9(s, 1H, NH)
g	H	CH_3	$CH_3C_6H_4$	1.50(s, 3H, CH_3), 2.25(s, 3H, CH_3) 2.3 (s, 3H, N-CH_3), 4.0 (s, 1H, Ar-NH), 6.8 (s, 1H, Ar-NH), 7.0-7.1(m, 9H, C_6H_4), 7.2 (s, 2H, NCH2CO), 7.4 (d, 2H, C_6H_4), 7.7(d, 2H, C_6H_4). 10.9(s, 1H, NH)
h	H	CH_3	ClC_6H_4	1.6(s, 3H, CH_3), 2.4(s, 3H, N-CH_3), 4.2 (s, 1H, Ar-NH), 6.8(s, 1H, Ar-NH), 7.0-7.1(m, 9H, C_6H_4), 7.2(s, 2H, NCH2CO), 7.4 (d, 2H, C_6H_4), 7.7(m, 4H, C_6H_4). 10.95(s, 1H, NH)
i	H	CH_3	$OCH_3C_6H_4$	1.50(s, 3H, CH_3), 3.9(s, 3H, OCH_3) 2.35 (s, 3H, N-CH_3), 4.1 (s, 1H, Ar-NH), 6.8 (s, 1H, Ar-NH), 7.0-7.1(m, 9H, C_6H_4), 7.2 (s, 2H, NCH2CO), 7.4(d, 2H, C_6H_4), 7.7 (d, 2H, C_6H_4), 10.9(s, 1H, NH)
j	H	CH_3	$NO_2C_6H_4$	1.5(s, 3H, CH_3), 2.3(s, 3H, N-CH_3), 4.0 (s, 1H, Ar-NH), 6.8(s, 1H, Ar-NH), 7.0-7.1(m, 1H, C_6H_4), 7.2(s, 2H, NCH2CO), 7.4(m, 2H, C_6H_4). 7.7 (d, 2H, C_6H_4), 10.90(s, 1H, NH)

III. Mass spectra

The mass spectra of (19Z)-2-{4-[(4E)-4-(2-phenyl hydrazono)-4,5-dihydro-3-methyl-5-oxopyrazol-1-yl] phenylamino}-N'-(1-phenylethylidene) acetohydrazide step-1 product (a) (R = H, R_1 = CH_3, R_2 =C_6H_5) showed molecular ion (M^+) peak at m/z 467.5.

The mass spectral fragmentation pattern of step-1 product (a) was analyzed. The molecular ion was observed at m/z 467 (12.5%) and the base peak was at m/z 334 (100%), other prominent peaks appeared at m/z 450(33.7%), 438 (16.4%), 390 (29.2%), 362 (13.6%), 347(26%) and 294(22.4%).

(19Z)-2-{4-[(4E)-4-(2-phenyl hydrazono)-4,5-dihydro-3-methyl-5-oxopyrazol-1-yl] phenylamino}-N'-(1-phenyl ethylidene) acetohydrazide step-1 product (a) on cyclization with excess acetic anhydride resulted in the formation of step-2 product (a).

To solution of starting material (0.01mol) in hot methanol (25 ml), acetophenone (0.01mol) and a drop of gL acetic acid were added. The solid that separated on refluxing for 3 hours filtered wash with cold methanol and recrystallized form methanol to give step-1 product (a). m.p. 236ºC, yield 84%.

Other compounds step-2 product (b-j) were synthesized similarly and their characterization data given in Table-3.

Table-3: Characterization data of step-1 product												
Step-I product	R	R_1	R_2	M.P ^{0}C	Yield (%)	Mol. Formula	Found (%) – Calcd (%)					
							C	H	N	O	Cl	Br
a	H	CH_3	C_6H_5	236	84	$C_{26}H_{25}N_7O_2$	66.87	5.42	21.04	6.92		
							(66.79)	(5.39)	(20.97)	(6.84)		
b	CH_3	CH_3	C_6H_5	220	60	$C_{27}H_{27}N_7O_2$	67.43	5.73	20.42	6.73		
							(67.34)	(5.65)	(20.36)	(6.64)		
c	OCH_3	CH_3	C_6H_5	215	75	$C_{27}H_{27}N_7O_3$	65.28	5.53	19.80	9.73		
							(65.18)	(5.47)	(19.71)	(9.65)		
d	OC_2H_5	CH_3	C_6H_5	200	65	$C_{28}H_{29}N_7O_3$	65.82	5.78	19.23	9.46		
							(65.74)	(5.71)	(19.17)	(9.38)		
e	Cl	CH_3	C_6H_5	195	63	$C_{26}H_{24}ClN_7O_3$	62.29	4.89	19.61	6.46	7.12	
							(62.21)	(4.82)	(19.53)	(6.37)	(7.06)	
f	Br	CH_3	C_6H_5	210	68	$C_{26}H_{24}BrN_7O_3$	57.21	4.49	18.02	5.96		14.71
							(57.15)	(4.43)	(17.94)	(5.86)		(14.62)
g	H	CH_3	$CH_3C_6H_4$	220	70	$C_{27}H_{25}N_7O_2$	67.42	5.74	20.45	6.72		
							(67.34)	(5.65)	(20.36)	(6.64)		
h	H	CH_3	ClC_6H_4	215	65	$C_{26}H_{24}ClN_7O_2$	62.28	4.88	19.59	6.43	7.13	
							(62.21)	(4.82)	(19.53)	(6.37)	(7.06)	
i	H	CH_3	$OCH_3C_6H_4$	235	70	$C_{27}H_{27}N_7O_3$	65.24	5.55	19.74	9.76		
							(65.18)	(5.47)	(19.71)	(9.65)		
j	H	CH_3	NO2C_6H_4	210	70	$C_{27}H_{27}N_7O_3$	61.02	4.80	21.95	12.56		
							(60.93)	(4.72)	(21.86)	(12.49)		

STEP-2 SYNTHESIS:

In a typical procedure a mixture of hydrazone step-1 product (a) and excess of acetic anhydride was refluxed for 2 hours and acetic anhydride was distilled off, and the residue was poured on to crushed ice. The solid thus obtained was filtered and recrystallized from aqueous DMF to give (4E)-4-(2-phenyl hydrazono)-1-{4-[(4-acetyl-4,5-dihydro-5-methyl-5-phenyl-1,3,4-oxadiazol-2-yl)aminomethyl]phenyl}-3-methyl-1H-pyrazol-5(4H)-one step-2 product (a) in 56% yield.

The cyclization reaction was extended to other hydrazones step-2 product (b-j) and in each case the respective 1,3,4 – oxadiazoles was isolated in 57-68% yields. The physical characteristics of step-2 product (a-j) are presented in Table-6.

The structure of step-2 product has been elucidated on the basis of their elemental analysis and spectra studies [IR, ^{1}HNMR, and MS data].

I. IR spectra

The IR (KBr) spectra data of (4E)-4-(2-substituted aryl hydrazono)-1-{4-[(4-acetyl-4,5-dihydro-5-methyl-5-phenyl-1,3,4-oxadiazol-2-yl)aminomethyl]phenyl}-3-methyl-1H-pyrazol-5(4H)-one (step-2 product).

Table-4: IR (KBr) spectra data of step-2 product

Step-2 product	R	R_1	R_2	ν_{max} in cm^{-1}		
				NH	C=O	C=N
a	H	CH_3	C_6H_5	3206	1685	1620
b	CH_3	CH_3	C_6H_5	3195	1690	1622
c	OCH_3	CH_3	C_6H_5	3230	1685	1625
d	OC_2H_5	CH_3	C_6H_5	3215	1695	1624
e	Cl	CH_3	C_6H_5	3230	1675	1630
f	Br	CH_3	C_6H_5	3210	1685	1627
g	H	CH_3	$CH_3C_6H_4$	3180	1695	1627
h	H	CH_3	ClC_6H_4	3195	1700	1629
i	H	CH_3	$OCH_3C_6H_4$	3245	1705	1630
j	H	CH_3	$NO_2C_6H_4$	3240	1685	1630

II. ^{1}HNMR spectra

The ^{1}HNMR (200 MHz) spectra of step-2 product (a, g, h, I, j) were measured in DMSO-d6 on data on presented in Table-5

Table-5 : ^{1}HNMR spectral data of step-2 product (a, g, h, I, j)

Step-2 product	R	R_1	R_2	^{1}HNMR (200MHz) (DMSO-d_6) (δppm)
a	H	CH_3	C_6H_5	2.1 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 2.6 (s, 3H, $COCH_3$), 5.0 (s, 1H, Ar-NH), 5.6(s, 2H, NCH_2), 6.8 (s,1H, Ar-NH), 7.1-7.3 (m, 9H, C_6H_4), 7.4(d, 2H,C_6H_4), 7.7(d, 2H, C_6H_4), 10.9 (s, 1H, NH).
g	H	CH_3	$CH_3C_6H_4$	2.15 (s, 3H, CH_3), 2.3 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 2.45 (s, 3H, $COCH_3$), 4.95 (s, 1H, Ar-NH), 5.2 (s, 2H, NCH_2), 6.8 (s, 1H, Ar-NH), 7.1-7.2 (m, 9H, C_6H_4), 7.4 (d, 2H, C_6H_4,), 7.7 (d, 2H, C_6H_4), 10.9 (s, 1H, NH).
h	H	CH_3	ClC_6H_4	2.2 (s, 3H, CH_3), 2.25(s, 3H, CH_3), 2.5 (s, 3H,$COCH_3$), 4.9 (s, 2H, NCH_2), 5.4 (s, 1H Ar-NH), 6.8 (s, 1H, Ar-NH), 7.1-7.3 (m, 9H, C_6H_4), 7.4 (d, 2H, C_6H_4,), 7.7 (m, 4H, C_6H_4), 10.9 (s, 1H, NH).
i	H	CH_3	$OCH_3C_6H_4$	2.2(s, 3H, CH_3), 2.4 (s, 3H, CH_3), 2.45 (s, 3H,$COCH_3$), 3.9 (s, 3H, OCH_3), 5.0 (s, 1H, Ar-NH), 5.2 (s, 2H, NCH_2), 6.8 (s, 1H, Ar-NH), 7.1-7.3 (m, 9H,C_6H_4), 7.4 (d, 2H, C_6H_4,), 7.7 (m, 4H, C_6H_4), 10.9 (s, 1H,NH).
j	H	CH_3	$NO_2C_6H_4$	2.15 (s, 3H, CH_3), 2.3(s, 3H, CH_3), 2.4 (s, 3H, $COCH_3$), 4.9 (s, 1H Ar-NH), 5.2 (s, 2H, NCH_2), 6.8 (s, 1H, Ar-NH), 7.1-7.3 (m, 4H, C_6H_4), 7.4 (m, 5H, C_6H_4,), 7.7 (m, 4H, C_6H_4), 10.9 (s, 1H, NH).

III. Mass spectra

The mass spectra of (4E)-4-(2-pehnyl hydrazono)-1-{4-[(4-acetyl-4,5-dihydro-5-methyl-5-phenyl-1,3,4-oxadiazol-2-yl)aminomethyl]phenyl}-3-methyl-1H-pyrazol-5(4H)-one step-2 product (a). (R = H, R_1 = CH_3, R_2 = C_6H_5) displayed molecular ion (M^+) at m/z 511.

The mass spectral fragmentation pattern of step-2 product (a) was analyzed. The molecular ion was observed at m/z 511 (16.5%) and the base peak was at m/z 392 (100%), other prominent peaks appeared at m/z 482 (13.8%), 434 (20.6%), 427 (30.7%), 416 (25.9%), and 406 (27.2%).

A mixture of step-1 product (a) (0.01 mol) and excessive acetic anhydride (10 ml) was refluxed for 2 hours. The excessive anhydride was distilled off and the residue was poured on to crushed ice. The solid thus obtained was filtered, washed with water and recrystallized from aq. methanol to furnish step-2 product (a), m.p. 185ºC, yield 56%.Other compounds step-2 product (b-j) were synthesized similarly and their characterization data given in Table-6.

Table-6: Characterization data of step-2 product

Step-2 product	R	R_1	R_2	M.P ^0C	Yield (%)	Mol. Formula	Found (%) – Calcd (%)					
							C	H	N	O	Cl	Br
a	H	CH_3	C_6H_5	200	62	$C_{28}H_{27}N_7O_3$	66.14	5.51	19.43	9.55		
							(66.00)	(5.34)	(19.24)	(9.42)		
b	CH_3	CH_3	C_6H_5	210	63	$C_{29}H_{29}N_7O_3$	66.67	5.74	18.90	9.35		
							(66.52)	(5.58)	(18.73)	(9.17)		
c	OCH_3	CH_3	C_6H_5	205	70	$C_{29}H_{29}N_7O_4$	64.74	5.59	18.33	12.01		
							(64.55)	(5.42)	(18.17)	(11.86)		
d	OC_2H_5	CH_3	C_6H_5	210	71	$C_{30}H_{31}N_7O_4$	65.23	5.77	17.89	11.66		
							(65.09)	(5.64)	(17.71)	(11.56)		
e	Cl	CH_3	C_6H_5	190	53	$C_{28}H_{26}ClN_7O_3$	61.97	4.95	18.22	8.85	6.65	
							(61.82)	(4.82)	(18.02)	(8.82)	(6.52)	
f	Br	CH_3	C_6H_5	200	62	$C_{28}H_{26}BrN_7O_3$	57.31	4.59	16.84	8.34		13.69
							(57.15)	(4.45)	(16.66)	(8.16)		(13.58)
g	H	CH_3	p- $CH_3C_6H_4$	210	65	$C_{29}H_{29}N_7O_3$	66.67	5.74	18.90	9.35		
							(66.52)	(5.58)	(18.73)	(9.17)		
h	H	CH_3	p-ClC_6H_4	210	68	$C_{28}H_{26}ClN_7O_3$	61.98	4.95	18.16	8.96	6.66	
							(61.82)	(4.82)	(18.02)	(8.82)	(6.52)	
i	H	CH_3	p-$OCH_3C_6H_4$	225	75	$C_{29}H_{29}N_7O_4$	64.70	5.58	18.33	12.03		
							(64.55)	(5.42)	(18.17)	(11.86)		
j	H	CH_3	p-NO2C_6H_4	215	71	$C_{28}H_{26}N_8O_5$	60.81	4.86	20.37	14.66		
							(60.64)	(4.73)	(20.21)	(14.43)		

MICROBIAL ACTIVITY:

Mannich bases *step-2 product (a,e,f)* have good antifungal activity against *Aspergillus Niger* NCCS 1196 and *Candida albicans* NCCS 2106. In this series chloro, bromo and nitro, p-phenyl syndronyl, p-tolyl syndronyl and N-phenyl syndronyl showed good antifungal activity against *Aspergillus Niger* and *Candida albicans* at the concentration of 250 µg/ml.

Table-7:Antibacterial activity by disc diffusion method for 1,3,4-oxadiazol compound-2(a-j)

S. No	Compd-2	R	R_1	R_2	Zone of inhibition (mm)			
					Staphylococus aureus NCCS 2079	*Bacillus Cereus* NCCS 2106	*Escherichia Coli* NCCS 2065	*Pseudomanas Aeruginos* NCCS 2200
1	a	H	CH_3	C_6H_5	6	5	5	4
2	b	CH_3	CH_3	C_6H_5	6	5	4	4
3	c	OCH_3	CH_3	C_6H_5	4	5	4	5
4	d	OC_2H_5	CH_3	C_6H_5	5	4	4	5
5	e	Cl	CH_3	C_6H_5	11	9	8	8
6	f	Br	CH_3	C_6H_5	10	8	8	8
7	g	H	CH_3	$CH_3C_6H_5$	4	5	4	5
8	h	H	CH_3	ClC_6H_4	11	8	8	8
9	i	H	CH_3	$OCH_3C_6H_4$	4	6	6	5
10	j	H	CH_3	$NO_2C_6H_4$	12	8	9	8
11	Amixcycillin				21	27	24	22
12	Cefaclor				19	22	19	20

CONCLUSION

In this research article, the synthesis of 1,3,4-oxadiazole pyrazol-3-one are presented. In the preceding part of this article the general methods of synthesis and biological importance of 1,3,4-oxadiazoles are briefly summarized.

Step-I.(19Z)-2-{4-[(4E)-4-(2-substituted aryl hydrazono)-4,5-dihydro-3-methyl-5- oxopyrazol-1- yl] phenylamino}-N'-(1-phenylethylidene) acetohydrazide

Step-II. (4E)-4-(2-substituted aryl hydrazono)-1-{4-[(4-acetyl-4,5-dihydro-5-methyl- 5-phenyl-1,3,4-oxadiazol-2-yl)aminomethyl]phenyl}-3-methyl-1H-pyrazol-5(4H)-one

A mixture of (4Z)-2-{4-[(15Z)-4-(2-phenylhydrazono)-4,5-dihydro-3-methyl-5-oxopyrazol-1-yl] phenylamino}-N'-(2-oxoindolin-3-ylidene) acetohydrazide (Starting Material) and acetophenone was refluxed in methanol containing a catalytic amount of glacial acetic acid for 4 hours furnished the corresponding (19Z)-2-{4-[(4E)-4-(2-substituted aryl hydrazono) -4,5 -dihydro-3 –methyl -5-oxopyrazol -1-yl] phenylamino}-N'-(1-phenylethylidene) acetohydrazide step-1 product in excellent yields. A mixture of step-I product and excessive acetic anhydride was

refluxed for 2 hours afforded (4E)-4-(2-substituted aryl hydrazono)-1-{4-[(4-acetyl-4,5-dihydro-5-methyl-5-phenyl-1,3,4-oxadiazol-2-yl) aminomethyl] phenyl}-3-methyl-1H-pyrazol-5(4H)-one step-II product. The structural assignments to compounds Step-I product and Step-II product were based on their elemental analysis and spectral (IR, ^{1}HNMR and MS) data. The synthetic approach to these compounds is profiled in Scheme II.

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