

RESEARCH ARTICLE



**SYNTHESIS OF PYRAZOLE DERIVATIVES AND EVALUATION OF ITS
ANTIMICROBIAL ACTIVITY**

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Abstract

The synthesis and characterization of new Quinolinoxyl acetyl pyrazole derivatives (3a-j) obtained from the reaction between 2,4-Diketo-3-(aryloxy) propane (1a-j) and 8-Quinolinoxylacetic acid hydrazide (2). The structures of these compounds have been established by ¹HNMR, (CDCl₃) and IR(KBr). The above compounds have been screened for their antimicrobial activity. The compounds showed significant antifungal activity. Ofloxacin and Ketoconazole were used as antimicrobial standard.

Keywords: antimicrobial, antifungal, pyrazole, Ofloxacin, Ketoconazole, ¹HNMR, (CDCl₃), IR(KBr).

Introduction

In recent year heterocyclic compounds analogues and derivatives have attracted strong interest due to their useful biological and pharmacological properties. Pyrazoles are one of the important members of heterocyclic compounds characterized by a five membered ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions.

The term Pyrazole was given by Ludwig Knorr in 1883 and its description was first made by Buchner in 1889. From 1889 to 1954, it was thought that pyrazoles could not be obtained naturally. However, in 1954, Kosuge and Okeda extracted the first natural pyrazole derivative 3-nonylpyrazole from a plant which is called as *Houttuynia Cordata*. In 1959, the first natural pyrazole, 1-pyrazolyl-alanine, which is *levo*- (1-pyrazolyl)alanine was isolated from *Citrullus Vulgaris* from seeds of watermelons.

Being so composed and having pharmacological effects on humans, they are classified as alkaloids, although they are rare in nature.

Pyrazoles are an important class of compounds for new drug development that attracted much attention. Several pyrazole derivatives have been synthesized as target structures and evaluated for their biological activities and have gained significant interest among the scientist.

This prevalence of pyrazole cores in biologically active molecules has stimulated the need for elegant and efficient ways to make these heterocyclic lead.

Pyrazole and its derivatives have shown wide range of biological activities such as antiviral (Joshi et al., 2010), ATP-competitive inhibitors of the mammalian target (Verheijen et al., 2009), antifungal (Tiwari et

al., 2013. Sharma et al., 2014.), antioxidant (Anand et al., 2012), antibacterial (Mogilaiah et al., 2013. Khan et al., 2013., Mogilaiah et al., 2008.), antitumor (El-Enany et al., 2011) and anticancer (Eman et al., 2013).

Materials and Methods

Synthesis of 2,4-Diketo-3-(aryloxy) propane (1a-j)

To substituted anilines dissolved in a mixture of concentrated hydrochloric acid (15 ml) and water (15 ml), cooled to 0° - 5 °C in an ice bath was added a cold, saturated solution of sodium nitrite (0.15 mole) with stirring. The diazonium salt thus formed was filtered into a cooled solution of acetylacetone (0.10 mole) in ethanol (50 ml) and sodium acetate (2.0 mole) in water (175 ml). The solids were filtered and washed with water and recrystallized from methanol.

Purity of the compound was checked by TLC on silica gel G plates using toluene: ethylacetate: formic acid (5:4:1) as solvent system and the spot was located by exposure to iodine vapours. The physical data of the compounds so obtained are given in Table-1.

IR (KBr): 3012-2993 (C-H), 1693-1661 (C=O), 1573-1536 (-N=N-).

¹HNMR (CDCl₃): 8d; 1.65 (s, 1H, CH), 2.47 (s, 3H, COCH₂), 2.59 (s, 3H, COCH₂), 3.83 (s, 3H, OCH₂), 6.93 (d, 2H, 2',6'-ArH), 7.35 (d, 2H, 3',5'-ArH).

8g; 1.78 (s, 1H, CH), 2.35 (s, 3H, COCH₂), 2.43 (s, 3H, COCH₂), 7.37 (d, 2H, 2',6'-ArH), 7.51 (d, 2H, 3',5'-ArH).

Synthesis of 8-Quinolinoxycetic acid hydrazide (2)

In a round bottom flask, a mixture of Ethyl-8-quinolinoxycetic acid (0.01 mole), hydrazine hydrate (0.20 mole) and absolute ethanol (50 ml) was added. A condenser with calcium chloride guard tube was attached to the flask and mixture was refluxed for 30 hours on water bath. The mixture was concentrated, cooled and poured in crushed ice. It was kept for 4-5 hours at room temperature and solid mass separated out was filtered, dried and recrystallized from ethanol.

Purity of the compound (2) was checked by TLC on silica gel G plates using methanol: acetone (1:1) as

solvent system and the spot was located by exposure to iodine vapours.

Yield: 84 %, m.p. 136 °C, R_f: 0.57, Molecular formula: C₁₁H₁₁N₃O₂, Molecular weight: 217.23.

%N: Found: 19.03%; Calcd: 19.34 %. IR (KBr): 3225 (N-H), 2980 (C-H), 1689 (C=O), 1578 (C=C).

¹HNMR (CDCl₃): 4.90 (s, 2H, OCH₂), 7.15-8.23 (m, 8H, 6-ArH & 2-NH₂), 8.95 (bs, 1H, NH).

Synthesis of 1-(8'-Quinolinoxycetyl)-3,5-dimethyl-4-(4''-carboxyphenylazo) pyrazole (3a-j)

In a 100 ml round bottom flask, a mixture of compounds (1a-j) (0.005 mole) and (2) (0.005 mole) in glacial acetic acid (25 ml) was refluxed for 12-18 hours. The mixture was concentrated, cooled and poured into crushed ice. The solid mass separated out was filtered, dried and recrystallized from methanol. This has been shown in scheme-1.

Purity of the compounds (3a-j) was checked by TLC on silica gel G plates using toluene: ethylacetate: formic acid (5:4:1) as solvent system and the spot was located by exposure to iodine vapours.

Yield of 1-(8'-Quinolinoxycetyl)-3,5-dimethyl-4-(4''-carboxyphenylazo) pyrazole (3a) is 74 %, m.p. >300°C, R_f: 0.69, Molecular formula: C₂₃H₁₉N₅O₄, Molecular weight: 429.433.

%N: Found: 16.20%; Calcd: 16.31 %.

IR (KBr):3520 (COOH), 2978 (C-H), 1693 (C=O), 1681 (C=N), 1594 (C=C),1530 (-N=N-).

¹HNMR (DMSO-d₆): 2.46 (s, 6H, 3, 5-CH₃), 3.42 (s, 2H, OCH₂), 7.62-8.08 (m, 10H, ArH), 13.69 (s, 1H, COOH).

Yield of 1-(8'-Quinolinoxycetyl)-3,5-dimethyl-4-(2''-carboxyphenylazo) pyrazole (3b)

is 73 %, m.p. 182 °C, R_f: 0.72, Molecular formula: C₂₃H₁₉N₅O₄, Molecular weight: 429.433.

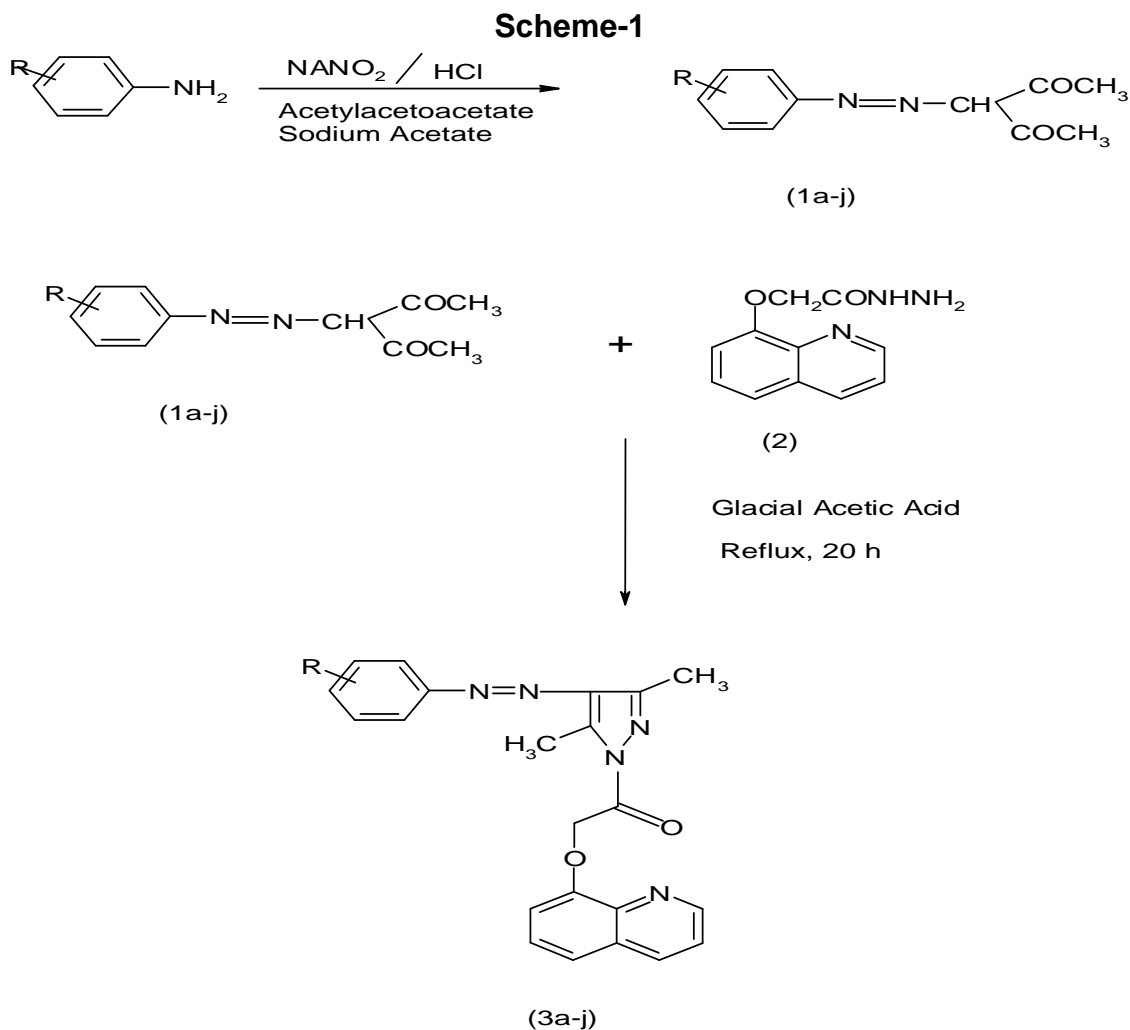
%N: Found: 16.52%; Calcd: 16.31 %.

IR (KBr):3537 (COOH), 2972 (C-H), 1679 (C=O), 1664 (C=N), 1603 (C=C),1542 (-N=N-).

Yield 1-(8'-Quinolinoxycetyl)-3,5-dimethyl-4-(2''-hydroxyphenylazo) pyrazole (3c)

51 %, m.p. 150°C, R_f: 0.60, Molecular formula: C₂₂H₁₉N₅O₃, Molecular weight: 401.423.

%N: Found: 17.27%; Calcd: 17.45 %.



R = 3a: 4-COOH, 3b: 2-COOH, 3c: 2-OH, 3d: 4-OMe, 3e: 4-Br, 3f: 4-F, 3g: 4-Cl,
3h: 2-Cl, 3i: 4-CH₃, 3j: 2-CH₃

Table-1 Physical data of the compounds (1a-j)

Compd	R	Yield (%)	m.p. (°C)	R _f	Mol. Weight	Mol. Formula	Nitrogen (%)	
							Found	Calcd.
1a	4-COOH	90.0	>350	0.69	248.24	C ₁₂ H ₁₂ N ₂ O ₄	10.98	11.28
1b	2-COOH	76.2	>350	0.80	248.24	C ₁₂ H ₁₂ N ₂ O ₄	11.13	11.28
1c	2-OH	67.3	142	0.78	220.23	C ₁₁ H ₁₂ N ₂ O ₃	12.56	12.72
1d	4-OCH ₃	90.0	108	0.86	234.25	C ₁₂ H ₁₄ N ₂ O ₃	12.08	11.96
1e	4-Br	83.4	130	0.91	283.12	C ₁₁ H ₁₁ N ₂ O ₂ Br	9.67	9.89
1f	4-F	62.5	126	0.87	222.22	C ₁₁ H ₁₁ N ₂ O ₂ F	12.51	12.61
1g	4-Cl	81.3	134	0.92	238.67	C ₁₁ H ₁₁ N ₂ O ₂ Cl	11.62	11.74
1h	2-Cl	76.4	110	0.89	238.67	C ₁₁ H ₁₁ N ₂ O ₂ Cl	11.66	11.74
1i	4-CH ₃	76.0	86	0.90	218.25	C ₁₂ H ₁₄ N ₂ O ₂	12.59	12.84
1j	2-CH ₃	71.8	105	0.90	218.25	C ₁₂ H ₁₄ N ₂ O ₂	12.75	12.84

IR (KBr): 3609 (OH), 2983 (C-H), 1688 (C=O), 1671 (C=N), 1578 (C=C), 1539 (-N=N-).

Yield of 1-(8'-Quinolinoxycetyl)-3,5-dimethyl-4-(4''-methoxyphenylazo) pyrazole (3d)

62 %, m.p. 164°C, R_f : 0.62, Molecular formula: $C_{23}H_{21}N_5O_3$, Molecular weight: 415.449.
%N: Found: 16.63%; Calcd: 16.86 %.

IR (KBr): 2977 (C-H), 1683 (C=O), 1669 (C=N), 1596 (C=C), 1556 (-N=N-).

Yield of 1-(8'-Quinolinoxycetyl)-3,5-dimethyl-4-(4''-bromophenylazo) pyrazole (3e)

70 %, m.p. 168 °C, R_f : 0.69, Molecular formula: $C_{22}H_{18}N_5O_2Br$, Molecular weight: 464.33.

%N: Found: 14.87%; Calcd: 15.08 %.

IR (KBr): 3008 (C-H), 1672 (C=O), 1665 (C=N), 1581 (C=C), 1551 (-N=N-), 550 (C-Br).

1H NMR ($CDCl_3$): 2.17 (s, 3H, 3- CH_3), 2.58 (merged s, 5H, 5- CH_3 & OCH_2), 7.56-7.68 (m, 10H, ArH).

Yield of 1-(8'-Quinolinoxycetyl)-3,5-dimethyl-4-(4''-fluorophenylazo) pyrazole (3f)

63 %, m.p. 172 °C, R_f : 0.82, Molecular formula: $C_{22}H_{18}N_5O_2F$, Molecular weight: 403.414.

%N: Found: 17.31%; Calcd: 17.36 %.

IR (KBr): 2986 (C-H), 1687 (C=O), 1677 (C=N), 1580 (C=C), 1538 (-N=N-), 1049 (C-F).

Yield of 1-(8'-Quinolinoxycetyl)-3,5-dimethyl-4-(4''-chlorophenylazo) pyrazole (3g)

60 %, m.p. 154°C, R_f : 0.90, Molecular formula: $C_{22}H_{18}N_5O_2Cl$, Molecular weight: 419.869.

%N: Found: 16.71%; Calcd: 16.68 %.

IR (KBr): 2981 (C-H), 1687 (C=O), 1675 (C=N), 1594 (C=C), 1534 (-N=N-), 723 (C-Cl).

1H NMR ($CDCl_3$): 2.17 (s, 3H, 3- CH_3), 2.58 (merged s, 5H, 5- CH_3 & OCH_2), 7.36-7.75 (m, 10H, ArH).

Mass (m/z): 420 (M^+), 233, 186, 139.

Yield of 1-(8'-Quinolinoxycetyl)-3,5-dimethyl-4-(2''-chlorophenylazo) pyrazole (3h)

54 %, m.p. 178°C, R_f : 0.68, Molecular formula: $C_{22}H_{18}N_5O_2Cl$, Molecular weight: 419.869.

%N: Found: 16.53%; Calcd: 16.68 %.

IR (KBr): 2993 (C-H), 1692 (C=O), 1669 (C=N), 1587 (C=C), 1547 (-N=N-), 719 (C-Cl).

Yield of 1-(8'-Quinolinoxycetyl)-3,5-dimethyl-4-(4''-methylphenylazo) pyrazole (3i)

58 %, m.p. 148°C, R_f : 0.71, Molecular formula: $C_{23}H_{21}N_5O_2$, Molecular weight: 399.45.

%N: Found: 17.44%; Calcd: 17.53 %.

IR (KBr): 2981 (C-H), 1689 (C=O), 1676 (C=N), 1591 (C=C), 1552 (-N=N-).

Yield of 1-(8'-Quinolinoxycetyl)-3,5-dimethyl-4-(2''-methylphenylazo) pyrazole (3j)

48 %, m.p. 136°C, R_f : 0.63, Molecular formula: $C_{23}H_{21}N_5O_2$, Molecular weight: 399.45.

%N: Found: 17.37%; Calcd: 17.53 %.

IR (KBr): 3006 (C-H), 1658 (C=O), 1673 (C=N), 1588 (C=C), 1539 (-N=N-), 723 (C-Cl).

1H NMR ($CDCl_3$): 2.39 (s, 3H, CH_3 -Ar), 2.50 (s, 3H, 3- CH_3), 2.58 (s, 3H, 5- CH_3), 2.62 (s, 2H, OCH_2), 7.24-7.60 (m, 10H, ArH).

Biological activity

The antimicrobial screening results of the pyrazole derivatives bearing quinoline moiety (**3a-j**) showed that some of the compounds exhibited significant antibacterial activity, at the same time, the compounds showed only moderate antifungal activity.

Out of all the synthesized pyrazole derivatives of the series, compound **3g** having chloro group at the 4th position of the phenyl ring exhibited remarkable antibacterial activity (MIC 25 $\mu\text{g mL}^{-1}$), against *E. coli* (gram negative bacteria), where as the compound **3h** having chloro group at the 2nd position of the phenyl ring showed similar antibacterial activity (MIC 25 $\mu\text{g mL}^{-1}$), against *S. aureus* (gram positive bacteria), as compared with the standard drug ofloxacin (MIC 10.0 $\mu\text{g mL}^{-1}$ against *S. aureus* and 12.5 $\mu\text{g mL}^{-1}$ against *E. coli*).

The result of antifungal activity in table-2 have shown that only the compounds **3a** and **3b** having COOH group at 4th and 2nd positions of the phenyl ring respectively, have shown moderate activity (MIC 50 $\mu\text{g mL}^{-1}$) against fungus *A. niger*, as

Table-2 Antimicrobial Activities of Quinoloinoxy acetyl Pyrazole Derivatives (3a-j)

Compound No.	MIC ($\mu\text{g/ml}$)		
	<i>S. aureus</i>	<i>E. coli</i>	<i>A. niger</i>
Ofloxacin	10.0	12.5	-
Ketoconazole	-	-	12.5
3a	50	>200	50
3b	100	200	50
3c	>200	100	200
3d	200	100	>200
3e	200	100	100
3f	100	50	100
3g	50	25	100
3h	25	50	100
3i	100	200	100
3j	200	100	>200

compared with the standard drug ketoconazole (MIC $12.5 \mu\text{g mL}^{-1}$).

Results and Discussion

2,4-Diketo-3-(aryloxy)-propane (1a-j)

The purity of the compounds (1a-j) was checked by TLC and its characterization on the basis of IR and NMR spectral data.

The IR spectrum of the compounds (1a-j) showed peaks at $3012-2993 \text{ cm}^{-1}$, CH stretching; $1693-1661 \text{ cm}^{-1}$, C=O stretching and $1573-1536 \text{ cm}^{-1}$, -N=N- stretching vibrations.

The NMR spectrum of the compounds (1d) and (1g) showed a singlet respectively at δ 1.65, δ 1.78 indicating the presence of N-CH proton.

compounds (1d) and (1g) showed two singlets as singlet respectively at δ 2.47, δ 2.59, δ 2.35, δ 2.43 of two COCH_3 protons and two doublets respectively at δ 6.93 and δ 7.35, δ 7.37 and δ 7.51 indicating the presence of 2',6'- and 3',5'- aromatic protons respectively.

Compound (1d) showed a singlet at δ 3.83 of OCH_3 protons attached to the phenyl ring.

8-Quinoloinoxyacetic acid hydrazide (2)

The IR spectrum of the compound (2) showed peaks at 3225 cm^{-1} , NH stretching; 2980 cm^{-1} , CH stretching; 1689 cm^{-1} , C=O stretching and 1578 cm^{-1} , C=C stretching vibrations of aromatic ring.

Its NMR spectrum which showed a singlet at δ 4.90 OCH_2 protons. In the aromatic region a multiplet of eight protons at δ 7.15-6.23 was observed indicating the presence of six aromatic and two NH_2 protons. The broad singlet of CONH proton was observed at δ 8.95.

1-(8-Quinoloinoxyacetyl)-3,5-dimethyl-4-(substituted phenylazo) pyrazoles (3a-j)

The IR spectrum of the compounds (3a-j) showed peaks at $3008-2972 \text{ cm}^{-1}$, CH stretching; $1693-1658 \text{ cm}^{-1}$, C=O stretching of pyrazoline ring; $1681-1664 \text{ cm}^{-1}$, C=N stretching; $1556-1530 \text{ cm}^{-1}$, -N=N- stretching and $1603-1578 \text{ cm}^{-1}$, C=C stretching vibrations of aromatic rings.

The NMR spectrum of the compounds (3a, 3e, 3g, 3j) showed a singlet respectively at δ 2.46 (merged singlet of 3 and 5 methyl groups attached to the pyrazole ring), δ 2.17, δ 2.17, δ 2.39 of methyl group at 3rd position attached to pyrazole ring of (3e, 3g) and to phenyl ring of (3j).

A singlet respectively at δ 3.42, δ 2.58, δ 2.58 [The signals of OCH_2 protons and 5- CH_3 protons were merged together of compounds (3e, 3g)], δ 2.62 of OCH_2 protons.

Compound (3j) showed two singlets at δ 2.57 and 2.58 of methyl groups attached to the 3rd and 5th position of the pyrazole ring.

A multiplet respectively at δ 7.62-8.08, δ 7.56-7.68, δ 7.36-7.75, δ 7.24-7.60 of 10 aromatic protons.

Mass spectral data of compound (3g) showed molecular ion peak M^+ at m/z 420, corresponding to the molecular formula $C_{22}H_{18}N_5O_2Cl$. Further peaks were observed at m/z 233, 186 and 139.

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