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Synthesis, characterization and antimicrobial evaluation of dicoumarinyl substituted pyrazolyl pyridines

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ABSTRACT

*The synthesis of various 2,6-di(coumarin-3-yl)-4-(1-phenyl-3–aryl-1H-pyrazol-4-yl)pyridines **(5a-r)** have been carried out by the reaction of 3-coumarinoyl methyl pyridinium bromide salts **(4a-c)** with various 3-[3-(1-phenyl-3-aryl-1H-pyrazol-4-yl) acryloyl] coumarins (coumarin chalcones) **(3a-f)** in the presence of ammonium acetate in refluxing acetic acid. The synthesized compounds were fully characterized by IR, ^{1}H-NMR, ^{13}C-APT and representative Mass spectral data. The synthesized compounds were screened for in vitro antimicrobial activity using Broth micro dilution method.*

Keywords: Coumarins, dicoumarinyl pyridines, pyridylcoumarins, pyrazole, Kröhnke reaction, antimicrobial activity

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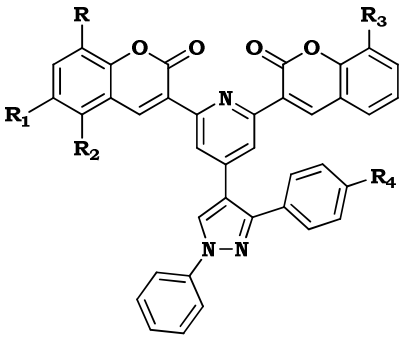
INTRODUCTION

Coumarins constitute an important class of benzopyrones, exhibiting a broad range of biological activities such as anticoagulants[1], antimicrobial[2], antibacterial[3], anticancer[4] and anti-HIV activity[5]. The interesting biological activities of the coumarins make them attractive targets in organic synthesis. Coumarins having pyridine substitution at C-3 are reported to have interesting biological activity. Many 3-(2-pyridyl)- and 3-(3-pyridyl)coumarins are known for their useful bioactivities viz. antifungal[6,9], bactericidal[7], fish toxicity[7] and moth proofing activity[8]. Some of them are also known for their CNS depressant activity[9]. Considering their biological importance, a variety of 3-pyridyl substituted coumarins were earlier synthesized from our laboratory[10]. Among five membered nitrogen containing heterocycles, pyrazole is a prominent moiety. A large number of compounds having pyrazole nucleus in their structure are reported to have wide range of biological activities viz., antioxidant[11], anti-invasive[12], antiviral[13], anti-inflammatory[14] and are also used as agrochemicals[15] and dyestuff[16]. Thus 3-(2-pyridyl)coumarins and pyrazole are very important heterocycles from bioactivity view point, therefore in the present work, some dicoumarinyl substituted pyrazolyl pyridines (A) have been synthesized, which are combination of both components i.e. pyrazole as well as pyridyl coumarin in a single scaffold.



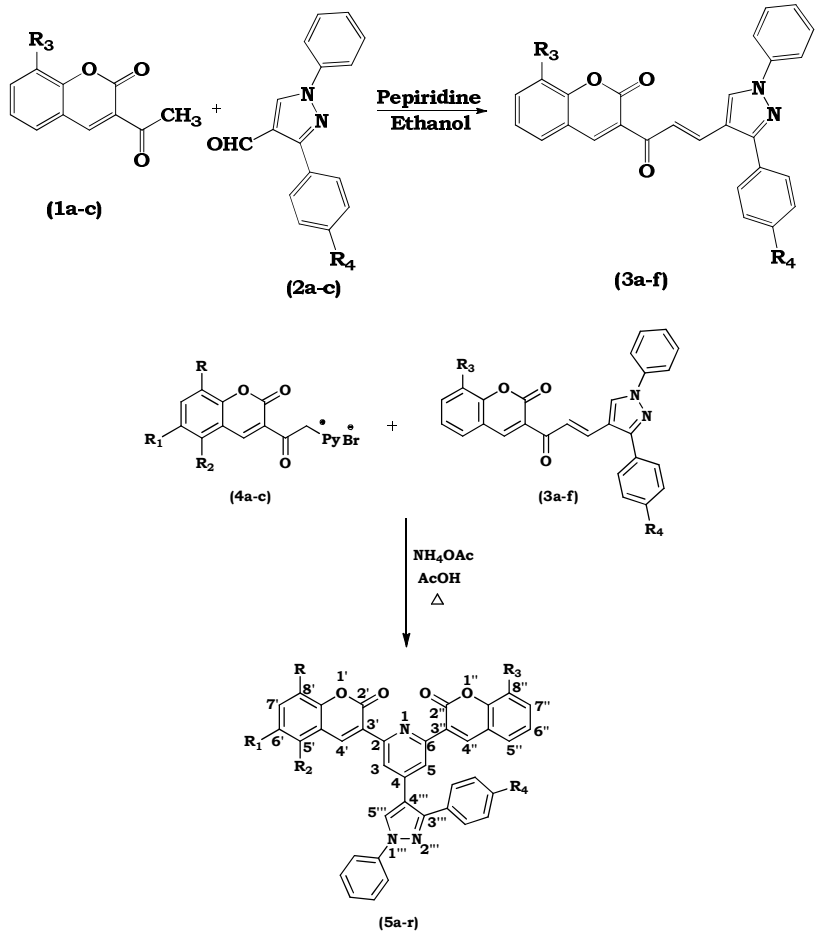
(A)

The purpose of synthesizing such type of target compounds was to have pyrazolyl pyridine nucleus flanked by two coumarin moieties. The structure seems as if two coumarins are having 3-(2-pyridyl) substitution pattern and has further substitution of pyrazole. One can expect a better biological properties from such type of compounds. With these objectives, in the present work synthesis of various 2,6-di(coumarin-3-yl)-4-(1-phenyl-3–aryl-1*H*-pyrazol-4-yl)pyridines has been carried out by *Krohnke's* reaction .

RESULTS AND DISCUSSION

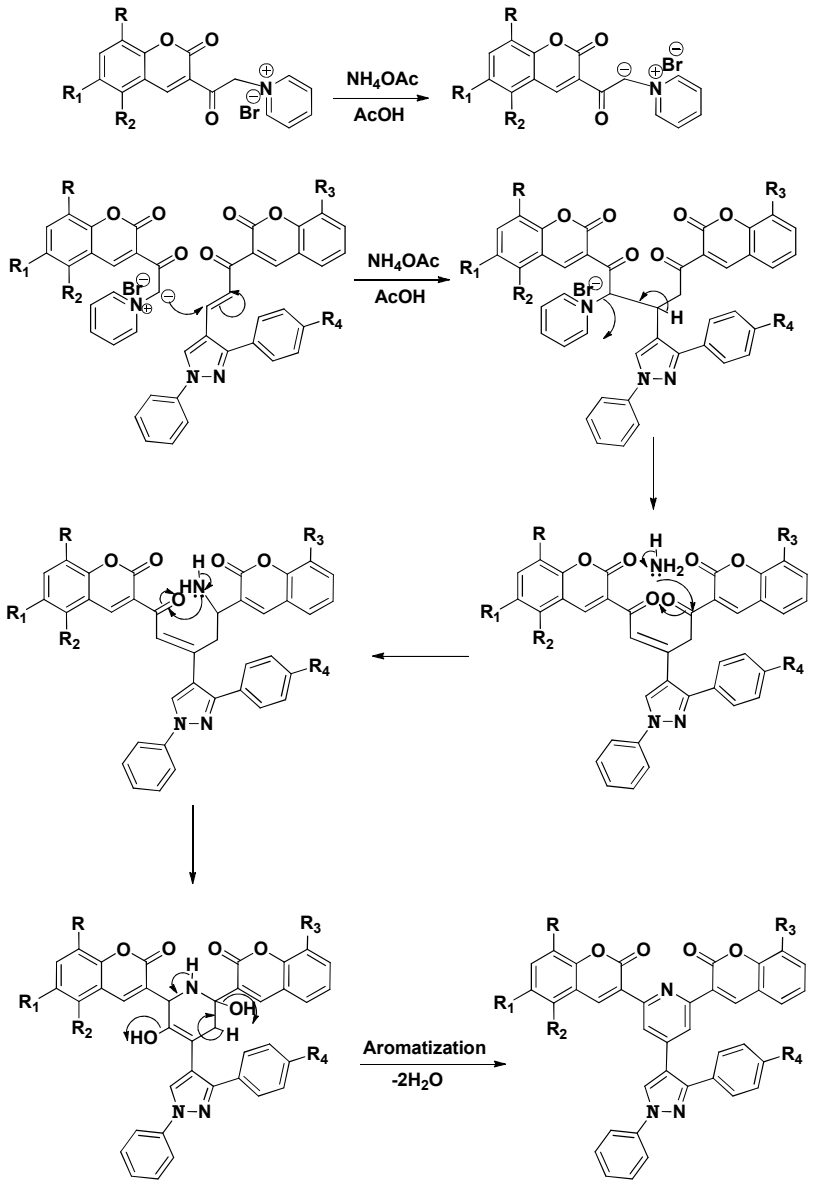
2.1. CHEMISTRY:

In the present work, various 2,6-di(coumarin-3-yl)-4-(1-phenyl-3–aryl-1*H*-pyrazol-4- yl)pyridines **(5a-r)** has been carried out by the reaction of 3-coumarinoyl methyl pyridinium bromide salts **(4a-c)** with various 3-[3-(1-phenyl-3-aryl-1*H*-pyrazol-4-yl) acryloyl] coumarins (coumarin chalcones) **(3a-f)** in the presence of ammonium acetate and acetic acid under Kröhnke reaction conditions [17] **(Scheme 1)**. The starting material 3-[3-(1-phenyl-3-aryl-1*H*-pyrazol-4-yl) acryloyl] coumarins (coumarin chalcones) **(3a-f)** were prepared by the reaction of various 3-acetyl coumarins **(1a-c)** and pyrazolaldehydes **(2a-c)** in the presence of piperidine in ethanol. The plausible mechanism for the synthesis of target compounds **(5a-r)** is demonstrated in **Scheme 2**.



	R	R_1	R_2	R_3	R_4		R	R_1	R_2	R_3	R_4
5a:	H	H	H	H	H	5j:	OCH_3	H	H	OCH_3	H
5b:	H	H	H	H	CH_3	5k:	OCH_3	H	H	OCH_3	CH_3
5c:	H	H	H	H	OCH_3	5l:	OCH_3	H	H	OCH_3	OCH_3
5d:	H	H	H	OCH_3	H	5m:	H	benzo		H	H
5e:	H	H	H	OCH_3	CH_3	5n:	H	benzo		H	CH_3
5f:	H	H	H	OCH_3	OCH_3	5o:	H	benzo		H	OCH_3
5g:	OCH_3	H	H	H	H	5p:	H	benzo		OCH_3	H
5h:	OCH_3	H	H	H	CH_3	5q:	H	benzo		OCH_3	CH_3
5i:	OCH_3	H	H	H	OCH_3	5r:	H	benzo		OCH_3	OCH_3

Scheme-1: Synthetic strategies adopted for the preparation of key precursors (3a-f) and title compounds (5a-r)



Scheme 2. Plausible mechanism for the synthesis of target compounds (5a-r)

The structures of all the synthesized compounds were confirmed on the basis of ^{1}H-NMR; ^{13}C-APT; IR; elemental analysis and representative mass spectral data.

Amongst the compounds **5a-r**, the IR spectrum of **5a** exhibited showed a strong band at 1724 cm^{-1} which is due to carbonyl stretching of δ-lactone ring present in coumarin nucleus. The bands observed at 1593 and 1546 cm^{-1} are due to aromatic C=C and C=N stretching vibrations respectively. The band observed at 3057 cm^{-1} is due to aromatic C-H stretching vibrations. The sharp bands observed at 691 and 748 cm^{-1} are due to C-H out of plane bending vibrations for mono substituted benzene ring. The ^{1}H-NMR spectrum of compound **5a** (in $CDCl_3$) showed twenty three protons in the aromatic region. Out of these, eighteen aromatic protons were observed between 7.34-7.88 δ as

a multiplet, five protons (C_3-H, C_5-H, C_4'-H, C_4''-H and C_5'''-H) were separated out from other aromatic protons and were observed in somewhat downfield region. The C_3 and C_5 protons of pyridine ring appeared as a singlet at 8.35 δ. The C_4' and C_4'' protons of coumarin rings appeared as a singlet at 8.76 δ. The C_5'''-H of pyrazole ring appeared as a sharp singlet at 8.28 δ. The C_3 and C_5 protons of pyridine ring appear in the downfield region due to the peri effect of carbonyl group of δ-lactone. The ^{13}C-APT spectrum of compound **5a** (in DMSO-d_6) showed signals at 116.43, 119.12, 119.58, 120.16, 122.55, 124.85, 125.24, 127.29, 128.69, 128.86, 129.09, 129.52, 130.03, 130.07, 132.64, 133.23, 139.62, 142.08, 143.64, 150.51, 151.64, 153.91 and 159.68 δ. Thus, total twenty three carbon signals are seen. The compound is having twenty three types of non equivalent carbon atoms and hence expected number of signals are observed. The most downfield signal appeared at 159.68 δ can be assigned to the carbonyl carbon of the δ-lactone ring of coumarin. The inverted signals observed in ^{13}C-APT spectrum at 116.43, 119.12, 122.55, 124.85, 127.29, 128.69, 128.86, 129.09, 129.52, 130.03, 130.07, 133.23 and 143.64 δ are due to thirteen non equivalent tertiary carbon atoms present in the compound. The mass spectrum of compound **5a** showed M^+ peak at m/z 585(74%) alongwith some other fragment peaks, which supported the structure of compound **5a**.

2.2. BIOLOGICAL RESULTS:

2.2.1. ANTIMICROBIAL ACTIVITY

The newly synthesized target compounds **(5a-r)** 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 suspention of 10 *μ*L from each well were further incubated and growth was noted at 37°C after 24 hour 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-1)** 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.

2.2.2. ANTIMICROBIAL EVALUTION

The compounds **(5a-r)** were screened for their *in vitro* antibacterial and antifungal evaluation against various bacterial and fungal pathogens by broth dilution method. Ampicillin, Chloramphenicol, Norfloxacin, Griseofulvin and Nystatin were used as standard drugs. The values of MIC are summarized in **Table-1**.

The assessment of antimicrobial screening data reveals that all the compounds **5a-r** exerted significant inhibitory activity against gram positive and gram negative bacteria. Compound **5g** (MIC = 62.5µg/mL) showed excellent activity compared to Ampicillin (MIC = 250µg/mL) and Norfloxacin (MIC = 100µg/mL) against *Bacillus subtilis*. Compounds **5d**, **5j, 5l,** and **5q** (MIC = 100µg/mL) exhibited excellent activity compared to Ampicillin (MIC = 250µg/mL) and equal activity to Norfloxacin (MIC = 100µg/mL) against *Bacillus subtilis* whereas, compounds **5q** (MIC = 62.5µg/mL) and **5l**, **5n**, **5o** (MIC = 100µg/mL) showed excellent activity against gram positive bacteria *Staphylococcus aureus* compared to Ampicillin. Compound **5k** (MIC = 62.5µg/mL) and compounds **5c, 5o** (MIC = 62.5µg/mL) have shown excellent activity in comparison of Ampicillin against *Escherichia coli* and *Salmonella typhi* respectively. Compounds **5h**, **5i, 5m** and **5p** (MIC = 200µg/mL) were found to be more potent against *Staphylococcus aureus* whereas compounds **5b, 5c, 5e, 5f, 5n** and **5r** (MIC = 200µg/mL) were found to be more active against *Bacillus subtilis* compared to Ampicillin. Compounds **5a, 5b, 5d, 5k** (MIC = 250µg/mL) and compounds **5a, 5h, 5i, 5k, 5m, 5o** (MIC = 250µg/mL) have shown equal activity to Ampicillin against gram positive bacteria *Staphylococcus aureus and Bacillus subtilis* respectively. Compounds **5c**, **5f, 5o** and **5r** (MIC = 100µg/mL) are found to be equipotent to Ampicillin against *Escherichia coli* while compounds **5j, 5p** and **5q** (MIC = 100µg/mL) showed equal activity to Ampicillin against *Salmonella typhi.* Compounds **5l** (MIC = 200µg/mL) and

compounds **5g, 5r** (MIC = 250µg/mL) were found to be more active than Griseofulvin (MIC = 500µg/mL) whereas, Compounds **5h, 5i, 5j** and **5m** are found to be equipotent to Griseofulvin (MIC = 500µg/mL) against *Candida albicans*. None of the tested compounds showed better activity against *Aspergillus niger* than standard drugs.

Table-1 : In *vitro* Antimicrobial activity of compounds (5a-f)

Compound	Minimum Inhibitory Concentration (MIC, $\mu g mL^{-1}$)					
	Gram +ve bacteria		Gram –ve bacteria		Fungi	
	B.s.	***S.a.***	***E.c.***	***S.t.***	***A.n.***	***C.a.***
5a	**250**	**250**	250	250	1000	1000
5b	**200**	**250**	200	200	1000	>1000
5c	**200**	**125**	**100**	**62.5**	500	1000
5d	**100**	**250**	200	200	500	>1000
5e	**200**	**125**	250	250	250	>1000
5f	**200**	**200**	**100**	200	1000	>1000
5g	**62.5**	**125**	200	100	1000	**250**
5h	**250**	**200**	125	250	100	**500**
5i	**250**	**200**	250	125	1000	**500**
5j	**100**	**125**	125	**100**	500	**500**
5k	**250**	**250**	**62.5**	200	500	>1000
5l	**100**	**100**	200	200	1000	**200**
5m	**250**	**200**	125	250	>1000	**500**
5n	**200**	**100**	200	250	250	1000
5o	**250**	**100**	**100**	**62.5**	>1000	1000
5p	**125**	**200**	250	**100**	250	1000
5q	**100**	**62.5**	200	**100**	500	1000
5r	**200**	**125**	**100**	250	1000	**250**
Ampicillin	250	250	100	100	-	-
Chloramphenicol	50	50	50	50	-	-
Ciprofloxacin	50	50	25	25	-	-
Norfloxacin	100	10	10	10	-	-
Gentamycin	1	0.25	0.05	5	-	-
Griseofulvin	-	-	-	-	100	500
Nystatin	-	-	-	-	100	100

B.s.: *Bacillus subtilis,* ***S.a.:*** *Staphylococcus aureus,* ***E.c.:*** *Escherichia coli,* ***S.t.:*** *Salmonella typhi,* ***A.n.:*** *Aspergillus niger,* ***C.a.:*** *Candida albicans*

MATERIALS AND METHODS

All the melting points are uncorrected. All reactions were performed with commercially available reagents and they were used without further purification. Organic solvents were purified by standard methods and stored over molecular sieves. All the IR spectra (KBr disc) were recorded on Shimadzu FT-IR 8400-S spectrometer. ^{1}H-NMR and ^{13}C APT spectra were recorded on Bruker Advance 400 spectrometer operating at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-APT. The chemical shift (δ) is reported in ppm using chloroform-d as a solvent and calibrated standard solvent signal. Mass spectra were recorded on Shimadzu QP 2010 spectrometer. Elemental analysis was carried out on Perkin- Elmer 2400 C-H-N-S-O Analyzer Series-II. Column chromatography was performed with silica gel 60–120 mesh (Merck, Mumbai, India.). All the compounds were routinely checked for completion of the reaction on silica gel 60 F254 TLC plates and their spots were visualized by exposure to a UV lamp, iodine vapour or $KMnO_4$ reagents.

Starting precursors 3-acetyl coumarins **(1-c)** [19], pyrazole aldehydes **(2a-c)** [20], 1-[2*H*-1-benzopyran-2-on-3-yl]-3-aryl-prop-2-en-1-ones (**3a-f)** [21] and 3-Coumarinoyl methyl pyridinium salts **(4a-c)** [22] were prepared using the reported procedures.

3.1. General procedure for the synthesis of 2,6-di(coumarin-3-yl)-4-(1-phenyl-3-aryl- 1*H*-pyrazol-4-yl)pyridines (5a-r).

In a 100 mL round bottom flask equipped with a dropping funnel, condenser, guard tube and magnetic needle, appropriate 3-coumarinoyl methyl pyridinium bromide salt **(4a-c)** (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 appropriate 3-[3-(1phenyl-3-aryl-1*H*-pyrazol-4-yl)acryloyl] coumarin (coumarin chalcone) **(3a-f)** (0.003 mol) in glacial acetic acid (15 mL) was added with stirring at room temperature during 15 minutes. The reaction mixture was further stirred for 1 hour and then refluxed for 8 hours 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 x 30 mL). The organic layer was washed with 5% sodium bicarbonate solution (3 x 20 mL), water (2 x 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 (6:4) as an eluent to give products **(5a-r)**. The compounds were recrystallized from chloroform-hexane.

The physical, analytical and spectral data for compounds **(5a-r)** are given below.

***Compound 5a* :** Yield 73%; mp 245-247 °C; yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1724 (C=O stretching of δ-lactone of coumarin), 1593 and 1546 (aromatic C=C and C=N stretchings), 3057 (aromatic C-H stretching), 691 and 748 (C-H bending of mono substituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 7.34-7.88 (18H, multiplet, aromatic protons), 8.28 (1H, singlet, C_5'''-H), 8.35 (2H, singlet, C_3-H and C_5-H), 8.76 (2H, singlet, C_4'-H and C_4''-H); ^{13}C APT (100MHz, DMSO-d_6, δ): 116.43(CH), 119.12(CH), 119.58(C), 120.16(C), 122.55(CH), 124.85(CH), 125.24(C), 127.29(CH), 128.69(CH), 128.86(CH), 129.09(CH), 129.52(CH), 130.03(CH), 130.07(CH), 132.64(C), 133.23(CH), 139.62(C), 142.08(C), 143.64(C), 150.51(C), 151.64(C), 153.91(C) and 159.68(CO of coumarin). Anal. Calcd. for $C_{38}H_{23}N_3O_4$: C, 77.94; H, 3.96; N, 7.18%. Found: C, 77.89; H, 3.91; N, 7.13%.

***Compound 5b* :** Yield 70%; mp >280°C; yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1720 (C=O stretching of δ-lactone of coumarin), 1605 and 1530 (aromatic C=C and C=N stretchings), 2930 (aliphatic C-H stretching), 3055 (aromatic C-H stretching), 825 (C-H bending vibrations of p-disubstituted benzene ring), 770 and 710 (C-H bending of mono substituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$+TFA, δ): 2.56 (3H, singlet, CH_3), 7.42-7.97 (17H, multiplet, aromatic protons), 8.41 (2H, singlet, C_3-H and C_5-H), 8.60 (2H, singlet, C_4'-H and C_4''-H), 8.95 (1H, singlet, C_5'''-H); ^{13}C APT (100MHz, $CDCl_3$+TFA, δ): 21.21(CH_3), 109.95(C), 112.78(C), 113.99(C), 115.21(CH), 115.61(C), 116.80(C), 117.10(CH), 118.11(C), 118.44(C), 120.24(CH), 121.48(CH), 126.98(CH), 129.27(CH), 130.18(CH), 130.43(CH), 131.06(CH), 131.96(CH), 137.06(CH), 145.48(C), 148.31(CH), 150.09(C), 153.99(C), and 160.22(CO of coumarin). Anal. Calcd. for $C_{39}H_{25}N_3O_4$: C, 78.12; H, 4.20; N, 7.01%. Found: C, 78.06; H, 4.24; N, 7.06%.

***Compound 5c* :** Yield 75%; mp 249-251°C; yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1715 (C=O stretching of δ-lactone of coumarin), 1610 and 1510 (aromatic C=C and C=N stretchings), 2934 (aliphatic C-H stretching), 3060 (aromatic C-H stretching), 825 (C-H bending vibrations of p-disubstituted benzene ring), 750 and 690 (C-H bending of mono substituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$+TFA, δ): 3.97 (3H, singlet, OCH_3), 7.19-7.90 (17H, multiplet, aromatic protons), 8.33 (2H, singlet, C_3-H and C_5-H), 8.56 (2H, singlet, C_4'-H and C_4''-H), 8.96 (1H, singlet, C_5'''-H); ^{13}C APT (100MHz, $CDCl_3$+TFA, δ): 55.64(OCH_3), 110.03(C), 112.86(C), 114.17(C), 115.24(CH), 115.69(C), 116.85(C), 117.17(CH), 118.17(C), 118.53(C), 120.01(CH), 121.11(CH), 126.81(CH), 129.63(CH), 130.09(CH), 130.37(CH), 131.06(CH), 131.93(CH), 136.72(CH), 145.38(C), 148.12(CH), 150.00(C), 154.05(C), and 160.66(CO of coumarin).Anal. Calcd. for $C_{39}H_{25}N_3O_5$: C, 76.09; H, 4.09; N, 6.83%. Found: C, 76.05; H, 4.06; N, 6.79%

***Compound 5d* :** Yield 69%; mp >280°C; yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1720 (C=O stretching of δ-lactone of coumarin), 1595 and 1485 (aromatic C=C and C=N stretchings), 2936 (aliphatic C-H stretching), 3055 (aromatic C-H stretching), 755 and 719 (C-H bending vibrations of mono substituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$+TFA, δ): 4.06 (3H, singlet, OCH_3), 7.27-7.87 (17H, multiplet, aromatic protons), 8.32 (2H, poorly resolved doublet, C_3-H and C_5-H), 8.48 and 8.53 (2H, two singlets, C_4'-H and C_4''-H), 9.05 (1H, singlet, C_5'''-H); ^{13}C APT (100MHz, $CDCl_3$+TFA, δ): 56.58(OCH_3), 110.24(C), 113.07(C), 114.23(C), 114.43(C), 115.91(CH), 116.86(C), 117.10(CH), 117.88(C), 118.19(C), 118.75(CH), 118.85(CH), 119.93(CH), 120.63(CH), 121.25(CH), 126.64(CH), 129.12(CH), 129.62(CH), 129.65(CH), 129.98(CH), 130.35(CH), 131.49(CH), 136.49(CH), 138.13(C), 143.62(C), 145.21(C), 145.32(C), 147.22(C), 147.74(CH), 147.99(CH), 150.08(C), 152.92(C), 154.05(C) and 159.79(CO of coumarin). Anal. Calcd. for $C_{39}H_{25}N_3O_5$: C, 76.09; H, 4.09; N, 6.83%. Found: C, 76.12; H, 4.06; N, 6.78%.

***Compound 5e* :** Yield 72%; mp 282-284°C; yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1715 (C=O stretching of δ-lactone of coumarin), 1595 and 1525 (aromatic C=C and C=N stretchings), 2937 (aliphatic C-H stretching), 3060 (aromatic C-H stretching), 825 (C-H bending vibrations of p-disubstituted benzene ring), 752 and 720 (C-H bending of mono substituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 2.38 (3H, singlet, CH_3), 3.98 (3H, singlet, OCH_3), 7.09-7.85 (16H, multiplet, aromatic protons), 8.27 (1H, singlet, C_5'''-H) 8.34 (2H, concealed doublet, C_3-H and C_5-H), 8.72 and 8.74 (2H, two singlets, C_4'-H and C_4''-H); ^{13}C APT (100MHz, $CDCl_3$, δ): 21.42(CH_3), 56.30(OCH_3), 113.84(CH), 116.38(CH), 119.25(CH), 119.47(C), 120.08(C), 120.16(CH), 120.21(C), 120.73(C), 122.75(CH), 124.39(CH), 124.56(CH), 125.55(C), 125.73(C), 126.77(CH), 127.61(CH), 128.53(CH), 128.86(CH), 128.97(CH), 129.28(CH), 129.48(CH), 132.17(CH), 138.13(C), 139.69(C), 142.43 (C), 142.61(CH), 142.72(CH), 143.58(C), 146.91(C) , 151.21(C) 151.28(C), 151.30(C), 153.93(C), 159.48(CO of coumarin) and 160.06(CO of coumarin).Anal. Calcd. for $C_{40}H_{27}N_3O_5$: C, 76.03; H, 4.32; N, 6.67%. Found: C, 75.98; H, 4.37; N, 6.61%.

***Compound 5f* :** Yield 74%; mp 248-250 °C; yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1715 (C=O stretching of δ-lactone of coumarin), 1590 and 1515 (aromatic C=C and C=N stretchings), 2942 (aliphatic C-H stretching), 3055 (aromatic C-H stretching), 830 (C-H bending vibrations of p-disubstituted benzene ring), 748 and 697 (C-H bending of mono substituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 3.85 and 4.02 (6H, two singlets, 2 x OCH_3), 6.93-7.86 (16H, multiplet, aromatic protons), 8.35 (3H, multiplet, C_3-H,C_5-H and C_5'''-H), 8.73 and 8.75 (2H, two singlets, C_4'-H and C_4''-H); ^{13}C APT (100MHz, $CDCl_3$+TFA, δ): 55.62(OCH_3), 56.61(OCH_3), 110.05(C), 112.75(C), 113.87(C), 115.58(C), 116.83(CH), 117.08(C), 118.41(C), 118.57(CH), 118.67(C), 118.73(C), 118.95(CH), 120.98(CH), 121.52(CH), 122.93(CH), 123.63(CH), 126.55(CH), 127.18(CH), 128.53(CH), 129.34(CH), 129.76(CH), 130.09(CH), 131.65(C), 131.72(CH), 134.51(CH), 137.81(C), 143.51(C), 144.60(C), 146.19(C), 147.07(C),147.78(CH),147.98(CH),152.78(C), 153.54(CO of coumarin), and 159.95(CO of coumarin)..Anal. Calcd. for $C_{40}H_{27}N_3O_6$: C, 74.41; H, 4.21; N, 6.51%. Found: C, 74.36; H, 4.18; N, 6.48%%.

***Compound 5g* :** Yield 75%; mp >280°C; yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1720 (C=O stretching of δ-lactone of coumarin), 1595 and 1485 (aromatic C=C and C=N stretchings), 2938 (aliphatic C-H stretching), 3055 (aromatic C-H stretching), 755 and 721 (C-H bending vibrations of mono substituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 4.02 (3H, singlet, OCH_3), 7.13-7.87 (17H, multiplet, aromatic protons), 8.35 (2H, poorly resolved doublet, C_3-H and C_5-H), 8.37 (1H, singlet, C_5'''-H) 8.74 and 8.75 (2H, two singlets, C_4'-H and C_4''-H); ^{13}C APT (100MHz, $CDCl_3$+TFA, δ): 56.58(OCH_3), 110.24(C), 113.07(C), 114.23(C), 114.43(C), 115.91(CH), 116.86(C), 117.10(CH), 117.88(C), 118.19(C), 118.75(CH), 118.85(CH), 119.93(CH), 120.63(CH), 121.25(CH), 126.64(CH), 129.12(CH), 129.62(CH), 129.65(CH), 129.98(CH), 130.35(CH), 131.49(CH), 136.49(CH), 138.13(C), 143.62(C), 145.21(C), 145.32(C), 147.22(C), 147.74(CH), 147.99(CH), 150.08(C), 152.92(C), 154.05(C) and 159.79(CO of coumarin).Anal. Calcd. for $C_{39}H_{25}N_3O_5$: C, 76.09; H, 4.09; N, 6.83%. Found: C, 76.11; H, 4.05; N, 6.79%.

***Compound 5h* :** Yield 70%; mp 281-283 °C; yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1715 (C=O stretching of δ-lactone of coumarin), 1595 and 1525 (aromatic C=C and C=N stretchings), 2932 (aliphatic C-H stretching), 3060 (aromatic C-H stretching), 825 (C-H bending vibrations of p-disubstituted benzene ring), 750 and 714 (C-H bending of mono substituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 2.38 (3H, singlet, CH_3), 3.98 (3H, singlet, OCH_3), 7.09-7.85 (16H, multiplet, aromatic protons), 8.27 (1H, singlet, C_5'''-H) 8.34 (2H, poorly resolved doublet, C_3-H and C_5-H), 8.72 and 8.74 (2H, two singlets, C_4'-H and C_4''-H); ^{13}C APT (100MHz, $CDCl_3$, δ): 21.42(CH_3), 56.30(OCH_3), 113.84(CH), 116.38(CH), 119.25(CH), 119.47(C), 120.08(C), 120.16(CH), 120.21(C), 120.73(C), 122.75(CH), 124.39(CH), 124.56(CH), 125.55(C), 125.73(C), 126.77(CH), 127.61(CH), 128.53(CH), 128.86(CH), 128.97(CH), 129.28(CH), 129.48(CH), 132.17(CH), 138.13(C), 139.69(C), 142.43 (C), 142.61(CH), 142.72(CH), 143.58(C), 146.91(C) , 151.21(C) 151.28(C), 151.30(C), 153.93(C), 159.48(C) and 160.06(CO of coumarin).Anal. Calcd. for $C_{40}H_{27}N_3O_5$: C, 76.30; H, 4.32; N, 6.67%. Found: C, 76.25; H, 4.28; N, 6.62%.

***Compound 5i* :** Yield 72%; mp 248-250°C; yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1715 (C=O stretching of δ-lactone of coumarin), 1610 and 1515 (aromatic C=C and C=N stretchings), 2930 (aliphatic C-H stretching), 3056 (aromatic C-H stretching), 830 (C-H bending vibrations of p-disubstituted benzene ring), 749 and 716 (C-H bending of mono substituted benzene ring); ^{1}H NMR (400MHz, DMSO-d_6, δ): 3.78 and 3.96 (6H, two singlets, 2 x OCH_3), 6.97-8.01 (16H, multiplet, aromatic protons),8.31(2H, meta coupled doublet *J = 2.0 Hz*, C_3-H, C_5-H), 9.01 (1H, singlet, C_5'''-H) 9.06 and 9.08 (2H, two singlets, C_4'-H and C_4''-H); ^{13}C APT (100MHz, $CDCl_3$+TFA, δ): 55.62(OCH_3), 56.61(OCH_3), 110.05(C), 112.75(C), 113.87(C), 115.58(C), 116.83(CH), 117.08(C), 118.41(C), 118.57(CH), 118.67(C), 118.73(C), 118.95(CH), 120.98(CH), 121.52(CH), 122.93(CH), 123.63(CH), 126.55(CH), 127.18(CH), 128.53(CH), 129.34(CH), 129.76(CH), 130.09(CH), 131.65(C), 131.72(CH), 134.51(CH), 137.81(C), 143.51(C), 144.60(C), 146.19(C), 147.07(C),147.78(CH), 147.98(CH), 152.78(C), 153.54(CO of coumarin), and 159.95(CO of coumarin).Anal. Calcd. for $C_{40}H_{27}N_3O_6$: C, 74.41; H, 4.21; N, 6.51%. Found: C, 74.35; H, 4.18; N, 6.47%.

***Compound 5j* :** Yield 69%; mp 270-272°C; yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1731 (C=O stretching of δ-lactone of coumarin), 1629 and 1516 (aromatic C=C and C=N stretchings), 2937 (aliphatic C-H stretching), 3050 (aromatic C-H stretching), 753 and 719 (C-H bending vibrations of mono substituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 4.02 (6H, singlet, 2 x OCH_3), 7.13-7.87 (16H, multiplet, aromatic protons), 8.35 (3H, multiplet, C_3-H,C_5-H and C_5'''-H), 8.74 (2H, singlet, C_4'-H and C_4''-H); ^{13}C APT (100MHz, $CDCl_3$+TFA, δ): 56.67(OCH_3), 110.03(C), 112.89(C), 114.17(C), 115.24(CH), 115.79(C), 116.85(C), 117.17(CH), 118.27(C), 118.63(C), 120.01(CH), 121.11(CH), 126.81(CH), 129.63(CH), 130.09(CH), 130.37(CH), 131.06(CH), 131.93(CH), 136.72(CH), 145.38(C), 148.12(CH), 150.00(C), 154.05(C), and 160.66(CO of coumarin).Anal. Calcd. for $C_{40}H_{27}N_3O_6$: C, 74.41; H, 4.21; N, 6.51%. Found: C, 74.45; H, 4.18; N, 6.48%.

***Compound 5k* :** Yield 70%; mp 268°C; yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1715 (C=O stretching of δ-lactone of coumarin), 1610 and 1540 (aromatic C=C and C=N stretchings), 2928 (aliphatic C-H stretching), 3066 (aromatic C-

H stretching), 825 (C-H bending vibrations of p-disubstituted benzene ring), 748 and 714 (C-H bending of mono substituted benzene ring); ^{1}H NMR (400MHz, DMSO-d_6, δ): 2.34 (3H, singlet, CH_3), 3.78 (6H, singlet, 2 x OCH_3), 6.97-7.99 (15H, multiplet, aromatic protons), 8.31 (2H, singlet, C_3-H and C_5-H), 9.00 (1H, singlet, C_5'''-H) 9.08 (2H, singlet, C_4'-H and C_4''-H); ^{13}C APT (100MHz, $CDCl_3$+TFA, δ): 21.21(CH_3), 55.61(OCH_3), 110.11(C), 112.94(C), 114.05(C), 115.77(C), 116.88(C), 117.19(CH), 118.12(C), 118.61(C), 120.06(CH), 121.10(CH), 126.88(CH), 129.59(CH), 129.64(CH), 130.13(CH), 130.33(CH), 131.91(CH), 136.86(CH), 145.41(C), 148.09(CH), 149.93(C), 153.01(C), 154.02(C), and 160.01(CO of coumarin).Anal. Calcd. for $C_{41}H_{29}N_3O_6$: C, 74.65; H, 4.43; N, 6.37%. Found: C, 74.60; H, 4.39; N, 6.34%.

***Compound 5l* :** Yield 69%; mp >300°C; yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1723 (C=O stretching of δ-lactone of coumarin), 1613 and 1516 (aromatic C=C and C=N stretchings), 2934 (aliphatic C-H stretching), 3040 (aromatic C-H stretching), 829 (C-H bending vibrations of p-disubstituted benzene ring), 750 and 690 (C-H bending of mono substituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 3.84 (3H, singlet, OCH_3), 4.01 (6H, singlet, 2 x OCH_3), 6.91-7.85 (15H, multiplet, aromatic protons), 8.28 (1H, singlet, C_5'''-H) 8.35 (2H, singlet, C_3-H and C_5-H), 8.73 (2H, singlet, C_4'-H and C_4''-H); ^{13}C APT (100MHz, $CDCl_3$, δ): 55.29(OCH_3), 56.29(OCH_3), 113.90(CH), 114.09(CH), 119.26(CH), 120.14(C), 120.24(CH), 122.88(CH), 124.39(CH), 124.92(C), 125.88(C), 126.75(CH), 127.63(CH), 129.04(CH), 129.49(CH), 129.94(C), 134.62(C), 142.54(C), 142.71(CH), 146.98(C), 147.21(C), 148.51(C), 151.39(C), 154.99(C), and 159.79(CO of coumarin).Anal. Calcd. for $C_{41}H_{29}N_3O_7$: C, 72.88; H, 4.33; N, 6.22%. Found: C, 72.82; H, 4.30; N, 6.18%.

***Compound 5m* :** Yield 74%; mp 259-261°C; yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1726 (C=O stretching of δ-lactone of coumarin), 1596 and 1560 (aromatic C=C and C=N stretchings), 3070 (aromatic C-H stretching), 750 and 690 (C-H bending of mono substituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$+TFA, δ): 7.23-8.38 (20H, multiplet, aromatic protons), 8.47 (2H, poorly resolved doublet, C_3-H and C_5-H), 8.77 (1H, singlet, C_5'''-H) 8.92 (1H, singlet,C_4''-H), 9.20 (1H, singlet, C_4'-H); ^{13}C APT (100MHz, $CDCl_3$+TFA, δ): 109.93(C), 112.06(C), 112.79(C), 113.53(C), 114.23(CH), 115.62(C), 116.09(CH), 116.91(C), 118.27(CH), 118.44(C), 118.89(C), 119.77(CH), 120.29(CH), 121.32(CH), 121.41(CH), 121.47(CH), 127.02(CH), 128.06(CH), 128.77(CH), 129.57(CH), 129.77(CH), 129.90(CH), 130.16(CH), 13O.71(CH), 130.82(C), 132.34(CH), 137.44(C), 139.57(CH), 142.94(CH), 143.46(C), 145.51(CH), 145.64(C), 147.07(C), 148.74(CH), 149.67(C), 152.72(C), 155.42(CO of coumarin) and 160.44(CO of coumarin).Anal. Calcd. for $C_{42}H_{25}N_3O_4$: C, 77.36; H, 3.96; N, 6.61%. Found: C, 77.30; H, 3.94; N, 6.57%.

***Compound 5n*:** Yield 73%; mp 278-280 °C; yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1728 (C=O stretching of δ-lactone of coumarin), 1597 and 1504 (aromatic C=C and C=N stretchings), 2934 (aliphatic C-H stretching), 3055 (aromatic C-H stretching), 825 (C-H bending vibrations of p-disubstituted benzene ring), 748 and 687 (C-H bending of mono substituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 2.40 (3H, singlet, CH_3), 7.22-8.46 (22H, multiplet, aromatic protons including C_3-H, C_5-H and C_5'''-H), 8.77 (1H, singlet,C_4''-H), 9.53 (1H, singlet, C_4'-H) ; ^{13}C APT (100MHz, $CDCl_3$, δ): 21.44(CH_3), 113.76(C), 116.47(CH), 116.66(CH), 119.26(CH), 119.53(C), 120.21(C), 121.80(CH), 122.71(CH), 122.77(CH), 124.42(C), 124.58(CH), 125.81(C), 126.17(CH), 126.80(CH), 127.65(CH), 128.45(CH), 128.56(CH), 128.84(CH), 129.13(CH), 129.30(CH), 129.49(CH), 129.54(C), 130.38(C), 132.22(CH), 133.69(CH), 138.17(C), 138.38(CH), 139.72(C), 142.54(C) 142.63(CH), 151.53(C), 151.76(C), 152.20(C), 153.93(C), 154.01(C), 160.06(CO of coumarin), and 160.15(CO of coumarin).Anal. Calcd. for $C_{43}H_{27}N_3O_4$: C, 77.49; H, 4.19; N, 6.96%. Found: C, 77.44; H, 4.23 N, 6.90%.

***Compound 5o*:** Yield 76%; mp >280°C; yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1728 (C=O stretching of δ-lactone of coumarin), 1597 and 1504 (aromatic C=C and C=N stretchings), 2939 (aliphatic C-H stretching), 3063 (aromatic C-H stretching), 825 (C-H bending vibrations of p-disubstituted benzene ring), 741 and 687 (C-H bending of mono substituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$+TFA, δ): 3.90 (3H, singlet, OCH_3), 7.23-8.38 (19H, multiplet, aromatic protons), 8.47 (2H, poorly resolved doublet, C_3-H and C_5-H), 8.77 (1H, singlet, C_5'''-H) 8.92 (1H, singlet,C_4''-H), 9.20 (1H, singlet, C_4'-H); ^{13}C APT (100MHz, $CDCl_3$+TFA, δ): 55.55(OCH_3), 109.96(C), 112.08(C), 112.78(C), 113.56(C), 115.28(CH), 115.61(C), 116.02(CH), 116.78(C), 117.16(CH), 118.18(C), 118.44(C), 119.92(CH), 120.36(CH), 120.93(CH), 121.57(CH), 127.01(CH), 128.09(CH), 128.83(C), 129.71(CH), 129.91(CH), 130.22(CH), 130.43(CH), 130.62(CH), 130.87(C), 131.00(CH), 137.05(CH), 139.55(CH), 141.59(CH), 143.09(CH) 145.78(C), 147.03(C), 148.63(CH), 149.66(C), 152.76(C), 154.01(C), 155.49(C), 161.24(CO of coumarin), and 161.67(CO of coumarin).Anal. Calcd. for $C_{43}H_{27}N_3O_5$: C, 77.58; H, 4.09; N, 6.31%. Found: C, 77.52; H, 4.06; N, 6.28%.

***Compound 5p*:** Yield 75%; mp 281-283°C; yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1728 (C=O stretching of δ-lactone of coumarin), 1597 and 1481 (aromatic C=C and C=N stretchings), 2931 (aliphatic C-H stretching), 3070 (aromatic C-H stretching), 741 and 687 (C-H bending of mono substituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 4.02

(3H, singlet, OCH_3), 7.14-8.46 (22H, multiplet, aromatic protons including C_3-H, C_5-H and C_5'''-H), 8.77 (1H, singlet,C_4''-H), 9.53 (1H, singlet, C_4'-H) ; ^{13}C APT (100MHz, $CDCl_3$+TFA, δ): 56.56(OCH_3), 109.96(C), 112.00(C), 112.79(C), 113.55(C), 114.23(CH), 115.62(C), 116.09(CH), 116.91(C), 118.27(CH), 118.44(C), 118.89(C), 119.77(CH), 120.29(CH), 121.30(CH), 121.41(CH), 121.47(CH), 127.00(CH), 128.06(CH), 128.77(C), 129.56(CH), 129.77(CH), 129.90(CH), 130.16(CH), 130.71(CH), 130.82(C), 132.34(CH), 137.44(C), 139.57(CH), 142.99(CH) 143.46(C), 145.51(CH), 145.64(C), 147.68(C), 148.78(CH), 149.65(C), 152.74(C), 155.41(CO of coumarin), and 160.41(CO of coumarin).Anal. Calcd. for $C_{43}H_{27}N_3O_5$: C, 77.58; H, 4.09; N, 6.31%. Found: 77.53; H, 4.05; N, 6.26%.

***Compound 5q*:** Yield 76%; mp >280°C; yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1736 (C=O stretching of δ-lactone of coumarin), 1619 and 1506 (aromatic C=C and C=N stretchings), 2940 (aliphatic C-H stretching), 3047 (aromatic C-H stretching), 818 (C-H bending vibrations of p-disubstituted benzene ring), 756 and 694 (C-H bending of mono substituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$+TFA, δ): 2.48 (3H, singlet, CH_3), 4.08 (3H, singlet, OCH_3), 7.34-8.46 (18H, multiplet, aromatic protons), 8.50 (2H, poorly resolved doublet, C_3-H and C_5-H), 8.71 (1H, singlet, C_5'''-H) 8.93 (1H, singlet,C_4''-H), 9.20 (1H, singlet, C_4'-H) ; ^{13}C APT (100MHz, $CDCl_3$+TFA, δ): 21.40(CH_3), 56.58(OCH_3), 109.97(C), 111.09(C), 112.94(C), 114.96(C), 115.57(C), 116.07(CH), 118.40(CH), 119.73(C), 119.86(CH), 121.65(CH), 121.89(CH), 122.40(C), 124.66(CH), 127.15(CH), 127.89(C), 128.37(CH), 129.01(CH), 129.20(CH), 129.39(CH), 129.47(CH), 129.91(CH), 130.15(CH), 130.76(CH), 131.09(CH), 132.28(C), 134.38(C), 135.20(C), 137.26(CH) 139.86(CH), 143.50(C), 148.30(C), 149.85(C), 153.67(C), 155.45(C), 160.53(CO of coumarin), and 161.05(CO of coumarin).Anal. Calcd. for $C_{44}H_{29}N_3O_5$: C, 77.55; H, 4.30; N, 6.18%. Found: 77.50; H, 4.26; N, 6.15%.

***Compound 5r*:** Yield 74%; mp >280°C; yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1728 (C=O stretching of δ-lactone of coumarin), 1597 and 1504 (aromatic C=C and C=N stretchings), 2932 (aliphatic C-H stretching), 3055 (aromatic C-H stretching), 833 (C-H bending vibrations of p-disubstituted benzene ring), 741 and 687 (C-H bending of mono substituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$+TFA, δ): 3.90 (3H, singlet, OCH_3), 4.18 (3H, singlet, OCH_3), 7.27-8.42 (18H, multiplet, aromatic protons), 8.51 (2H, poorly resolved doublet, C_3-H and C_5-H), 8.81 (1H, singlet, C_5'''-H) 8.96 (1H, singlet,C_4''-H), 9.24 (1H, singlet, C_4'-H); ^{13}C APT (100MHz, $CDCl_3$+TFA, δ): 55.61(OCH_3), 56.61(OCH_3), 109.92(C), 112.06(C), 112.76(C), 113.58(C), 115.24(CH), 115.61(C), 116.02(CH), 116.78(C), 117.16(CH), 118.18(C), 118.44(C), 119.92(CH), 120.36(CH), 120.93(CH), 121.57(CH), 127.01(CH), 128.09(CH), 128.83(C), 129.71(CH), 129.91(CH), 130.22(CH), 129.43(CH), 130.63(CH), 130.87(C), 131.02(CH), 137.02(C), 139.55(CH), 141.59(CH), 143.09(CH) 145.78(C), 147.03(C), 148.63(CH), 149.68(C), 152.76(C), 154.03(C), 155.48(C), 161.24(CO of coumarin) and 161.66(CO of coumarin). Anal. Calcd. for $C_{44}H_{29}N_3O_6$: C, 75.96; H, 4.20; N, 6.04%. Found: C, 75.90; H, 4.16; N, 6.00%.

In case of compounds **5m-r**, in ^{1}H-NMR spectra, the C_4' proton of coumarin ring appeared in the most downfield region due to diamagnetic anisotropic effect of additional benzene ring fused to coumarin nucleus.

In case of the compounds **5d,5g,5n** and **5q** the number of carbon signals in ^{13}C-APT spectra are less than expected (in case of compounds **5d,5g,5n** one signal and in **5q** two signals). This may be due to identical chemical shifts of certain carbons which may appear at same position.

CONCLUSION

From present study, we summarized that employed synthetic strategy provide efficient route for the synthesis 2,6-di(coumarin-3-yl)-4-(1-phenyl-3–aryl-1*H*-pyrazol-4-yl)pyridines 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 **5c, 5g, 5k, 5o** and **5q** were found to be the most efficient members of the series.

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