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# Antimicrobial Evaluation of Some New Pyrazolyl Pyridinyl Coumarins

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## ABSTRACT

*The synthesis of various pyrazolyl pyridinyl substituted coumarins 3-{4'-[1''-phenyl -3''- (pyridin-3''' -yl)-1H- pyrazol- 4''-yl]- 6'-aryl -pyridin -2'-yl} coumarins (4a-f) and 3-{4'-[1''-phenyl-3''-(pyridin-4'''-yl)-1H-pyrazol-4''-yl]-6'-aryl-pyridin-2'-yl}coumarins (5a-f) have been carried out by the reaction of 3-(3-(1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl)acryloyl)coumarins or 3-(3-(1-phenyl–3- (pyridin-4-yl)-1H–pyrazol-4-yl) acryloyl) coumarins with appropriate aroyl methyl pyridinium salts under Krohnke's reaction condition. The synthesized compounds were fully characterized by Infrared (IR), Proton Nuclear Magnetic Resonance ($^{1}$H-NMR), Carbon-13 Nuclear Magnetic Resonance ($^{13}$C-NMR), DEPT-135, DEPT-90 and representative Mass spectral data. The synthesized compounds were screened for in vitro antimicrobial activity using Broth micro dilution method.*

**Keywords:** Coumarins, Pyridinylcoumarins, Pyrazole, Kröhnke reaction, Antimicrobial activity.

## INTRODUCTION

Among five membered nitrogen containing heterocycles, pyrazole is a prominent moiety. A large number of compounds having pyra-zole nucleus in their structure are reported to have wide range of biological activities viz., antioxidant [1], anti-invasive [2], antiviral [3], anti-inflammatory [4,5] and are also used as agrochemicals [6] and dyestuffs [7,8]. Celecoxib [9] was the first to market this class of compounds used as anti-inflammatory and analgesic agents. Phenylbutazone [9] has been used in the treatment of severe arthritis. The pyrazole derivative like Tartrazine is used as food colourant [9]. Soliman [10] and Shatik [11] have reported some pyrazole derivatives, which were found to be useful as anticancer agents. Singh et al. [12] have synthesized series of pyrazole derivatives by the condensation of appropriate 2-hydrazino-4-arylthiazoles with various α-cyanoacetophenones. The compounds exhibited anti-inflammatory and anti-antagonist activities. Wariishi [13,14] has reported some pyrazole derivatives having indole moiety. These compounds were reported to possess properties of photographic dye. Lange et al. [15,16] have synthesized antagonistic active pyrazole derivatives. Armand et al. [17,18] have synthesized analgesic and anti-inflammatory pyrazole derivatives. Some selected medicinally useful pyrazoles are shown in **(**Chart 1).

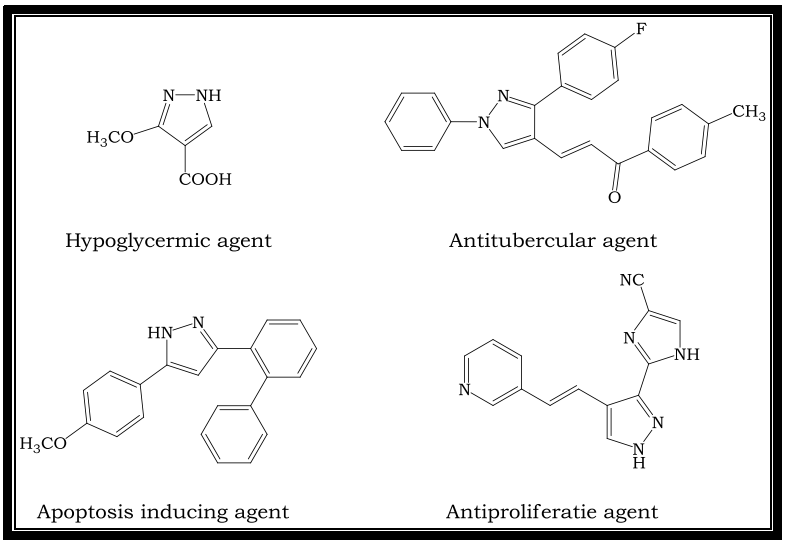



**Chart 1: Medicinally useful pyrazoles**

Moffett tested the synthesized pyridyl coumarin derivatives for various physiological activities. Most of them showed a central nervous system depression property [17,18]. 3-(3-Pyridyl)coumarin inhibited monoamine oxidase (MAO) *in vitro* and hence was considered as possible stimulant. 6-Bromo-3-(2-pyridyl) and 6-bromo-3-(3-pyridyl) coumarins were found to be antifungal agents *in vitro* [19,20]. Certain derivatives of these class of compounds were also found to be useful UV screening agents and as optical brightening agents for textile. When certain pyridyl derivatives having $–NH_2$ function in the benzene ring were reacted with fluosilicic acid, they formed amine-fluosilicate salts which were found to be effective as moth-proofing agents [21,22].

Srenivasulu et al. [23] have synthesized some 3-(3-pyridyl)coumarin derivatives. The compounds were synthesized by reacting substituted salicylaldehyde/o-hydroxy acetophenone with 3-pyridine acetic acid or its sodium salt under Perkin reaction conditions. These compounds were reported to have fish toxicity and bactericidal activities. Bragg and Wibberely [24] have synthesized 3-(2-pyridyl) and 3-(4-pyridyl) coumarins using Knoevenagel reaction, in which salicylaldehyde was treated with ethyl-2-pyridyl acetate or ethyl-4-pyridyl acetate in the presence of piperidine. Mohareb et al. [25] have synthesized 3-(2-pyridyl) coumarin with substituents in pyridine nucleus. Some selected biologically active pyridyl substituted coumarins are shown in (Chart 2).

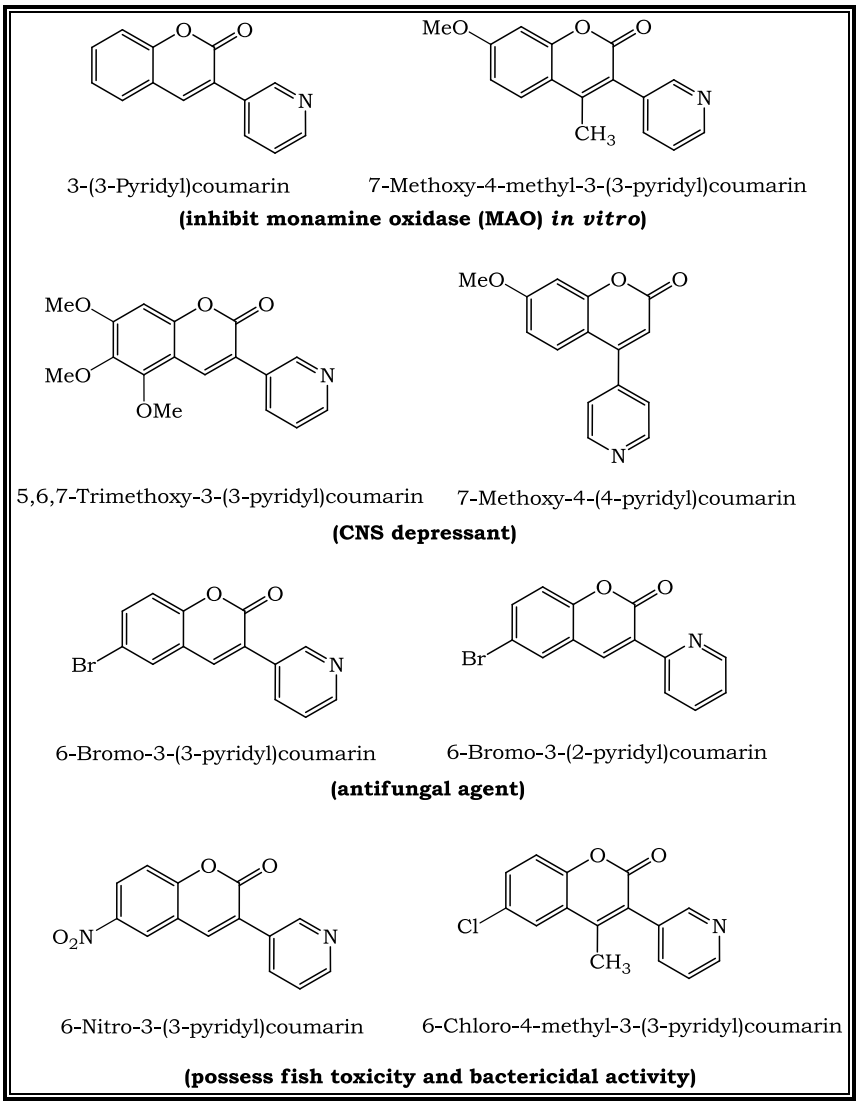



**Chart 2: Selected biologically active pyridyl substituted coumarins**

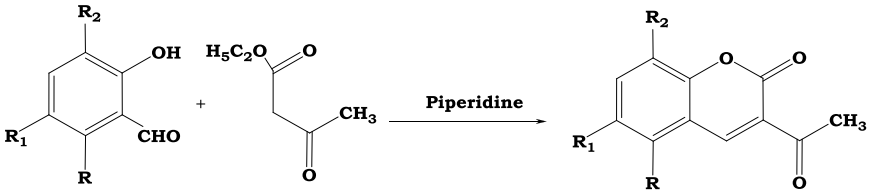
Thus pyridinyl coumarins and pyrazole are very important heterocycles from bioactivity view point, therefore in the present work, some pyrazolyl pyridinyl substituted coumarins 4a-f and 5a-f have been carried out by Krohnke's reaction [26,27], which are combination of both components i.e. pyrazole as well as pyridinyl coumarin in a single scaffold.

## MATERIALS AND METHODS

All melting points (ºC) are uncorrected. The yields of all compounds reported are of crystallized. All solvents used were distilled and dried. The purity of the compounds was checked by TLC (TLC aluminium sheet silica gel 60 $F_{254}$, Merck). Column chromatography was performed on silica gel (60-120 mesh). C, H, N analysis were carried out on Parkin-Elmer 2400 C-H-N-S-O Analyzer Series II. Infrared (IR) spectra were recorded in KBr pellets on Shimadzu FT-IR 8400-S spectrometer or Parkin-Elmer Frontier Fourier Transform Infrared (FTIR) /FIR spectrometer. Mass spectra were recorded using a Perkin-Elmer Clarus 500 spectrometer. NMR spectra were recorded using a Bruker Avance 400 spectrometer or Varian 400 spectrometer, operating at 400 MHz for Proton Nuclear Magnetic Resonance ($^1$H-NMR) and 100 MHz for Carbon-13 Nuclear Magnetic Resonance ($^{13}$C-NMR /DEPT-90/DEPT-135. The chemical shift (δ) is reported in ppm using $CDCl_3$/DMSO as a solvent, and calibrated standard solvent signal.

**Experimental Section**

*Preparation of 3-acetyl coumarins (Scheme 1)*

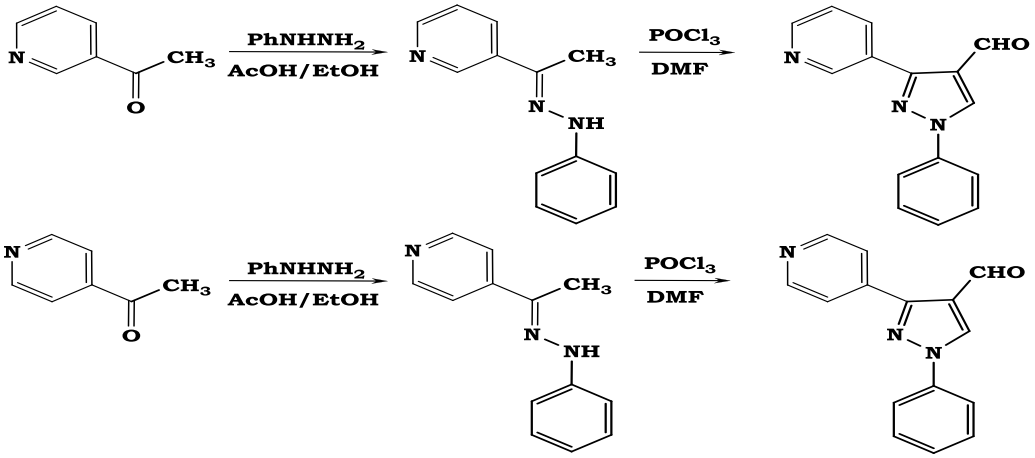



**Scheme 1: Synthesis of 3-acetyl coumarins**

In a 100 ml round bottom flask, a mixture of an appropriate salicylaldehyde (0.01 mol), ethyl acetoacetate (0.01 mol) and 3-4 drops of piperidine was stirred for 10 min at room temperature. It was then heated for 30 min in water bath. On cooling, a yellow solid product was obtained, which was filtered out and washed with cold ether. It was recrystallized from chloroform-hexane.

**3-Acetyl coumarin**: R=$R_1$=$R_2$=H, Yield: 97%, M. p. 119°C (lit. [28] mp 120°C)
**8-Methoxy-3-acetyl coumarin**: R=$R_1$= H, $R_2$=$OCH_3$, Yield: 95%, M. p. 171°C (lit. [28] M. p. 174°C)
**5,6-Benzo-3-acetyl coumarin**: R=$R_1$=benzo, $R_2$=H, Yield: 86%, M. p. 185°C (lit. [26,27] M. p. 186°C)

*Preparation of 1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-alde-hyde and 1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-aldehyde (Scheme 2)*




**Scheme 2: Synthesis of Preparation of 1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-alde-hyde and 1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-aldehyde**

The following general procedure was used.
In a 100 ml round bottom flask, a mixture of an appropriate acetylpyridine (0.01 mol), phenyl hydrazine (0.01 mol) and ethanol (5 ml) containing 1-2 drops of glacial acetic acid was warmed on a steam cone for 15 min. The separated phenyl hydrazone was filtered off, washed with cold ethanol (5 ml) and dried. It was recrystallized from ethanol.

The freshly prepared acetyl pyridine phenyl hydrazone (0.06 mol) was taken in a 250 ml three necked round bottom flask fitted with addition funnel and guard tube. Then anhydrous Dimethyl Formamide (DMF) (0.6 mol) was added and the reaction mixture was cooled to 0°C with stirring. To this reaction mixture, phosphorous oxychloride ($POCl_3$) (0.18 mol) was added dropwise with stirring during one hour at 0°C. The reaction mixture was further stirred at 0°C for 1 h and then heated at 65°C-70°C for two hours. It was then poured into crushed ice (200 g) and left overnight in a refrigerator, during which a solid product was separated out which was filtered off, washed with sodium carbonate (5%, 3 × 30 ml) and water. It was then dried and recrystallized from ethanol.

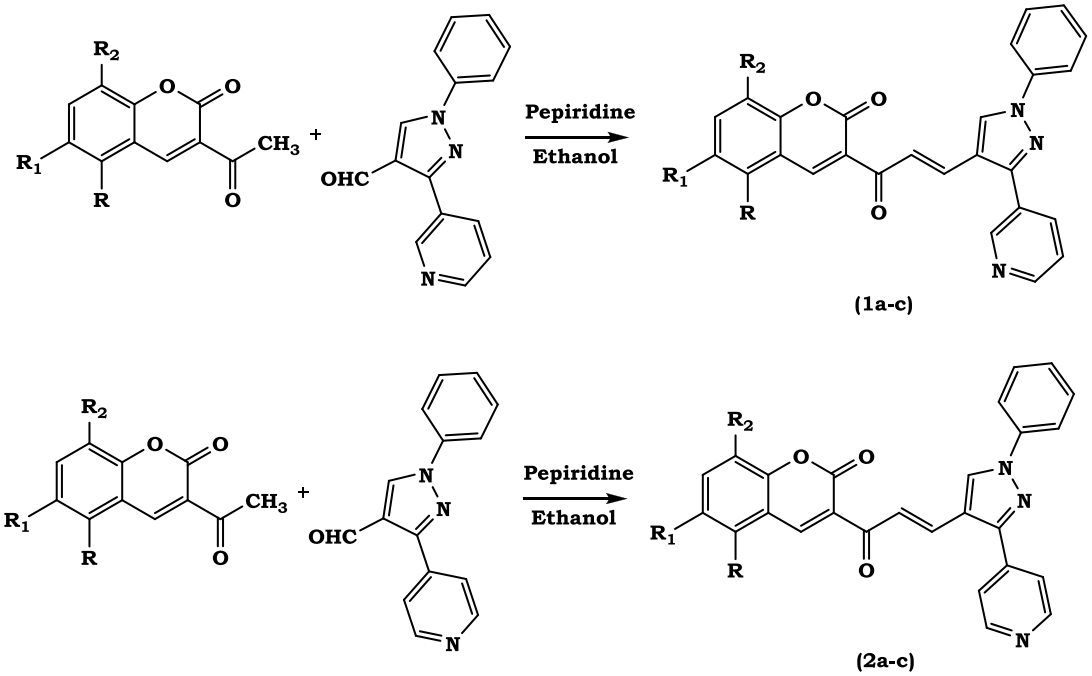
*1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-aldehyde*

| **Molecular Formula** | $C_{15}H_{11}N_3O$ | | |
|---|---|---|---|
| **Yield** | 82% | | |
| **M. p.** | 158°C | | |
| **Analysis** | %C | %H | %N |
| **Found** | 72.17 | 4.38 | 16.77 |
| **Calculated** | 72.28 | 4.45 | 16.86 |
| **IR ($cm^{-1}$)** | $V_{max}$ 1681 (C=O stretching of aldehyde), 1599 and 1531 (aromatic C=C and C=N stretchings), 681 and 754 (C-H out of plane bending vibrations of mono substituted benzene ring), 3132 (aromatic C-H stretching). | | |
| **$^1$H-NMR $CDCl_3$ (δ, ppm)** | 7.42-9.14 (10H, multiplet, aromatic protons), 10.08 (1H, singlet, proton of CHO). | | |

*1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-aldehyde*

| **Molecular Formula** | $C_{15}H_{11}N_3O$ | | |
|---|---|---|---|
| **Yield** | 81% | | |
| **M. P.** | 176°C | | |
| **Analysis** | %C | %H | %N |
| **Found** | 72.18 | 4.35 | 16.78 |
| **Calculated** | 72.28 | 4.45 | 16.86 |
| **IR ($cm^{-1}$)** | $V_{max}$ 1681 (C=O stretching of aldehyde), 1599 and 1518 (aromatic C=C and C=N stretchings), 684 and 759 (C-H out of plane bending vibrations of mono substituted benzene ring), 3094 (aromatic C-H stretching). | | |
| **$^1$H-NMR $CDCl_3$ (δ, ppm)** | 7.45-9.56 (10H, multiplet, aromatic protons), 10.06 (1H, singlet, proton of CHO). | | |

*Preparation of 3-(3-(1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl) -acryloyl)coumarins (1a-c) and 3-(3-(1-phenyl-3-(pyridin-4-yl) -1H-pyrazol-4-yl)acryloyl)coumarins (2a-c) (Scheme 3)*




**Scheme 3: Synthesis of compounds (2a-c)**

The following general procedure was used.
In a 100 ml round bottom flask, an appropriate 3-acetyl coumarin (0.01 mol) and an appropriate pyrazole aldehyde (0.015 mol) were taken in 50 ml of ethanol. Catalytic amount of piperidine (1.0 ml) was added and the reaction mixture was stirred for 10 min at room temperature. The reaction mixture was then refluxed on water bath for 4 h. It was allowed to cool to room temperature. A solid product separated out was filtered off, washed with cold ethanol and dried. It was recrystallized from ethanol.

**Compound 1a**: $R=R_1=R_2=H$

| **Molecular Formula** | $C_{26}H_{17}N_3O_3$ | | |
|---|---|---|---|
| **Yield** | 83% | | |
| **M. P.** | 220°C | | |
| **Analysis** | %C | %H | %N |
| **Found** | 74.29 | 4.07 | 9.89 |
| **Calculated** | 74.45 | 4.09 | 10.02 |
| **IR ($cm^{-1}$)** | $V_{max}$ 1724 (C=O stretching of δ-lactone of coumarin), 1685 (C=O stretching of α,β unsaturated carbonyl group), 1609 and 1539 (aromatic C=C and C=N stretchings), 697 and 755 (C-H out of plane bending vibrations of mono substituted benzene ring), 3046 (aromatic C-H stretching). | | |
| **$^1$H-NMR $CDCl_3$ (δ, ppm)** | 7.35-9.00 (17H, multiplet, fourteen aromatic protons+C4 proton of coumarin+two olefinic protons). | | |

**Compound 1b**: R=$R_1$=H, $R_2$=$OCH_3$

| **Molecular Formula** | $C_{27}H_{19}N_3O_4$ | | |
|---|---|---|---|
| **Yield** | 80% | | |
| **M. P.** | 210°C | | |
| **Analysis** | %C | %H | %N |
| **Found** | 72.08 | 4.22 | 9.26 |
| **Calculated** | 72.15 | 4.26 | 9.35 |
| **IR ($cm^{-1}$)** | $V_{max}$ 1720 (C=O stretching of δ-lactone of coumarin), 1662 (C=O stretching of α,β unsaturated carbonyl group), 1604 and 1534 (aromatic C=C and C=N stretchings), 694 and 761 (C-H out of plane bending vibrations of mono substituted benzene ring), 3037 (aromatic C-H stretching). | | |
| **$^1$H-NMR $CDCl_3$ (δ, ppm)** | 4.03 (3H, singlet, OCH3), 7.38-8.99 (16H, multiplet, thirteen aromatic protons+C4 proton of coumarin+two olefinic protons). | | |

**Compound 1c**: R=$R_1$=Benzo, $R_2$=H

| **Molecular Formula** | $C_{30}H_{19}N_3O_3$ | | |
|---|---|---|---|
| **Yield** | 78% | | |
| **M. P.** | 218°C | | |
| **Analysis** | %C | %H | %N |
| **Found** | 76.64 | 4.05 | 8.9 |
| **Calculated** | 76.75 | 4.08 | 8.95 |
| **IR ($cm^{-1}$)** | $V_{max}$ 1717 (C=O stretching of δ-lactone of coumarin), 1684 (C=O stretching of α,β unsaturated carbonyl group), 1597 and 1577 (aromatic C=C and C=N stretchings), 781 and 691 (C-H out of plane bending vibrations of mono substituted benzene ring), 3050 (aromatic C-H stretching). | | |
| **$^1$H-NMR $CDCl_3$ (δ, ppm)** | 7.52-9.45 (19H, multiplet, sixteen aromatic protons+C4 proton of coumarin+two olefinic protons). | | |

**Compound 2a**: R=$R_1$=$R_2$=H

| **Molecular Formula** | $C_{26}H_{17}N_3O_3$ | | |
|---|---|---|---|
| **Yield** | 83% | | |
| **M. P.** | 220°C | | |
| **Analysis** | %C | %H | %N |
| **Found** | 74.41 | 3.86 | 9.88 |
| **Calculated** | 74.45 | 4.09 | 10.02 |
| **IR ($cm^{-1}$)** | $V_{max}$ 1717 (C=O stretching of δ-lactone of coumarin), 1685 (C=O stretching of α,β unsaturated carbonyl group), 1607 and 1576 (aromatic C=C and C=N stretchings), 688 and 761 (C-H out of plane bending vibrations of mono substituted benzene ring), 3033 (aromatic C-H stretching). | | |
| **$^1$H-NMR $CDCl_3$ (δ, ppm)** | 7.38-8.78 (17H, multiplet, fourteen aromatic protons+C4 proton of coumarin+two olefinic protons). | | |

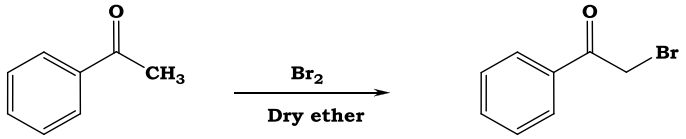
**Compound 2b**: R=$R_1$=H, $R_2$=$OCH_3$

| **Molecular Formula** | $C_{27}H_{19}N_3O_4$ | | |
|---|---|---|---|
| **Yield** | 85% | | |
| **M. P.** | 160°C | | |
| **Analysis** | %C | %H | %N |
| **Found** | 72.11 | 4.24 | 9.33 |
| **Calculated** | 72.15 | 4.26 | 9.35 |
| **IR ($cm^{-1}$)** | $V_{max}$ 1717 (C=O stretching of δ-lactone of coumarin), 1636 (C=O stretching of α,β unsaturated carbonyl group), 1607 and 1576 (aromatic C=C and C=N stretchings), 688 and 761 (C-H out of plane bending vibrations of mono substituted benzene ring), 3034 (aromatic C-H stretching). | | |
| **$^1$H-NMR $CDCl_3$ (δ, ppm)** | 4.03 (3H, singlet, OCH3), 7.21-8.78 (16H, multiplet, thirteen aromatic protons+C4 proton of coumarin+two olefinic protons). | | |

**Compound 2c**: R=$R_1$=Benzo, $R_2$=H

| **Molecular Formula** | $C_{30}H_{19}N_3O_3$ | | |
|---|---|---|---|
| **Yield** | 82% | | |
| **M. P.** | 188°C | | |
| **Analysis** | %C | %H | %N |
| **Found** | 76.73 | 3.92 | 8.87 |
| **Calculated** | 76.75 | 4.08 | 8.95 |
| **IR ($cm^{-1}$)** | $V_{max}$ 1717 (C=O stretching of δ-lactone of coumarin), 1685 (C=O stretching of α,β unsaturated carbonyl group), 1607 and 1576 (aromatic C=C and C=N stretchings), 761 and 663 (C-H out of plane bending vibrations of mono substituted benzene ring), 3033 (aromatic C-H stretching). | | |
| **$^1$H-NMR $CDCl_3$ (δ, ppm)** | 7.40-9.31 (19H, multiplet, sixteen aromatic protons+C4 proton of coumarin+two olefinic protons). | | |

*Preparation of phenacyl bromide (Scheme 4)*

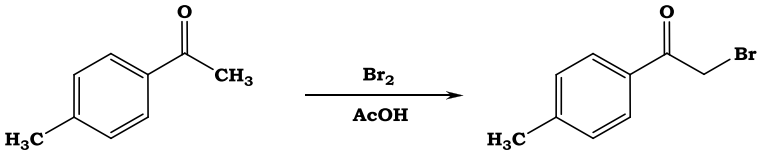



**Scheme 4: Synthesis of Phenacyl bromide**

A solution of acetophenone (0.33 mol) in anhydrous ether (25 m; l was placed in a three necked flask equipped with a dropping funnel and gas absorption tube. The flask was placed in an ice bath and was allowed to cool to 0ºC-5ºC. A trace amount of anhydrous $AlCl_3$ (0.1 g) was introduced and bromine (0.33 mol) was added dropwise with stirring during 30 min. The reaction mixture was allowed to come to room temperature and was stirred for further 1 h. Then pet. ether 40-60 (50 ml) was added. The phenacyl bromide was separated out as white solid. It was filtered out and was washed with a mixture of pet. ether 40-60 and water (100 ml, 1: 1). It was dried and crystallized from rectified spirit.

**Phenacyl bromide**: Yield: 85%, M. p. 47°C (lit. [29] M. p. 49°C)

*Preparation of 4-methyl phenacyl bromide (Scheme 5)*

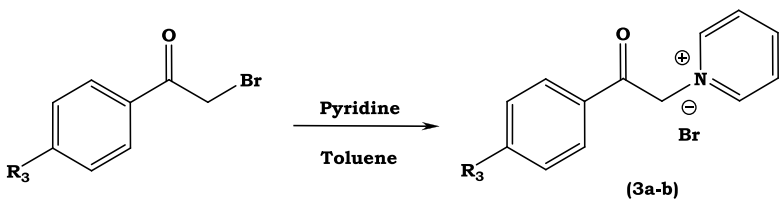



**Scheme 5: Synthesis of 4-methyl phenacyl bromide**

A solution of a p-methyl acetophenone (0.5 mol) in acetic acid (100 ml) was placed in a three necked flask equipped with a dropping funnel and a condenser with gas absorption tube. Bromine (0.5 mol) was added gradually from dropping funnel with stirring at room temperature during 30 min. The reaction mixture was stirred for 2 h at room temperature. It was then poured into ice cold water (500 ml). The product was separated out as a white solid. It was filtered out and was washed with water and dried. The product was crystallized from rectified spirit.

**4-Methyl phenacyl bromide**: R=$CH_3$; Yield: 86%, M. p. 48°C (lit. [29] mp 45°C-49°C)

*Preparation of aroyl methyl pyridinium bromide salts (3a-b) (Scheme 6)*




**Scheme 6: Synthesis of compounds (3a-b)**

The following general procedure was used.
An appropriate phenacyl bromide (0.25 mol) was dissolved in dry toluene (100 ml) in a 250 ml three necked flask equipped with a dropping funnel and magnetic needle at room temperature. Dry pyridine (0.25 mol) was added slowly to the above solution with stirring. The reaction mixture was refluxed at 100ºC for 30 min. It was then allowed to come to room temperature. The aroyl methyl pyridinium bromide salt was separated out as a white solid. It was filtered out and washed with dry toluene. It was then dried at 60°C-70ºC for 4-5 h in vacuum oven.

**Compound 3a**: $R_3$=H; Yield: 80%, mp 193ºC (lit. [29] M. p. 194°C-197ºC)
**Compound 3b**: $R_3$=$CH_3$; Yield: 85%, mp 203ºC (lit. [30,31] M. p. 205ºC)

*Synthesis of 3-{4'-[1''-phenyl-3''-(pyridin-3'''-yl)-1H-pyrazol-4''-yl]-6'-aryl-pyridin-2'-yl}coumarins 4a-f) and 3-{4'-[1''-phe-nyl-3''-(pyridin-4'''-yl)-1H-pyrazol-4''-yl]-6'-aryl-pyridin-2'-yl}coumarins(5a-f) (Figure 1)*

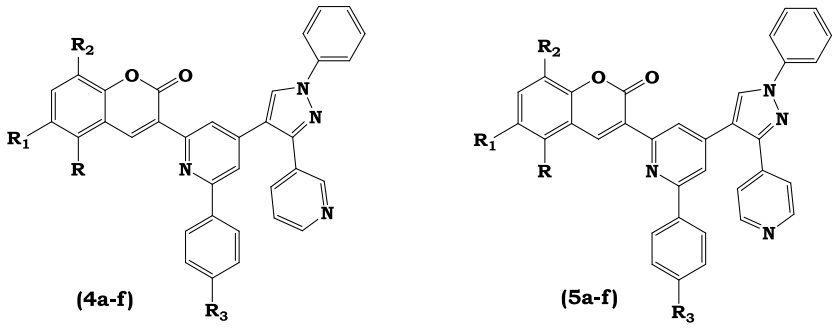


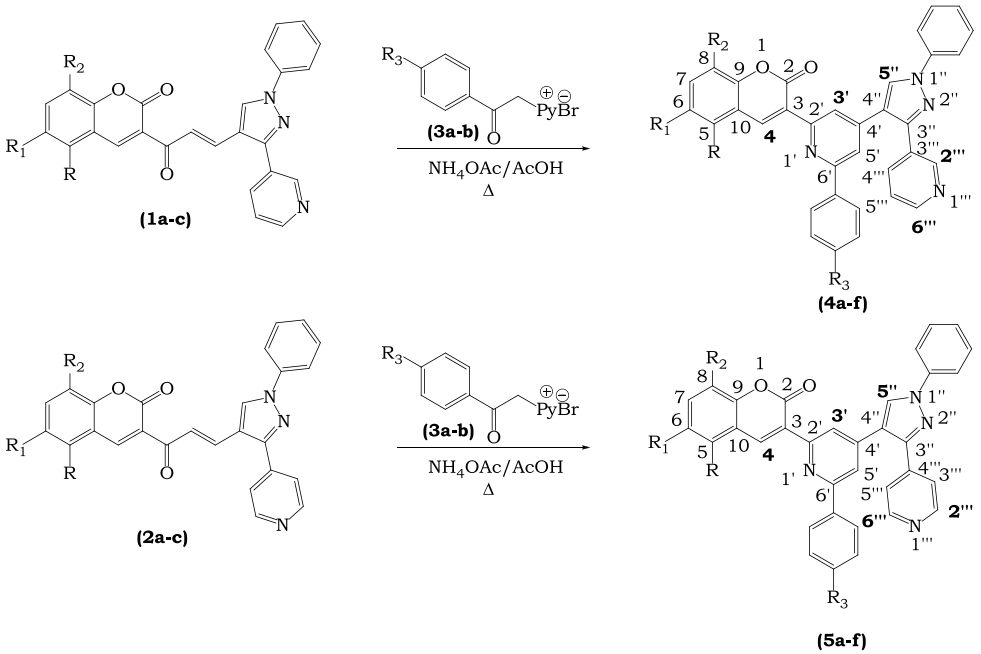

**Figure 1: Structures of compounds (4a-f) and (5a-f)**

The following general procedure was used.

In a 100 ml round bottom flask equipped with a condenser, guard tube and magnetic needle, an appropriate aroyl methyl pyridinium bromide salts (3a-b) (0.003 mol) in glacial acetic acid (15 ml) was taken. To this, ammonium acetate (0.03 mol) was added with stirring at room temperature. Then a solution of an appropriate 3-(3-(1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazol-4-yl)acryloyl)coumarin(1a-c) or 3-(3-(1-phenyl-3-(pyridin-4-yl)-1*H*-pyrazol-4-yl)acryloyl)coumarin (2a-c) (0.003 mol) in glacial acetic acid (15 ml) was added with stirring at room temperature and the reaction mixture was further stirred for 1 h at room temperature. It was then refluxed for 12 h at 140°C. It was then allowed to come to room temperature and was poured into ice-cold water (75 ml). A crude solid obtained was extracted with chloroform (3 × 30 ml). The organic layer was washed with 5% sodium bicarbonate solution (3 × 20 ml), water (2 × 20 ml) and dried over anhydrous sodium sulfate. The removal of chloroform under reduced pressure gave crude material which was subjected to column chromatography using silica gel and chloroform-ethyl acetate (7: 3) as an eluent to give compounds (4a-f) and (5a-f). The compounds were recrystallized from chloroform-hexane.

## RESULTS AND DISCUSSION

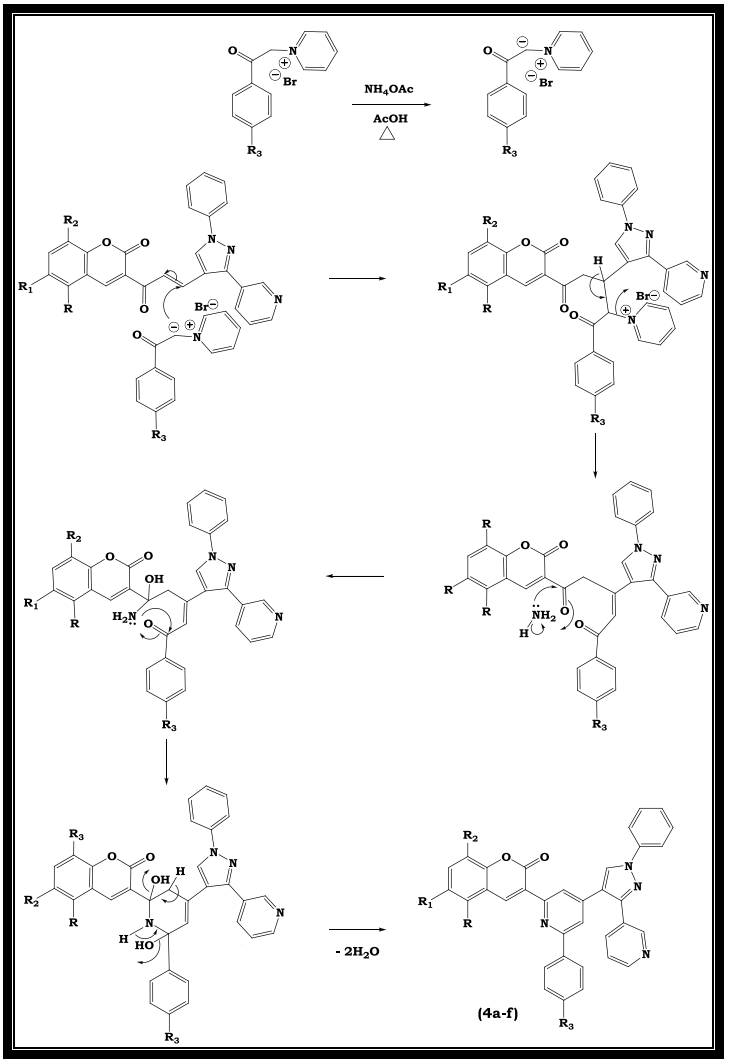
The condensation of 3-(3-(1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazol-4-yl)acryloyl)coumarins (1a-c) and 3-(3-(1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazol-4-yl)acryloyl)coumarins (2a-c) with aroyl methyl pyridinium salts (3a-b) under Krohnke's reaction condition proceeded smoothly and gave the expected products (4a-f) and (5a-f) (Scheme 7 and Table 1) in 65%-75% yield. The detailed mechanism for the formation of compounds (4a-f) is shown in Scheme 8. Formation of compounds (5a-f) follows similar mechanism.




**Scheme 7: Formation of the compounds (4a-f) and (5a-f) by the Krohnke's reaction**

**Table 1: Synthesis data of the compounds (4a-f) and (5a-f)**

| | R | $R_1$ | $R_2$ | $R_3$ | | R | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|---|---|---|---|---|---|
| **4a** | H | H | H | H | **5a** | H | H | H | H |
| **4b** | H | H | $OCH_3$ | H | **5b** | H | H | $OCH_3$ | H |
| **4c** | Benzo | | H | H | **5c** | Benzo | | H | H |
| **4d** | H | H | H | $CH_3$ | **5d** | H | H | H | $CH_3$ |
| **4e** | H | H | $OCH_3$ | $CH_3$ | **5e** | H | H | $OCH_3$ | $CH_3$ |
| **4f** | Benzo | | H | $CH_3$ | **5f** | Benzo | | H | $CH_3$ |




**Scheme 8: Mechanism of the formation of compounds (4a-f)**

**Compound 4a**: R=$R_1$=$R_2$=$R_3$=H

| **Molecular Formula** | $C_{34}H_{22}N_4O_2$ | | |
|---|---|---|---|
| **Yield** | 68% | | |
| **M. p.** | 230°C-234°C | | |
| **Analysis** | %C | %H | %N |
| **Found** | 78.62 | 4.21 | 10.73 |
| **Calculated** | 78.75 | 4.28 | 10.8 |

**Compound 4b:** R=R1=R3=H, R2=$OCH_3$

| **Molecular Formula** | $C_{35}H_{24}N_4O_3$ | | |
|---|---|---|---|
| **Yield** | 72% | | |
| **M. p.** | 218°C-220°C | | |
| **Analysis** | %C | %H | %N |
| **Found** | 76.58 | 4.37 | 10.15 |
| **Calculated** | 76.63 | 4.41 | 10.21 |

**Compound 4c:** R=R1=Benzo, R2=R3=H

| **Molecular Formula** | $C_{38}H_{24}N_4O_2$ | | |
|---|---|---|---|
| **Yield** | 69% | | |
| **M. p.** | 240ºC | | |
| **Analysis** | %C | %H | %N |
| **Found** | 80.19 | 4.22 | 9.77 |
| **Calculated** | 80.27 | 4.25 | 9.85 |

**Compound 4d:** R=R1=R2=H, R3=$CH_3$

| **Molecular Formula** | $C_{35}H_{24}N_4O_2$ | | |
|---|---|---|---|
| **Yield** | 67% | | |
| **M. p.** | 194°C-196°C | | |
| **Analysis** | %C | %H | %N |
| **Found** | 78.87 | 4.46 | 10.45 |
| **Calculated** | 78.93 | 4.54 | 10.52 |

**Compound 4e**: R=$R_1$=H, $R_2$=$OCH_3$, $R_3$=$CH_3$

| **Molecular Formula** | $C_{36}H_{26}N_4O_3$ | | |
|---|---|---|---|
| **Yield** | 72% | | |
| **M. p.** | 223ºC | | |
| **Analysis** | %C | %H | %N |
| **Found** | 76.80 | 4.57 | 9.90 |
| **Calculated** | 76.85 | 4.66 | 9.96 |

**Compound 4f**: R=$R_1$=Benzo, $R_2$=H, $R_3$=$CH_3$

| **Molecular Formula** | $C_{39}H_{26}N_4O_2$ | | |
|---|---|---|---|
| **Yield** | 74% | | |
| **M. p.** | 235°C-237°C | | |
| **Analysis** | %C | %H | %N |
| **Found** | 80.31 | 4.44 | 9.57 |
| **Calculated** | 80.39 | 4.5 | 9.62 |

**Compound 5a**: R=$R_1$=$R_2$=$R_3$=H

| **Molecular Formula** | $C_{34}H_{22}N_4O_2$ | | |
|---|---|---|---|
| **Yield** | 75% | | |
| **M. p.** | 225°C-227°C | | |
| **Analysis** | %C | %H | %N |
| **Found** | 78.67 | 4.23 | 10.71 |
| **Calculated** | 78.75 | 4.28 | 10.8 |

**Compound 5b**: R=$R_1$=$R_3$=H, $R_2$=$OCH_3$

| **Molecular Formula** | $C_{35}H_{24}N_4O_3$ | | |
|---|---|---|---|
| **Yield** | 73% | | |
| **M. p.** | 195°C-197°C | | |
| **Analysis** | %C | %H | %N |
| **Found** | 76.58 | 4.36 | 10.16 |
| **Calculated** | 76.63 | 4.41 | 10.21 |

**Compound 5c**: R=$R_1$=Benzo, $R_2$=$R_3$=H

| **Molecular Formula** | $C_{38}H_{24}N_4O_2$ | | |
|---|---|---|---|
| **Yield** | 65% | | |
| **M. p.** | 230˚C | | |
| **Analysis** | %C | %H | %N |
| **Found** | 80.21 | 4.2 | 9.77 |
| **Calculated** | 80.27 | 4.25 | 9.85 |

**Compound 5d**: R=$R_1$=$R_2$=H, $R_3$=$CH_3$

| **Molecular Formula** | $C_{35}H_{24}N_4O_2$ | | |
|---|---|---|---|
| **Yield** | 66% | | |
| **M. p.** | 207°C-209°C | | |
| **Analysis** | %C | %H | %N |
| **Found** | 78.88 | 4.48 | 10.46 |
| **Calculated** | 78.93 | 4.54 | 10.52 |

**Compound 5e**: R=$R_1$=H, $R_2$=$OCH_3$, $R_3$=$CH_3$

| **Molecular Formula** | C36H26N4O3 | | |
|---|---|---|---|
| **Yield** | 72% | | |
| **M. p.** | 237°C | | |
| **Analysis** | %C | %H | %N |
| **Found** | 76.82 | 4.6 | 9.91 |
| **Calculated** | 76.85 | 4.66 | 9.96 |

**Compound 5e**: R=$R_1$=H, $R_2$=$OCH_3$, $R_3$=$CH_3$

| **Molecular Formula** | $C_{39}H_{26}N_4O_2$ | | |
|---|---|---|---|
| **Yield** | 74% | | |
| **M. p.** | 236°C-238°C | | |
| **Analysis** | %C | %H | %N |
| **Found** | 80.35 | 4.43 | 9.58 |
| **Calculated** | 80.39 | 4.5 | 9.62 |

The structures of all the compounds (4a-f) and (5a-f) were confirmed by analytical and spectral data.
Thus, the reaction of 3-(3-(1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazol-4-yl)acryloyl)coumarin 1a with phenacyl pyridinium bromide salt 3a in the presence of ammonium acetate in refluxing acetic acid gave a compound 4a as a yellow solid product in 68% yield.

The IR spectrum of 4a showed a strong band at 1729 $cm^{-1}$ which is due to carbonyl stretching of δ-lactone ring present in coumarin nucleus. The bands observed at 1596 and 1499 $cm^{-1}$ are due to aromatic C=C and C=N stretching vibrations respectively. The band observed at 3031 $cm^{-1}$ is due to aromatic C-H stretching vibrations. The sharp bands observed at 691 and 751 $cm^{-1}$ are due to C-H out of plane bending vibrations for mono substituted benzene ring.

The $^1$H-NMR spectrum of compound 4a (in $CDCl_3$) showed twenty two protons in the region 7.26-9.00 δ. The $C_4$ proton of coumarin ring appeared as a singlet in the most down field region at 9.00 δ. This proton appears in the most down field region due to peri effect of nitrogen of the pyridine nucleus present at 3$^{rd}$ position of coumarin. The $C_2'''$ proton of pyridine nucleus which is attached to pyrazolyl ring appeared as a meta coupled doublet at 8.94 δ *(J=2.0 Hz)*. The $C_6'''$ proton of this pyridine appeared as doublet of a doublet at 8.64 δ *(J=4.6 and 1.6 Hz)*. The $C_2'''$ and $C_6'''$ protons appeared in the down field region due to their attachment to the carbons which are directly attached to $N_1'''$-atom. The $C_3'$ proton of pyridine ring attached to coumarin appeared as meta coupled doublet at 8.48 δ *(J=1.2 Hz)*. The $C_5''$ proton of pyrazole ring appeared as a sharp singlet at 8.34. The remaining other seventeen protons appeared as a multiplet between 7.26-7.97 δ.

The $^{13}$C-NMR spectrum of compound 4a (in $CDCl_3$) showed signals at 102.33, 110.66, 116.16, 118.16, 118.18, 119.29, 120.96, 124.03, 125.43, 126.26, 127.37, 127.64, 127.99, 128.33, 128.79, 129.24, 129.30, 130.33, 132.91, 133.04, 134.49, 143.62, 145.83, 147.28, 147.55, 147.95, 153.60, 154.95, 156.44 and 161.95 δ. The compound is having thirty types of non-equivalent carbon atoms and hence expected number of signals is observed. The most downfield signal appeared at 161.95 δ can be assigned to the carbonyl carbon of the δ-lactone ring of coumarin. The DEPT-90 spectrum of compound 4a (in $CDCl_3$) showed signals at 110.62, 116.96, 118.11, 118.29, 119.96, 124.03, 125.44, 126.26, 127.37, 127.62, 127.97, 128.34, 129.20, 129.36, 130.53, 145.83, 147.55, and 147.95 δ which are due to eighteen tertiary carbons.

The mass spectrum of compound 4a showed $M^+$ peak at 518(75%) (m/z %) along with some other fragments peaks at 474 (100%), 476 (23%), 248 (52%), 220 (28%), 77 (71%), 44 (25%), etc. The appearance of molecular ion peak at 518 mass units supports the structure of compound 4a.

The IR and NMR data for other compounds (4b-f) and (5a-f) are given below (Tables 2-12).

**Table 2: IR and NMR data for Compound 4b**

| | |
|---|---|
| IR ($cm^{-1}$) | $\nu_{max}$ 1719 (C=O stretching of δ-lactone of coumarin), 1600 and 1477 (aromatic C=C and C=N stretching), 3057 (aromatic C-H stretching), 758 and 693 (C-H bending vibrations of mono substituted benzene ring), 2935 (aliphatic C-H stretching). |
| $^1$H-NMR (d, ppm) (CDCl3) | 3.87 (3H, singlet, $OCH_3$), 7.17-7.98 (16H, multiplet, aromatic protons except protons at $C_4$, $C_3'$, $C_5''$, $C_2'''$ and $C_6'''$), 8.35 (1H, singlet, $C_5''$ proton of pyrazole ring), 8.50 (1H, meta coupled doublet, proton at $C_3'$, *J=1.2 Hz*), 8.66 (1H, doublet of the doublet, proton at $C_6'''$, *J=4.6 Hz* and *1.6 Hz*), 8.95 (1H, meta coupled doublet, proton at $C_2'''$, *J=2.0 Hz*), 9.01 (1H, singlet, $C_4$ proton of coumarin ring). |
| $^{13}$C-NMR (d, ppm) (CDCl3) | 55.85 ($OCH_3$), 102.34 (C), 110.65 (CH), 113.96 (CH), 118.10(CH), 119.96 (CH), 120.29 (CH), 123.20 (C), 124.00(CH), 126.29 (CH), 126.42 (CH), 127.37 (CH), 127.69 (CH), 128.71 (C), 129.20 (CH), 129.37 (CH), 130.50 (CH), 132.96 (C), 133.00 (C), 134.00 (CH), 134.42 (C), 140.65 (C), 143.65 (C), 145.86 (CH), 147.42 (C), 147.50 (CH), 147.91 (CH), 148.59 (C), 154.99 (C), 156.45 (C), 160.95 (CO of coumarin). |

**Table 3: IR and NMR data for Compound 4c**

| | |
|---|---|
| IR ($cm^{-1}$) | $\nu_{max}$ 1727 (C=O stretching of δ-lactone of coumarin), 1595 and 1498 (aromatic C=C and C=N stretchings), 3049 (aromatic C-H stretching), 757 and 689 (C-H bending vibrations of mono substituted benzene ring), 2926 (aliphatic C-H stretching). |

| | |
|---|---|
| $^{1}$H-NMR (d, ppm) (CDCl3) | 7.26-8.22 (19H, multiplet, aromatic protons except protons at $C_4$, $C_3'$, $C_5''$, $C_2'''$ and $C_6'''$), 8.34 (1H, singlet, $C_5''$ proton of pyrazole ring), 8.49 (1H, meta coupled doublet, proton at $C_3'$, *J=1.2 Hz*), 8.84 (1H, doublet of the doublet, proton at $C_6'''$, *J=4.6 Hz* and *1.6 Hz*), 9.06 (1H, meta coupled doublet, proton at $C_2'''$, *J=2.0 Hz*), 9.87 (1H, singlet, $C_4$ proton of coumarin ring). |
| $^{13}$C-NMR (d, ppm) (CDCl3) | 102.34(C), 110.62 (CH), 115.54 (CH), 117.73 (C), 118.18 (CH), 119.97 (CH), 122.23 (CH), 123.58 (CH), 124.02 (CH), 126.30 (CH), 126.84 (CH), 127.36 (CH), 127.64 (CH), 128.49 (CH), 128.74 (C), 128.86 (C), 129.25 (CH), 129.35 (CH), 130.14 (CH), 130.28 (C), 130.47 (CH), 132.95 (C), 133.00 (C), 134.00 (CH), 134.46 (C), 143.64 (C), 145.77 (CH), 147.38 (C), 147.49 (CH), 147.88(CH), 150.65 (C), 154.96 (C), 156.43 (C), 160.94 (CO of coumarin). |

**Table 4: IR and NMR data for Compound 4d**

| | |
|---|---|
| IR ($cm^{-1}$) | $\nu_{max}$ 1719 (C=O stretching of δ-lactone of coumarin), 1600 and 1477 (aromatic C=C and C=N stretchings), 3057 (aromatic C-H stretching), 758 and 693 (C-H bending vibrations of mono substituted benzene ring), 815 (C-H bending vibrations of p-disubstituted benzene ring), 2935 (aliphatic C-H stretching). |
| $^{1}$H-NMR (d, ppm) (CDCl3) | 2.43 (3H, singlet, $CH_3$), 7.26-8.12 (16H, multiplet, aromatic protons except protons at $C_4$, $C_3'$, $C_5''$, $C_2'''$ and $C_6'''$), 8.32 (1H, singlet, $C_5''$ proton of pyrazole ring), 8.59 (1H, meta coupled doublet, proton at $C_3'$, *J=1.2 Hz*), 8.75 (1H, doublet of the doublet, proton at $C_6'''$, *J=4.6 Hz* and *1.6 Hz*), 9.05 (1H, meta coupled doublet, proton at $C_2'''$, *J=2.0 Hz*), 9.10 (1H, singlet, $C_4$ proton of coumarin ring). |
| $^{13}$C-NMR (d, ppm) (CDCl3) | 21.34 ($CH_3$), 102.32 (C), 110.62 (CH), 116.13(CH), 118.18(CH), 119.91(CH), 120.98(CH), 123.31(CH), 124.00(CH), 125.41(CH), 126.23(CH), 127.92(CH), 128.31(C), 128.73(CH), 129.36(CH), 129.51(CH), 130.32(C), 130.54(CH), 132.97(C), 133.09(C), 134.01(C), 136.04(C), 143.61(C), 145.88(CH), 147.44(C), 147.53(CH), 147.91(CH), 153.00(C), 154.92(C), 156.43(C), 161.94(CO of coumarin). |

**Table 5: IR and NMR data for Compound 4e**

| | |
|---|---|
| IR ($cm^{-1}$) | $\nu_{max}$ 1726 (C=O stretching of δ-lactone of coumarin), 1596 and 1477 (aromatic C=C and C=N stretchings), 3033 (aromatic C-H stretching), 742 and 695 (C-H bending vibrations of mono substituted benzene ring), 827 (C-H bending vibrations of p-disubstituted benzene ring), 2929 (aliphatic C-H stretching), |
| $^{1}$H-NMR (d, ppm) (CDCl3) | 2.43 (3H, singlet, $CH_3$), 3.88 (3H, singlet, $OCH_3$), 7.14-8.12 (15H, multiplet, aromatic protons except protons at $C_4$, $C_3'$, $C_5''$, $C_2'''$ and $C_6'''$), 8.32 (1H, singlet, $C_5''$ proton of pyrazole ring), 8.42 (1H, meta coupled doublet, proton at $C_3'$, *J=1.2 Hz*), 8.58 (1H, doublet of the doublet, proton at $C_6'''$, *J=4.6 Hz* and *1.6 Hz*), 8.87 (1H, meta coupled doublet, proton at $C_2'''$, *J=2.0 Hz*), 8.93 (1H, singlet, $C_4$ proton of coumarin ring). |
| $^{13}$C-NMR (d, ppm) (CDCl3) | 21.37($CH_3$), 55.84($OCH_3$), 102.33(C), 110.64(CH), 113.64(CH), 118.09(CH), 119.95(CH), 120.28(CH), 123.21(C), 123.36(CH), 124.00(CH), 126.30(CH), 126.43(CH), 128.72(CH), 129.36(C), 129.49(CH), 130.32(C), 130.52(CH), 132.96(C), 133.00(C), 134.01(CH), 136.03(C), 140.63(C), 143.64(C), 145.85(CH), 147.43(C), 147.49(CH), 147.89(CH), 148.60(C), 154.90(C), 156.43(C), 160.93(CO of coumarin). |

**Table 6: IR and NMR data for Compound 4f**

| | |
|---|---|
| IR ($cm^{-1}$) | $\nu_{max}$ 1726 (C=O stretching of δ-lactone of coumarin), 1599 and 1457 (aromatic C=C and C=N stretchings), 3067 (aromatic C-H stretching), 755 and 689 (C-H bending vibrations of mono substituted benzene ring), 832 (C-H bending vibrations of p-disubstituted benzene ring), 2927 (aliphatic C-H stretching). |
| $^{1}$H-NMR (d, ppm) (CDCl3) | 2.43 (3H, singlet, $CH_3$), 7.26-8.22 (18H, multiplet, aromatic protons except protons at $C_4$, $C_3'$, $C_5''$, $C_2'''$ and $C_6'''$), 8.35 (1H, singlet, $C_5''$ proton of pyrazole ring), 8.49 (1H, meta coupled doublet, proton at $C_3'$, *J=1.2 Hz*), 8.83 (1H, doublet of the doublet, proton at $C_6'''$, *J=4.6 Hz* and *1.6 Hz*), 9.06 (1H, meta coupled doublet, proton at $C_2'''$, *J=2.0 Hz*), 9.87 (1H, singlet, $C_4$ proton of coumarin ring). |
| $^{13}$C-NMR (d, ppm) (CDCl3) | 21.21 ($CH_3$), 102.34 (C), 110.63 (CH), 115.48 (CH), 117.72 (C), 118.17 (CH), 119.97 (CH), 122.19 (CH), 123.34 (CH), 123.64 (CH), 124.01 (CH), 126.23 (CH), 126.83 (CH), 128.54 (CH), 128.74 (C), 128.85 (C), 129.36 (CH), 129.55 (CH), 130.29 (CH), 130.36 (C), 130.46 (C), 130.47 (CH), 132.96(C), 133.00 (C), 134.07 (CH), 136.04 (C), 143.63 (C), 145.86 (CH), 147.39 (C), 147.52 (CH), 147.92(CH), 150.64 (C), 154.97 (C), 156.43(C), 160.87 (CO of coumarin). |

**Table 7: IR and NMR data for Compound 5a**

| | |
|---|---|
| IR ($cm^{-1}$) | $\nu_{max}$ 1727 (C=O stretching of δ-lactone of coumarin), 1598 and 1499 (aromatic C=C and C=N stretchings), 3066 (aromatic C-H stretching), 756 and 691 (C-H bending vibrations of mono substituted benzene ring), 2928 (aliphatic C-H stretching). |
| $^{1}$H-NMR (d, ppm) (CDCl3) | 7.26-7.96 (17H, multiplet, aromatic protons except protons at $C_4$, $C_3'$, $C_5''$, $C_2'''$ and $C_6'''$), 8.31 (1H, singlet, $C_5''$ proton of pyrazole ring), 8.50 (1H, meta coupled doublet, proton at $C_3'$, *J=1.2 Hz*), 8.64 (2H, ortho coupled doublet, protons at $C_2'''$ and $C_6'''$, *J=4.4 Hz*), 9.03 (1H, singlet, $C_4$ proton of coumarin ring). |
| $^{13}$C-NMR (d, ppm) (CDCl3) | 102.34 (C), 110.61 (CH), 116.18 (CH), 118.20 (CH), 119.96 (CH), 120.99 (CH), 125.36 (CH), 125.43 (C), 126.28 (CH), 127.33 (CH), 127.68 (CH), 127.98 (CH), 128.28 (CH), 128.70 (C), 129.25(CH), 129.36 (CH), 130.49 (CH), 132.95 (C), 134.48 (C), 140.34 (C), 145.64 (C), 145.85(CH), 147.40(CH), 153.63 (C), 153.79 (C), 154.97 (C), 156.43 (C), 160.92 (CO of coumarin). |

**Table 8: IR and NMR data for Compound 5b**

| | |
|---|---|
| IR ($cm^{-1}$) | $\nu_{max}$ 1718 (C=O stretching of δ-lactone of coumarin), 1598 and 1478 (aromatic C=C and C=N stretchings), 3049 (aromatic C-H stretching), 758 and 729 (C-H bending vibrations of mono substituted benzene ring), 2935 (aliphatic C-H stretching). |
| $^{1}$H-NMR (d, ppm) (CDCl3) | 3.88 (3H, singlet, $OCH_3$), 7.16-7.96 (16H, multiplet, aromatic protons except protons at $C_4$, $C_3'$, $C_5''$, $C_2'''$ and $C_6'''$), 8.31 (1H, singlet, $C_5''$ proton of pyrazole ring), 8.51 (1H, meta coupled doublet, proton at $C_3'$, *J=1.2 Hz*), 8.65 (2H, ortho coupled doublet, protons at $C_2'''$ and $C_6'''$, *J=4.4 Hz*), 9.01 (1H, singlet, $C_4$ proton of coumarin ring). |
| $^{13}$C-NMR (d, ppm) (CDCl3) | 55.78 ($OCH_3$), 102.33 (C), 110.27 (CH), 113.95 (CH), 118.09 (CH), 119.96 (CH), 120.31 (CH), 121.36 (CH), 123.21 (C), 126.20 (CH), 126.43 (CH), 127.36 (CH), 127.69 (CH), 128.72 (C), 129.19 (CH), 129.36 (CH), 130.50 (CH), 132.89 (C), 134.42 (C), 140.37 (C), 140.64 (C), 143.64 (C), 145.79 (CH), 147.43 (C), 148.58 (C), 149.84 (CH), 155.01 (C), 156.43 (C), 161.03 (CO of coumarin). |

**Table 9: IR and NMR data for Compound 5c**

| | |
|---|---|
| IR ($cm^{-1}$) | $\nu_{max}$ 1735 (C=O stretching of δ-lactone of coumarin), 1598 and 1406 (aromatic C=C and C=N stretchings), 3074 (aromatic C-H stretching), 756 and 690 (C-H bending vibrations of mono substituted benzene ring), 2927 (aliphatic C-H stretching). |
| $^{1}$H-NMR (d, ppm) (CDCl3) | 7.26-7.86 (19H, multiplet, aromatic protons except protons at $C_4$, $C_3'$, $C_5''$, $C_2'''$ and $C_6'''$), 8.30 (1H, singlet, $C_5''$ proton of pyrazole ring), 8.49 (1H, meta coupled doublet, proton at $C_3'$, *J=1.2 Hz*), 8.62 (2H, ortho coupled doublet, protons at $C_2'''$ and $C_6'''$, *J=4.4 Hz*), 9.81 (1H, singlet, $C_4$ proton of coumarin ring). |
| $^{13}$C-NMR (d, ppm) (CDCl3) | 102.34 (C), 110.58 (CH), 115.48 (CH), 117.74 (C), 118.27 (CH), 119.98 (CH), 121.34 (CH), 122.23 (CH), 123.60(CH), 126.23 (CH), 126.83 (CH), 127.34 (CH), 127.66 (CH), 128.54 (CH), 128.74 (C), 128.78 (C), 129.25 (CH), 129.36 (CH), 130.12 (C), 130.30 (CH), 130.50 (CH), 132.96 (C), 134.48(C), 140.30 (C), 143.64 (C), 145.86 (CH), 147.41 (C), 149.87 (CH), 150.55 (C), 154.96 (C), 156.44(C), 160.89 (CO of coumarin). |

**Table 10: IR and NMR data for Compound 5d**

| | |
|---|---|
| IR ($cm^{-1}$) | $\nu_{max}$ 1726 (C=O stretching of δ-lactone of coumarin), 1599 and 1490 (aromatic C=C and C=N stretchings), 3067 (aromatic C-H stretching), 755 and 689 (C-H bending vibrations of mono substituted benzene ring), 832 (C-H bending vibrations of p-disubstituted benzene ring), 2927 (aliphatic C-H stretching). |
| $^{1}$H-NMR (d, ppm) (CDCl3) | 2.43 (3H, singlet, $CH_3$), 7.26-7.96 (16H, multiplet, aromatic protons except protons at $C_4$, $C_3'$, $C_5''$, $C_2'''$ and $C_6'''$), 8.32 (1H, singlet, $C_5''$ proton of pyrazole ring), 8.51 (1H, meta coupled doublet, proton at $C_3'$, *J=1.2 Hz*), 8.65 (2H, ortho coupled doublet, protons at $C_2'''$ and $C_6'''$, *J=4.8 Hz*), 9.03 (1H, singlet, $C_4$ proton of coumarin ring). |

| | |
|---|---|
| $^{13}$C-NMR (d, ppm) (CDCl3) | 21.29 ($CH_3$), 102.29 (C), 110.56 (CH), 116.20 (CH), 118.20 (CH), 119.94 (CH), 120.99 (C), 121.33 (CH), 123.32 (CH), 125.42 (CH), 126.31 (CH), 127.96 (CH), 128.28 (CH), 128.73 (C), 129.34 (CH), 129.57 (CH), 130.38 (C), 130.46 (CH), 132.49 (C), 136.00 (C), 140.34 (C), 143.65 (C), 145.85 (CH), 147.44 (C), 149.78 (CH), 153.63 (C), 154.99 (C), 156.45 (C), 160.94 (CO of coumarin). |

**Table 11: IR and NMR data for Compound 5e**

| | |
|---|---|
| IR ($cm^{-1}$) | $\nu_{max}$ 1726 (C=O stretching of δ-lactone of coumarin), 1594 and 1477 (aromatic C=C and C=N stretchings), 3057 (aromatic C-H stretching), 776 and 695 (C-H bending vibrations of mono substituted benzene ring), 827 (C-H bending vibrations of p-disubstituted benzene ring), 2935 (aliphatic C-H stretching). |
| $^1$H-NMR (d, ppm) (CDCl3) | 2.44 (3H, singlet, $CH_3$), 3.88 (3H, singlet, $OCH_3$), 7.12-7.95 (15H, multiplet, aromatic protons except protons at $C_4$, $C_3'$, $C_5''$, $C_2'''$ and $C_6'''$), 8.30 (1H, singlet, $C_5''$ proton of pyrazole ring), 8.49 (1H, meta coupled doublet, proton at $C_3'$, *J=1.2 Hz*), 8.63 (2H, ortho coupled doublet, protons at $C_2'''$ and $C_6'''$, *J= 4.8 Hz*), 8.99 (1H, singlet, $C_4$ proton of coumarin ring). |
| $^{13}$C-NMR (d, ppm) (CDCl3) | 21.34 ($CH_3$), 55.85 ($OCH_3$), 102.33 (C), 110.58 (CH), 113.97 (CH), 118.09 (CH), 119.88 (CH), 120.34 (CH), 121.36 (CH), 123.26 (CH), 123.40 (C), 126.19 (CH), 126.44 (CH), 128.72 (C), 129.24 (C), 129.28 (CH), 129.56 (CH), 130.40 (CH), 132.87 (C), 136.05 (C), 140.35 (C), 140.64(C), 143.65 (C), 145.87 (CH), 147.38 (C), 148.58 (C), 149.85 (CH), 154.98 (C), 156.44 (C), 160.97 (CO of coumarin). |

**Table 12: IR and NMR data for Compound 5f**

| | |
|---|---|
| IR ($cm^{-1}$) | $\nu_{max}$ 1735 (C=O stretching of δ-lactone of coumarin), 1508 and 1406 (aromatic C=C and C=N stretchings), 3074 (aromatic C-H stretching), 756 and 690 (C-H bending vibrations of mono substituted benzene ring), 824 (C-H bending vibrations of p-disubstituted benzene ring), 2927 (aliphatic C-H stretching). |
| $^1$H-NMR (d, ppm) (CDCl3) | 2.44 (3H, singlet, $CH_3$), 7.26-7.9.81 (18H, multiplet, aromatic protons except protons at $C_4$, $C_3'$, $C_5''$, $C_2'''$ and $C_6'''$), 8.31 (1H, singlet, $C_5''$ proton of pyrazole ring), 8.48 (1H, meta coupled doublet, proton at $C_3'$, *J=1.2 Hz*), 8.63 (2H, ortho coupled doublet, proton at $C_2'''$ and $C_6'''$, *J=5.6 Hz*), 9.81 (1H, singlet, $C_4$ proton of coumarin ring). |
| $^{13}$C-NMR (d, ppm) (CDCl3) | 21.32 ($CH_3$), 102.34 (C), 110.61 (CH), 115.54 (CH), 117.76 (C), 118.27 (CH), 119.96 (CH), 121.47 (CH), 122.34(CH), 123.30 (CH), 123.61 (CH), 126.30 (CH), 126.83 (CH), 128.49 (CH), 128.78 (C), 128.89 (C), 129.35 (CH), 129.56 (CH), 129.87 (C), 130.17 (CH), 130.30 (C), 130.35 (CH), 130.47 (C), 132.89 (C), 136.03 (C), 143.31 (C), 145.85 (CH), 147.39 (C), 149.88 (CH), 150.54 (C), 154.96 (C), 156.45 (C), 160.95 (CO of coumarin). |

The newly synthesized target compounds 4a-f and 5a-f were evaluated for their *in vitro* antibacterial activity against two Gram positive bacteria *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 441) and two Gram negative bacteria *Escherichia coli* (MTCC 443) and *Salmonella typhi* (MTCC 98). They were also evaluated for their *in vitro* antifungal activity against *Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 282) as fungal strains. Broth dilution method was used for the determination of the antibacterial and antifungal activity as recommended by NCCLS [18]. Ampicillin, Chloramphenicol and Norfloxacin were used as standard antibacterial drugs, whereas Griseofulvin and Nystatin were used as standard antifungal drugs. All MTCC cultures were collected from Institute of Microbial Technology, Chandigarh and tested against above mentioned known drugs. Mueller-Hinton broth was used as the nutrient medium for the test bacteria and Sabouraud Dextrose broth was used for the test fungi. Inoculum size for the test strains was adjusted to $10^8$ CFU (Colony Forming Unit per milliliter) per milliliter by comparing the turbidity. Each synthesized compound was diluted with DMSO so as to have the stock solution of 2000 µg/ml concentration as a stock solution. The results were recorded in the form of primary and secondary screening. The synthesized compounds (5a-r) were screened for their antibacterial and antifungal activity at the concentration of 1000, 500 and 250 µg/ml for the primary screening. The synthesized compound showing activity against microbes in the primary screening were further screened in a second set of dilution at concentrations of 200, 100, 62.5, 50 and 25 µg/ml. The suspension of 10 µl from each were further well incubated and growth was noted at 37°C after 24 h for bacteria and 48 hour for fungi. The lowest concentration which showed no visible growth (turbidity) after spot subculture was considered as the minimum inhibitory concentration (MIC) for each compound.

The investigation of the data summarized in (Table 13) reveals that many compounds were found to be active against Gram-positive bacteria while some of the compounds were found to be active against Gram-negative bacterial and fungal species as compared to that of the standard antimicrobial drugs.

**Table 13: Estimation of the minimum inhibitory concentration (MIC)**

| **Compound** | **Minimum Inhibitory Concentration (MIC, µgml$^{-1}$)** | | | | | |
|---|---|---|---|---|---|---|
| | **Gram +ve bacteria** | | **Gram –ve bacteria** | | **Fungi** | |
| | ***Bacillus subtillus*** | ***Staphylocossus aureus*** | ***Escherichia coli*** | ***Salmonella typhii*** | ***Asperigillus niger*** | ***Candida albicans*** |

| | | | | | | |
|---|---|---|---|---|---|---|
| 4a | 250 | 250 | 250 | 200 | >1000 | 250 |
| 4b | 100 | 100 | 62.5 | 100 | >1000 | 1000 |
| 4c | 200 | 250 | 100 | 125 | >1000 | 1000 |
| 4d | 250 | 200 | 250 | 200 | >1000 | >1000 |
| 4e | 100 | 100 | 200 | 125 | 500 | 500 |
| 4f | 125 | 100 | 250 | 200 | 250 | >1000 |
| 5a | 125 | 200 | 250 | 250 | 500 | >1000 |
| 5b | 62.5 | 200 | 100 | 100 | >1000 | 250 |
| 5c | 250 | 250 | 125 | 200 | 1000 | 1000 |
| 5d | 200 | 125 | 200 | 250 | >1000 | 500 |
| 5e | 200 | 200 | 100 | 100 | >1000 | 1000 |
| 5f | 125 | 125 | 250 | 500 | >1000 | 500 |
| Ampicillin | 250 | 250 | 100 | 100 | - | - |
| Chloramphenicol | 50 | 50 | 50 | 50 | - | - |
| Ciprofloxacin | 50 | 50 | 25 | 25 | - | - |
| Norfloxacin | 100 | 10 | 10 | 10 | - | - |
| Gentamycin | 0.5 | 0.25 | 0.05 | 1 | - | - |
| Griseofulvin | - | - | - | - | 100 | 500 |
| Nystatin | - | - | - | - | 100 | 100 |

The assessment of antimicr.obial screening data reveals that almost all the compounds 4a-f and 5a–f, exerted significant inhibitory activity against gram positive bacteria compared to Ampicillin (MIC=250 µg/ml) against *Bacillus subtilis*. Compounds 5b (MIC = 62.5 µg/ml), 4b and 4e (MIC=100 µg/ml) exhibited excellent activity compared to Ampicillin (MIC=250 µg/ml) and comparable activity to Norfloxacin (MIC=100 µg/ml) against *Bacillus subtilis*. Compounds 4f, 5a, and 5f (MIC=125 µg/ml) displayed better inhibition against *B. subtilis* compared to Ampicillin (MIC=250 µg/ml). Compounds 4c, 5d, and 5e (MIC=200 µg/ml) displayed comparable activity to Ampicillin (MIC=250 µg/ml). Compounds 4a and 4d (MIC=250µg/ml) were found to have equipotent activity compared to Ampicillin (MIC=250 µg/ml).

Compounds 4b, 4e and 4f (MIC=100 µg/ml), 5d and 5f (MIC=125 µg/ml) were found to be more potent against *S. aureus* compared to Ampicillin (MIC=250 µg/ml). Compounds 5d and 5f (MIC=200 µg/ml) were found to be more potent against gram positive bacteria *S. aureus* compared to Ampicillin (MIC=250 µg/ml). Compounds 4a, 4c and 5c (MIC=250 µg/ml) were found to have equipotent activity compared to Ampicillin (MIC=250 µg/ml).

Compounds 4b (MIC=62.5 µg/ml) exerted excellent inhibition against gram negative bacteria *E. coli* compared to Ampicillin (MIC=100 µg/ml) whereas compounds 4c and 5b (MIC=100 µg/ml) were found to be equipotent against *E. coli* compared to Ampicillin (MIC=100 µg/ml).

Compounds 4b, 5b and 5e (MIC=100 µg/ml) showed equal inhibition to Ampicillin against gram negative bacteria *S. typhi*.
Compounds 4a and 5b (MIC=250 µg/ml) were found to be more active than Griseofulvin (MIC=500 µg/ml) whereas, Compounds 4e, 5d and 5f (MIC=500 µg/ml) were found to be equipotent to Griseofulvin (MIC=500 µg/ml) against *C. albicans*. None of the tested compounds showed better activity than standard drugs against *A. niger*.

Among the compounds 4a-f and 5a-f compounds 4b and 5b exhibited excellent inhibitory activity than other derivatives. The enhanced activity of the above compounds can be attributed to the presence of $-OCH_3$ group in coumarin ring. All the compounds exhibited excellent activity against *B. subtilis* and *S. aureus* compared to the standard drugs. Examining the antimicrobial data, it has been observed that the derivatization of the parent molecule moderately changed the antimicrobial potency of the synthesized analogs. Thus compounds 4b and 5b were found to be the most efficient members of the series.

## CONCLUSION

From present study, we summarized that employed synthetic strategy provide efficient route for the synthesis of 3-{4'-[1"-phenyl-3"-(pyridin-3"'-yl)-1*H*-pyrazol-4"-yl]-6'-aryl-pyridin-2'-yl} coumarins (4a-f) and 3-{4'-[1"-phenyl-3"-(pyridin-4"'-yl)-1*H*-pyrazol-4"-yl]-6'-aryl-pyridin-2'-yl}coumarins (5a-f) by Krohnke's protocol in good yield. Moreover the starting precursors were also easy to prepare from synthesis point of view. Antimicrobial study on target compounds concluded that the all the compounds exerted promising activity against gram positive bacteria and gram negative. The target compounds showed feeble activity against fungal pathogens. Compounds 4b and 5b were found to be the most efficient members of the series.

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