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Synthesis and antibacterial studies of some novel isoxazoline derivatives

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Abstract: A series of 3-[3-(2,4-dichloro-5-fluorophenyl)-5-(2-furyl)-4,5-dihydro-1*H*-pyrazol-1-yl]-5-(substituted phenyl/2-thienyl)isoxazolines (**4a-j**) were prepared. The structures of the isoxazoline derivatives were confirmed on the bases of elemental analysis and spectral data. The compounds were screened for their *in vitro* antibacterial activity using gram-positive bacteria and gram-negative bacteria.

Keywords: isoxazoline, chalcone, antibacterial activity, heterocyclic synthesis.

INTRODUCTION

The classical synthesis of the title compounds involves the base-catalyzed condensation of substituted aromatic ketone and substituted aldehydes to give α,β-unsaturated ketones (chalcones), which on cyclization with hydroxylamine hydrochloride in alkaline medium give the corresponding isoxazoline derivatives.

In recent years, attention has increasingly been given to the synthesis of isoxazoline derivatives as a source of new antibacterial agents. The synthesis of novel isoxazoline derivatives remains a main focus of medicinal research. Isoxazoline derivatives have been reported to possess antifungal,[1] antibacterial,[2] anticonvulsant,[3] antiinflammatory,[4] antiviral[5] and analgesic[6] activity. In recent years, fluorinated acetophenones have found an important place in the manufacture of drugs, such as ciprofloxacin.[7] Moreover, incorporation of fluorine can alter the course of the reaction as well as the biological activities. In addition, isoxazoline derivatives have played a crucial role in the theoretical development of heterocyclic chemistry and are also used extensively in organic synthesis.[8–12]

Encouraged by the diverse biological activities of fluorinated isoxazoline and pyrazoline compounds, it was decided to prepare a new series of fluorine containing 3-[3-(2,4-dichloro-5-fluorophenyl)-5-(2-furyl)-4.5-dihydro-1*H*-pyrazol-1-yl]-5-(substituted phenyl/2-thienyl)isoxazolines (**4a-j**) thus bringing both types of compounds to-

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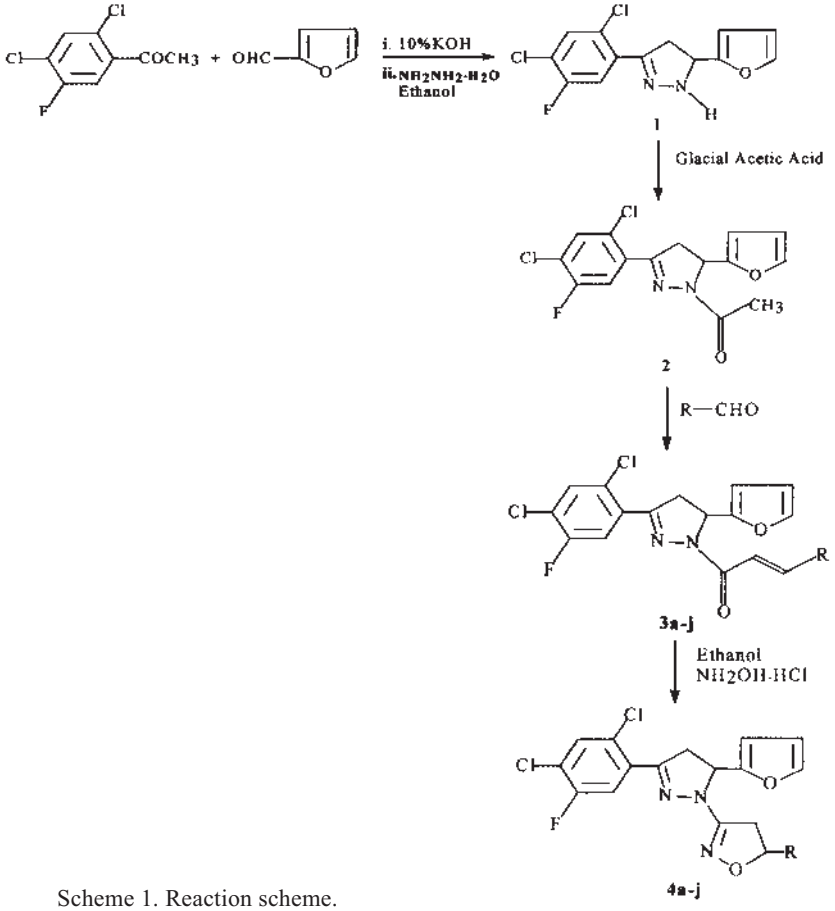
gether in a single molecule, and to screen the newly synthesized compounds for their antibacterial activities.

EXPERIMENTAL

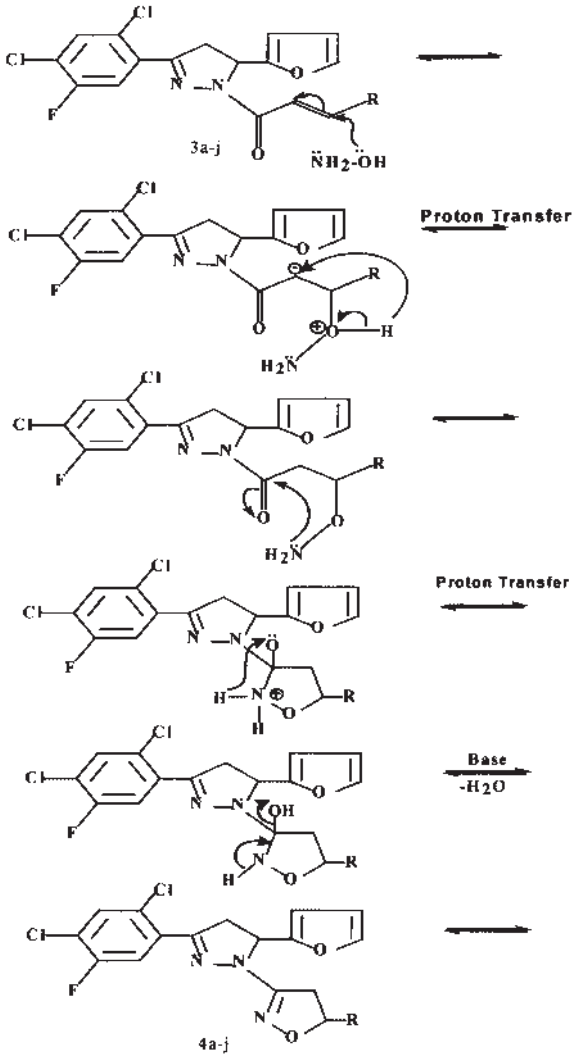
All melting points were determined in an open capillary and are uncorrected. Elemental analysis was performed by C.D.R.I., Lucknow and results are within ±0.4% of the calculated values. The infrared (IR) spectra were recorded on a FTIR-8400 Shimadzu spectrometer using potassium bromide pellets. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Bruker Avance dpx-200 spectrometer (at 200 MHz) using tetramethylsilane (TMS) as the internal standard. All reagents were of the highest purity commercially available. The chemical shifts are expressed in part per million (ppm) downfield from the internal standard; the coupling constants are in Hz, and signals are quoted as *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet), or *m* (multiplet). Thin layer chromatography analytical separation were conducted with silica gel 60 F-254 (Merck) plates of 0.25 mm thickness eluted with either benzene:CH_3OH (9:1) or $CHCl_3$:CH_3OH (8:2) and were visualized with UV (254 nm) or iodine to check the purity of the compounds.

In this study 2,4-dichloro-5-fluoroacetophenone on condensation with furan-2-carbaldehyde according to the Claisen-Schmidt condensation[13–15] gave 1-(2,4-dichloro-5-fluorophenyl)-3-(2-furyl)-2-propen-1-one, which on reaction with hydrazine hydrate (80%) yielded 3-(2,4-dichloro-5-flu-



Scheme 1. Reaction scheme.

orophenyl)-5-(2-furyl)pyrazoline (**1**) (Scheme 1). This compound on acetylation with glacial acetic acid[16–18] gave the 1-acetylpyrazoline derivative (**2**), which on condensation with a substituted aromatic aldehyde afforded the corresponding chalcones (**3a-j**). These chalcones were further reacted with hydroxylamine hydrochloride in alkaline medium to yield the corresponding isoxazoline derivatives (**4a-j**) by the following reaction mechanism (Scheme 2).



Scheme 2. Reaction mechanism.

*Preparation of 3-(2,4-dichloro-5-fluorophenyl)-5-(2-furyl)pyrazoline (**1**)*

2,4-Dichloro-5-fluoroacetophenone (0.01 mol) and furan-2-carbaldehyde (0.01 mol) in ethanol (50 mL) were stirred at 32 °C for 30 min. Then, 10 % KOH (3 mL) was added in it and the reaction mixture was stirred for 4 h and then kept for 24 h at room temperature. The reaction mixture was then poured into ice-cold water and acidified with dilute HCl. The formed precipitate was filtered off, washed with distilled water and recrystallised from ethanol to afford the chalcone. This chalcone (0.01 mol) and hydrazine hydrate (0.02 mol) in ethanol (25 mL) were refluxed on a water bath for 6 h. The reaction mixture was then cooled, poured into ice-cold water and acidified with dil.

HCl. The separated solid was filtered, washed with water, dried and recrystallised from ethanol, m.p. 112 °C. Yield 71 %. Found: C: 52.11; H: 3.06; N: 9.29, Calcd. for $C_{13}H_9Cl_2FN_2O$: C: 52.17; H: 3.01; N: 9.36 %. IR (KBr): =CH str. (3080 cm^{-1}), C–H bending [1,4-substituted (848 cm^{-1})], C–Cl str. (794 cm^{-1}), C–F str. (1018 cm^{-1}), C=N (pyrazoline ring, 1604 cm^{-1}), –NH (3280 cm^{-1}). ^{1}H NMR ($CDCl_3$); δ 3.3 (1H, *dd*, $–CH_{2pyraz}$), δ 3.92 (1H, *dd*, $–CH_{2pyraz}$), δ 6.3 (1H, *dd*, $–CH_{pyraz}$), δ 7.55–7.70 (4H, *m*, Ar-H) and δ 9.25 (1H, *s*, –NH) confirms the presence of compound **1**.

*Preparation of 1-acetyl-3-(2,4-dichloro-5-fluorophenyl)-5-(2-furyl)pyrazoline (**2**)*

3-(2,4-Dichloro-5-fluorophenyl)-5-(2-furyl)pyrazoline (**1**) was refluxed in glacial acetic acid (25 mL) for 5 h. Then the reaction mixture was concentrated. On cooling, a solid formed which was filtered, washed with water until neutral pH and recrystallised using chloroform/methanol m.p. 158 °C. Yield: 67 %. Found: C: 52.75; H: 3.21; N: 8.25, Calcd. for $C_{15}H_{11}Cl_2FN_2O_2$: C: 52.79; H: 3.23; N: 8.21%. IR (KBr): C=C str. (1575 cm^{-1}), C–H bending [1,2,4,5-substituted (870 cm^{-1})], C–Cl str. (750 cm^{-1}), C–F str. (1040 cm^{-1}), C=N (pyrazoline ring, 1612 cm^{-1}), $–NCOCH_3$ (1662 cm^{-1}), –C–O–C– (1270 cm^{-1}). ^{1}H NMR ($CDCl_3$); δ 3.2 (1H, *dd*, $–CH_{2pyraz}$), δ 3.89 (1H, *dd*, $–CH_{2pyraz}$), δ 6.1 (1H, *dd*, CH_{pyraz}), δ 2.7 (3H, *s*, $–COCH_3$), and δ 7.40–7.55 (*m*, 4H, Ar-H) confirms the presence of compound **2**.

*Preparation of 1-[3-(2,4-dichloro-5-fluorophenyl)-5-(2-furyl)-4,5-dihydro-1*H*-pyrazol-1-yl]-3-(3,4,5-trimethoxyphenyl)-2-propen-1-one (**3a**)*

A mixture of 1-acetyl-3-(2,4-dichloro-5-fluorophenyl)-5-(2-furyl)pyrazoline (**2**) (0.01 mol) and 3,4,5-trimethoxybenzaldehyde (0.01 mol) in ethanol (50 mL) was stirred at 32 °C for 30 min. Then 10 % KOH (3 mL) was added and the stirring continued for a further 4 h. After standing at room temperature for 24 h, the reaction mixture was poured into ice-cold water and acidified with dilute HCl. The formed precipitate was filtered off, washed with water, dried and recrystallised from dimethyl formamide/water. The crude product was recrystallised using dimethyl formamide/water. All the substituted chalcones were prepared by the same procedure. M.p. 142 °C. Yield: 74 %. Found: C: 57.84; H: 4.09; N: 5.35, Calcd. for $C_{25}H_{21}Cl_2FN_2O_5$: C: 57.80; H: 4.05; N: 5.39 %. IR (KBr): =CH str. (3060 cm^{-1}), C=C str. (1564 cm^{-1}), C–Cl str. (794 cm^{-1}), C–F str. (1045 cm^{-1}), C=N (pyrazoline ring, 1604 cm^{-1}), –C–O–C– (sym, 1255 cm^{-1}), –C=C–C=O (1668 cm^{-1}). ^{1}H NMR ($CDCl_3$): δ 3.12 (1H, *dd*, $–CH_{2pyraz}–$), δ 3.86 (1H, *dd*, $–CH_{2pyraz}–$), δ 5.9 (1H, *dd*, CH_{pyraz}), δ 3.9 (*s*, 9H, 3,4,5-OCH_3) and δ 7.25–7.55 (4H, *m*, Ar-H) confirms the presence of compound **3a**.

*Preparation of 3-[3-(2,4-dichloro-5-fluorophenyl)-5-(2-furyl)-4,5-dihydro-1*H*-pyrazol-1-yl]-5-(3,4,5-trimethoxyphenyl)isoxazoline (**4a**)*

A mixture of 1-[3-(2,4-dichloro-5-fluorophenyl)-5-(2-furyl)-4,5-dihydro-1*H*-pyrazol-1-yl]-3-(3,4,5-trimethoxyphenyl)-2-propen-1-one (**3a**) (0.01 mol), hydroxylamine hydrochloride (0.01 mol) and distilled water (2 mL) in ethanol (25 mL) was refluxed on a water bath for 5 h in the presence of 30 % KOH. The reaction mixture was then poured into ice cold water and acidified with dilute HCl. The formed solid was filtered, washed with water until neutral pH, dried and recrystallised from ethanol. M.p. 141 °C Yield 69 %. Found: C: 56.14; H: 3.10; N: 7.80, Calcd. for $C_{25}H_{22}Cl_2FN_3O_5$: C: 56.18; H: 3.12; N: 7.87 %. IR (KBr): C=C str. (1525 cm^{-1}), C–Cl str. (778 cm^{-1}), C–F str. (1035 cm^{-1}), C=N (pyrazoline ring, 1635 cm^{-1}), –C–O–C– (sym., 1245 cm^{-1}), –C–O–N– (1231 cm^{-1}). ^{1}H NMR ($CDCl_3$); δ 3.15 (1H, *dd*, CH_{2isox}), δ 3.95 (1H, *dd*, CH_{2isox}), δ 6.2 (1H, *dd*, CH_{isox}), δ 3.81 (*s*, 9H, 3,4,5-OCH_3), and δ 7.35–7.65 (4H, *m*, Ar-H) confirms the presence of compound **4a**.

All compounds were synthesized by the same procedure and their formulas, melting points, yields and analytical data are shown in Table I.

TABLE I. Formulas, melting points, yields and analytical data of the isoxazolines **4a-j**

Compd.	R	Molecular formula	M.p. °C	Yield %	Found (Calcd.)/%		
					C	H	N
4a	3,4,5-Trimethoxyphenyl	$C_{25}H_{22}Cl_2FN_3O_5$	141	69	56.14 (56.18)	4.10 (4.12)	7.80 (7.87)
4b	3-Phenoxyphenyl	$C_{28}H_{20}Cl_2FN_3O_3$	138	55	62.71 (62.69)	3.69 (3.73)	7.80 (7.84)
4c	4-Methoxyphenyl	$C_{23}H_{18}Cl_2FN_3O_3$	167	72	58.26 (58.23)	3.77 (3.80)	8.89 (8.86)
4d	2-Chlorophenyl	$C_{22}H_{15}Cl_3FN_3O_2$	161	70	55.23 (55.17)	3.10 (3.14)	8.82 (8.78)
4e	4-Hydroxyphenyl	$C_{22}H_{16}Cl_2FN_3O_3$	159	63	57.42 (57.39)	3.53 (3.48)	9.17 (9.13)
4f	4-Fluorophenyl	$C_{22}H_{15}Cl_2F_2N_3O_2$	147	67	57.21 (57.14)	3.30 (3.25)	9.12 (9.09)
4g	2-Hydroxy-4-methoxyphenyl	$C_{23}H_{18}Cl_2FN_3O_4$	144	66	56.40 (56.33)	3.69 (3.67)	8.53 (8.57)
4h	2-Nitrophenyl	$C_{22}H_{15}Cl_2FN_4O_4$	127	71	53.97 (53.99)	3.09 (3.07)	11.39 (11.45)
4i	4-*N,N*-Dimethylaminophenyl	$C_{24}H_{21}Cl_2FN_4O_2$	124	62	59.20 (59.14)	4.35 (4.31)	11.55 (11.50)
4j	2-Thienyl	$C_{20}H_{14}Cl_2FN_3O_2S$	156	65	53.40 (53.33)	3.14 (3.11)	7.15 (7.11)

RESULTS AND DISCUSSION

The structure of compounds **4a-j** were confirmed on the basis of spectral and elemental analysis. The IR spectrum of **4a-j** exhibited a band due to =CH str. (3100–3000 cm^{-1}), C=C str. (1635–1495 cm^{-1}), C–H bending [1,2,4,5-substituted (900–860 cm^{-1})], C–H bending [1,4-substituted (840–800 cm^{-1})], C–Cl str. (750–700 cm^{-1}), C–F str. (1100–1000 cm^{-1}), C=N (ring) (1650–1580 cm^{-1}) stretching vibration band which indicates the presence of the isoxazoline ring. Further, in their ^{1}H-NMR ($CDCl_3$) spectrum, the appearance of a signal at δ 3.3 (*d*, 2H, –C**H**$_2$–CH, isoxazoline) and δ 6.3 (*s*, 1H, –CH$_2$–C**H**, isoxazoline) confirms the presence of the isoxazoline ring.

Antibacterial activity

The target molecules were tested for antibacterial activity against the variety of test organisms *Escherichia coli*, *Pseudomonas aeruginosa* (gram-negative bacteria) and *Staphylococcus aureus, Salmonella paratyphi* A (gram-positive bacteria).

The screening results indicate that compounds **4a** and **4f** show promising activity and compounds **4b** and **4e** poor activity against *E. coli*. Compounds **4a** and **4f** show good activity and compounds **4b** and **4e** low activity against *P. aeruginosa.* Compounds **4a** and **4i** show high activity and compounds **4b** and **4e** low activity against *S. aureus*. Compounds **4a** and **4j** show high activity and compounds **4b** and **4c** low activity against *S. paratyphi* A.

TABLE II. Antibacterial activity of the compounds **4a-j**

Compounds	Antibacterial activity			
	Diameter of zone of inhibition (in mm)			
	S. aureus	*S. paratyphi* A	*E. coli*	*P. aeruginosa*
4a	13	12	14	10
4b	7	4	6	5
4c	8	7	5	8
4d	6	8	8	6
4e	5	4	5	6
4f	10	6	12	11
4g	8	7	6	9
4h	6	8	8	7
4i	12	5	8	8
4j	8	11	5	4
Ampicillin	20	24	21	18

The zones of inhibition of the reference compound ampicillin are also given in Table II. The result indicates that the presence of methoxy-, chloro-, and fluoro-groups enhanced the antibacterial activity. However, no specific structure–activity relationship could be established.

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ИЗВОД

СИНТЕЗА И ИСПИТИВАЊЕ АНТИМИКРОБНЕ АКТИВНОСТИ НЕКИХ НОВИХ ИЗОКСАЗОЛИНСКИХ ДЕРИВАТА

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Добијена је серија 3-[3-(2,4-дихлоро-5-флуорофенил)-5-(2-фурил)-4,5-дихидро-1*H*-пиразол-1-ил]-5-(супституисани фенил/2-тијенил)-изоксазолина (**4a–j**). Структуре изоксазолинских деривата су потврђене на основу елементалне анализе и спектралних података. Испитана је антибактеријска активност ових једињења према грам-позитивним и грам-негативним бактеријама.

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