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Synthesis, Characterization and *In Vitro* Antimicrobial Screening of Some PyrazolylPyridyl Substituted Dicoumarins

Dr. Neha N. Gohil, Dr. D. I. Brahmbhatt
Department of Chemistry, Sardar Patel University,
Vallabh Vidyanagar, Gujarat, India

ABSTRACT
In the present study, a series of pyrazolyl pyridyl substituted dicoumarins has been synthesized. The synthesis of various 3',3"-(4-(1'"-phenyl-3'"-(pyridin-3""-yl)-1*H*-pyrazol-4'"-yl)pyridine-2,6-diyl) dicoumarins and 3',3"-(4-(1'"-phenyl-3'"-(pyridin-4""-yl)-1*H*-pyrazol-4'"-yl) pyridine-2,6-diyl)dicoumarins has been carried out by the reaction of various 3-coumarinoyl methyl pyridinium bromide salts with 3-(3-(1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazol-4-yl)acryloyl)coumarins and 3-(3-(1-phenyl-3-(pyridin-4-yl)-1*H*-pyrazol-4-yl)acryloyl)coumarins (coumarin chalcones) respectively under *Krohnke's* reaction condition. Structural assignments were based on spectroscopic methods (IR, ^{1}H-NMR, ^{13}C-APT, Mass spectral data and elemental analysis). The compounds were subjected to *in vitro* antimicrobial screening against representative panel of bacteria (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Salmonella typhi*) and fungi (*Aspergillus niger and Candida albicans*).

Keywords: *Coumarins, dicoumarins,krohnke synthesis, antimicrobial screening, broth dilution method, spectral data.*

INTRODUCTION
Coumarins (2*H*-1-benzopyran-2-ones) are well known aromatic *δ*-lactones isolated from variety of plant sources[1].Coumarins have a wide range of applications in the field of pharmaceuticals due to their diverse pharmacological and biological propertiessuch as antimicrobial[2], anticoagulant[3], antitumor[4], anti-inflammatory[5], anti-HIV[6], anticonvulsant[7], antidiabetic[8], analgesic[9], antianxiety[10] etc.A wide number ofcoumarin derivatives have been found in literature which contain nucleus like pyridine, indole, imidazole, diazole, thiazole, and triazole etc. as a substituent group possess important biological activities[11-15].

Among variety of heterocyclic substituted coumarins, pyridyl substituted coumarins draw a special attention in synthetic as well as medicinal field due to their diverse pharmacological activities. A number of coumarin derivatives having pyridine substitution mainly at 3- or 4- position of the coumarin, possess various biological properties *viz*antifungal[16], anticoagulant[17], antihyperglycemic[18], CNS depressant activity[19]and also shown electrochemical and photophysical properties[20].

In literature pyrazolyl substituted pyridine derivatives are well documented and reported to possess severalmedicinal properties like antimicrobial activity[21], cytotoxic activity, DNA binding property[22], anticancer activity[23], used as a potent inhibitor of the transforming growth factor-*β* type I receptor kinase domain[24]and exhibited appreciable cyclooxygenase-2 (COX-2) potency and selectivity[25]. They also showed some photo-physical and electro chemiluminescence properties[26].

In dicoumarinyl pyridines, the pyridine nucleus is flanked between two coumarin moieties at C-2 and C-6 position and the structure seems as if two coumarins have 3-(2-pyridyl) substitution. We had earlier synthesized various dicoumarinyl pyridines in our laboratory by using well-known *Krohnke's* pyridine synthesis[27-28].Thus considering the importance of pyridylcoumarins, pyrazolyl substituted pyridines and

in continuation of our synthesis work on dicoumarinyl pyridines, we have synthesized some new pyrazolylpyridyl substituted dicoumarins in present work.

EXPERIMENTAL

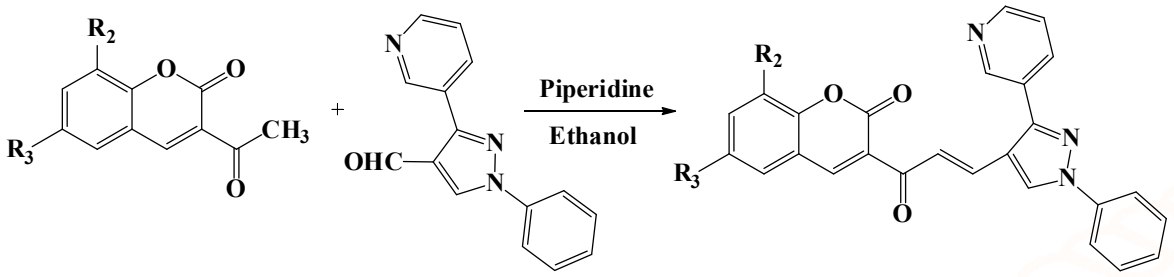
All the melting points are uncorrected. All reactions were performed with commercially available reagents and they were used without further purification. Organic solvents were purifiedby standard methods and stored over molecular sieves. All the IR spectra (KBr disc) were recorded on Shimadzu FTIR 8400-S 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 Analyser Series-II. 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. In the present work, the synthesis of various 3',3''- (4-(1'''-phenyl-3'''-(pyridin-3''''-yl)-1*H*-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarins **(6a-i)** and 3',3''-(4-(1'''-phenyl-3'''-(pyridin-4''''-yl)-1*H*-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarins**(7a-i)** has been carried out by reacting appropriate 3-coumarinoyl methyl pyridinium bromide salt **(3a-c)**with various coumarinchalcones3-(3-(1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazol-4yl)acryloyl)coumarins**(4a-c)** and 3-(3-(1-phenyl-3-(pyridin-4-yl)-1*H*-pyrazol-4-yl)acryloyl)coumarins**(5a-c)**respectively in the presence of ammonium acetate in refluxing acetic acid.Compounds **(3a-c)**were prepared according to literature procedure[29,30,31].

1) General procedure for the preparation of 3-(3-(1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl)acryloyl)coumarins (4a-c) and 3-(3-(1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)acryloyl)coumarins (5a-c):

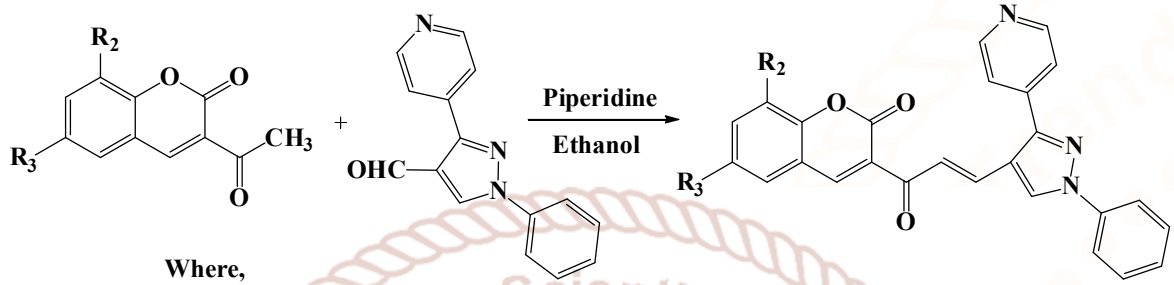
In a 100 mL round bottom flask, an appropriate 3-acetyl coumarin(0.01 mol) and appropriate pyrazole aldehyde (0.015 mol) were taken in 50 mL of ethanol. Catalytic amount of piperidine (1.0 mL) was added and the reaction mixture was stirred for 10 minutes at room temperature. The mixture was then refluxed on water bath for 4 hours. 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. Chalcones**4a**, **4b**, **5a** and **5b** were prepared according to literature procedure[32].

2) General procedure for the synthesis of Synthesis of 3',3''-(4-(1'''-phenyl-3'''-(pyridin-3''''-yl)-1H-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarins (6a-i) and 3',3''-(4-(1'''-phenyl-3'''-(pyridin-4''''-yl)-1H-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarins (7a-i).

In a 100 mL round bottom flask equipped with a condenser, guard tube and magnetic needle, an appropriate 3-coumarinoyl methyl pyridinium bromide salt **(3a-c)**(0.003 mol) was taken in glacial acetic acid (15 mL). To this, ammonium acetate (0.03 mol) was added with stirring at room temperature. Then a solution of appropriate 3-(3-(1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazol-4-yl)acryloyl)coumarin **(4a-c)**or 3-(3-(1-phenyl-3-(pyridin-4-yl)-1*H*-pyrazol-4-yl)acryloyl)coumarin **(5a-c)**(0.003 mol) in acetic acid (15 mL) was added with stirring at room temperature and reaction mixture was further stirred for 45 minutes and then refluxed for 8 hours at 140°C. It was then allowed to come to room temperature. The reaction mixture was poured into ice-cold water (75 mL). A crude solid obtained was extracted with chloroform (3 x 30 mL). The combined chloroform extract was washed with 10% sodium bicarbonate solution (3 x 20 mL), with water (3 x 20 mL) and dried over anhydrous sodium sulphate. The removal of chloroform under vacuum gave a solid product. This was purified by column chromatography using silica gel and chloroform-pet.ether (60-80) (8:2) as an eluent to give product **(6a-i)** and **(7a-i)**. The compounds were recrystallized from chloroform-hexane.

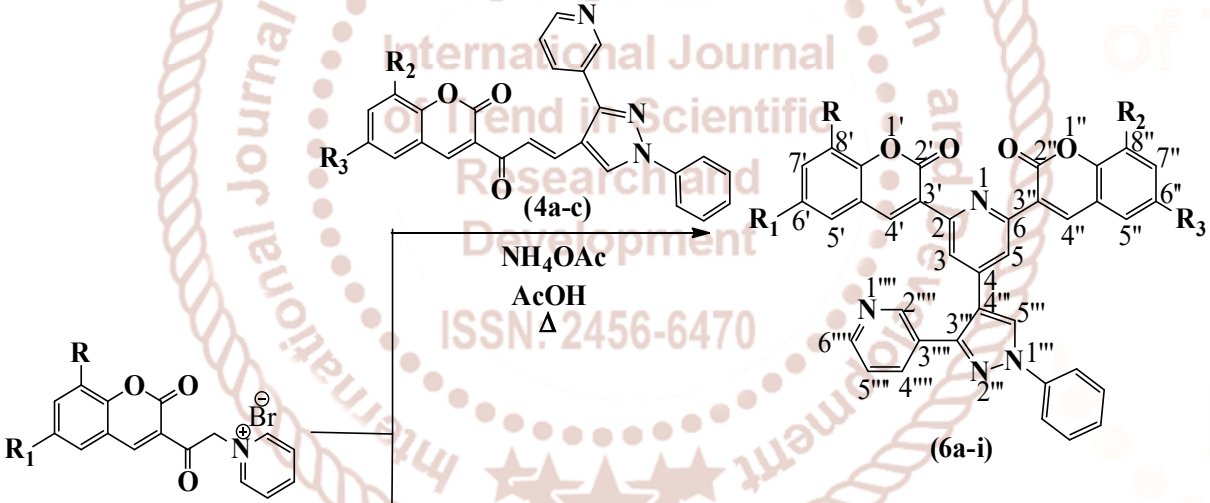


(4a-c)



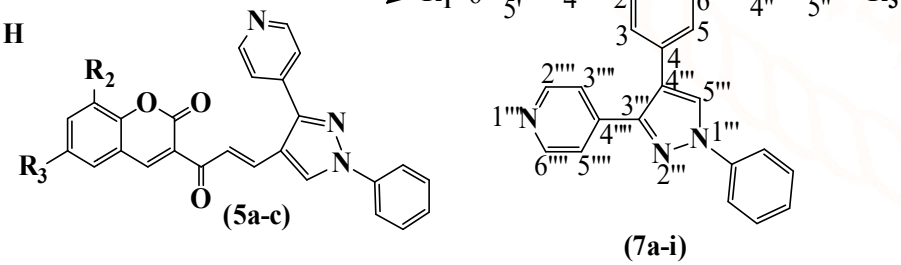
(5a-c)

Where,
4a=5a: R_2=R_3=H
4b=5b: R_2=OCH_3, R_3=H
4c=5c: R_2=H, R_3=Br



(3a-c)

Where,
3a : R= R_1= H
3b : R= OCH_3, R_1= H
3c : R= H, R_1= Br



Scheme 1. Synthetic pathway for the synthesis of target compounds **(6a-i)** and **(7a-i)**

Compounds	R	R_1	R_2	R_3
6a : 7a	H	H	H	H
6b : 7b	H	H	OCH_3	H
6c : 7c	H	H	H	Br
6d : 7d	OCH_3	H	H	H
6e : 7e	OCH_3	H	OCH_3	H
6f : 7f	OCH_3	H	H	Br
6g : 7g	H	Br	H	H
6h : 7h	H	Br	OCH_3	H
6i : 7i	H	Br	H	Br

Spectral Interpretation

6-Bromo-3-(3-(1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-4-yl)acryloyl)coumarins (4c): IR (KBr, ν_{max}, cm^{-1}):1728(C=O stretching of δ-lactone of coumarin), 1684(α,β unsaturated carbonyl group), 1605 and 1543(aromatic C=C and C=N stretchings), 756(C-H bending vibration of mono substituted benzene ring), 3047(aromatic C-H stretching).^{1}H-NMR (400 MHz, $CDCl_3$, δ):7.41-8.99 (16H, multiplet, thirteen aromatic protons + C_4proton of coumarin+ two olefinicprotons).

6-Bromo-3-(3-(1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)acryloyl)coumarins (5c): IR (KBr, ν_{max}, cm^{-1}):1728 (C=O stretching of δ-lactone of coumarin), 1674 (α,β unsaturated carbonyl group), 1605 and 1543(aromatic C=C and C=N stretchings), 764 (C-H bending vibration of mono substituted benzene ring), 3047 (aromatic C-H stretching). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.29-8.99 (16H, multiplet, thirteen aromatic protons + C_4 proton of coumarin+ two olefinic protons).

3',3''-(4-(1'''-phenyl-3'''-(pyridin-3''''-yl)-1H-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarin (6a): Yield: 71%., m.p.250-252˚C., IR (KBr, ν_{max}, cm^{-1}): 1726 (C=O stretching of δ-lactone of coumarin), 1608 and 1457 (aromatic C=C and C=N stretchings), 691 and 759 (C-H bending vibrations of mono substituted benzene ring), 3062 (aromatic C-H stretching). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 7.35-8.08 (15H, multiplet, Ar-H except C_5'''-H, C_3-H,C_5-H, C_6''''-H, C_4'-H, C_4''-H and C_2''''-H), 8.36 (2H, singlet, C_5'''-H), 8.38(2H, singlet,C_3-HandC$_5$-H), 8.66 (1H, doublet of doublet, *J = 4.8 Hz* and *1.6 Hz*, C_6''''-H), 8.82 (2H, singlet, C_4'-H and C_4''-H), 8.85 (1H, doublet, *J = 0.8 Hz*, C_2''''-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ): 116.47(C), 119.39(C), 119.47(CH), 120.78(CH), 122.03(CH), 122.68(C), 123.59(C), 124.63(C), 125.33(CH), 127.25(C), 127.89(C), 128.58(CH), 128.89(C), 129.62(C), 132.34(C), 135.99(C), 139.53(CH), 141.64(CH), 142.72(C), 149.41(C), 149.49(C), 151.54(CH), 154.05(CH), 160.08(CO of coumarin). Anal.Calcd. for $C_{37}H_{22}N_4O_4$: C, 75.76; H, 3.78; N, 9.55 %. Found: C, 75.82; H, 3.85; N, 9.62 %.

8''-Methoxy-3',3''-(4-(1'''-phenyl-3'''-(pyridin-3''''-yl)-1H-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarin (6b): Yield: 73%., m.p.230-232˚C., IR (KBr, ν_{max}, cm^{-1}): 1728 (C=O stretching of δ-lactone of coumarin), 1612 and 1481 (aromatic C=C and C=N stretchings), 687 and 756 (C-H bending vibrations of mono substituted benzene ring), 2978 (aliphatic C-H stretching), 3062 (aromatic C-H stretching). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 4.02 (3H, singlet, OCH_3), 7.14-8.07 (14H, multiplet, Ar-H except C_5'''-H, C_3-H,C_5-H, C_6''''-H, C_4'-H, C_4''-H and C_2''''-H), 8.36 (2H, multiplet, C_5'''-H and C_3-H), 8.40 (1H, doublet, *J = 1.2 Hz*, C_5-H), 8.64 (1H, doublet of doublet, *J = 4.8 Hz* and *1.6 Hz*, C_6''''-H), 8.80 (1H, singlet, C_4'-H), 8.82 (1H, singlet,C_4''-H), 8.84 (1H, doublet, *J = 2.0 Hz*, C_2''''-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ): 56.23(OCH_3), 114.00(CH), 116.38(CH), 118.48(CH), 119.22(C), 119.74(CH), 120.04(CH), 120.28(C), 120.68(C) 122.56(CH), 123.59(CH), 124.43(CH), 124.59(CH), 124.90(C), 125.33(C), 127.20(CH), 127.89(CH), 128.25(CH), 128.93(C), 129.59(CH), 129.78(CH), 132.28(CH), 136.01(CH), 136.26(CH), 139.50(C), 141.56(C), 142.71(CH), 142.86(CH), 146.94(C), 148.01(C), 149.32(C) 149.60(C), 150.17(C), 151.38(C), 159.75(CO of coumarin), 160.05(CO of coumarin). Anal.Calcd. for $C_{38}H_{24}N_4O_5$: C, 74.02; H, 3.92; N, 9.09 %. Found: C, 74.11; H, 4.09; N, 9.16 %.

6''-Bromo-3',3''-(4-(1'''-phenyl-3'''-(pyridin-3''''-yl)-1H-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarin (6c): Yield: 68%., m.p.145-147˚C., IR (KBr, ν_{max}, cm^{-1}):1720 (C=O stretching of δ-lactone of coumarin), 1620 and 1496 (aromatic C=C and C=N stretchings), 694 and 771 (C-H bending vibrations of mono substituted benzene ring), 3055 (aromatic C-H stretching).^{1}H-NMR (400 MHz, $CDCl_3$, δ): 7.38-8.08 (14H, multiplet, Ar-H except C_5'''-H, C_3-H,C_5-H, C_6''''-H, C_4'-H, C_4''-H and C_2''''-H), 8.36 (1H, singlet, C_5'''-H), 8.37 (1H, doublet, *J = 1.6 Hz*, C_3-H), 8.41 (1H, doublet, *J = 1.6 Hz*, C_5-H), 8.65 (1H, doublet of

doublet, *J = 4.8 Hz* and *1.2 Hz*, C_6''''-H), 8.76 (1H, singlet, C_4'-H), 8.81 (1H, singlet, C_4''-H), 8.83 (1H, doublet, *J = 1.6 Hz*, C_2''''-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ): 110.09(C), 112.93(C), 115.65(C), 115.76(C), 117.14(CH), 118.06(C), 118.59(C), 118.70(CH) 118.82(CH), 119.31(C), 120.08(CH), 122.25(CH), 122.67(CH), 126.92(CH), 128.08(CH), 129.41(CH), 130.01(CH), 130.65(CH), 132.44(CH), 137.11(CH), 138.09(C), 139.28(CH), 140.65(CH), 141.08(CH), 143.62(C), 145.77(C), 146.26(CH), 146.65(CH), 146.82(C), 148.68(CH), 149.63(C), 152.91(C), 154.13(C), 160.72(CO of coumarin), 161.15(CO of coumarin). Anal.Calcd. for $C_{37}H_{21}BrN_4O_4$: C, 66.78; H, 3.18; N, 8.42 %. Found: C, 66.82; H, 3.22; N, 8.49 %.

8'-Methoxy-3',3''-(4-(1'''-phenyl-3'''-(pyridin-3''''-yl)-1H-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarin (6d): Yield: 73%., m.p.230-232˚C., IR (KBr, ν_{max}, cm^{-1}): 1728 (C=O stretching of δ-lactone of coumarin), 1612 and 1481 (aromatic C=C and C=N stretchings), 687 and 756 (C-H bending vibrations of mono substituted benzene ring), 2978 (aliphatic C-H stretching), 3062 (aromatic C-H stretching).^{1}H-NMR (400 MHz, $CDCl_3$, δ): 4.12 (3H, singlet, OCH_3), 7.15-8.06 (14H, multiplet, Ar-H except C_5'''-H, C_3-H,C_5-H, C_6''''-H, C_4'-H, C_4''-H and C_2''''-H), 8.36 (2H, multiplet, C_5'''-H and C_3-H), 8.40 (1H, doublet, *J = 1.2 Hz*, C_5-H), 8.64 (1H, doublet of doublet, *J = 4.0 Hz* and *1.6 Hz*, C_6''''-H), 8.81 (1H, singlet, C_4'-H), 8.82 (1H, singlet,C_4''-H), 8.85 (1H, doublet, *J = 2.0 Hz*, C_2''''-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ): 56.28(OCH_3), 114.05(CH), 116.43(CH), 118.58(CH), 119.20(C), 119.78(CH), 120.04(C), 120.24(CH), 120.66(C) 122.58(CH), 123.59(CH), 124.40(CH), 124.50(CH), 124.93(C), 125.33(C), 127.29(CH), 127.89(CH), 128.23(CH), 128.95(C), 129.59(CH), 129.76(CH), 132.28(CH), 136.00(CH), 136.26(CH), 139.51(C), 141.56(C), 142.71(CH), 142.86(CH), 146.98(C), 148.02(C), 149.30(C), 149.65(C), 150.17(C), 151.34(C), 159.70(CO of coumarin), 160.05(CO of coumarin). Anal.Calcd. for $C_{38}H_{24}N_4O_5$: C, 74.02; H, 3.92; N, 9.09 %. Found: C, 74.11; H, 4.09; N, 9.16 %.

8',8''-Dimethoxy-3',3''-(4-(1'''-phenyl-3'''-(pyridin-3''''-yl)-1H-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarin (6e):Yield: 78%., m.p.290-292˚C., IR (KBr, ν_{max}, cm^{-1}): 1712 (C=O stretching of δ-lactone of coumarin), 1620 and 1496 (aromatic C=C and C=N stretchings), 663 and 748 (C-H bending vibrations of mono substituted benzene ring), 2970 (aliphatic C-H stretching), 3055 (aromatic C-H stretching). ^{1}H-NMR (400 MHz, $CDCl_3$, δ):4.00 (6H, singlet, 2 x OCH_3), 7.11-8.03 (13H, multiplet, Ar-H except C_5'''-H, C_3-H,C_5-H, C_6''''-H, C_4'-H, C_4''-H and C_2''''-H), 8.34 (1H, singlet, C_5'''-H), 8.37 (2H, singlet, C_3-H andC_5-H), 8.61 (1H, doublet of doublet, *J = 4.8 Hz* and *1.6 Hz*, C_6''''-H), 8.79 (2H, singlet, C_4'-H and C_4''-H), 8.81 (1H, doublet, *J = 2.0 Hz*, and C_2''''-H).^{13}C-APT (100 MHz, $CDCl_3$, δ): 56.21(OCH_3), 114.07(CH), 119.50(CH), 120.14(C), 120.39(CH), 120.77(C) 122.72(CH), 123.67(CH), 124.56(CH), 125.50(C), 127.27(CH), 128.03(CH), 128.66(C), 129.67(CH), 135.99(CH), 139.65(C), 141.67(C), 142.94(CH), 143.63(C), 146.98(C), 149.51(CH), 151.53(C), 159.61(CO of coumarin). Anal.Calcd. for $C_{39}H_{26}N_4O_6$: C, 72.44; H, 4.05; N, 8.66 %. Found: C, 72.59; H, 4.15; N, 8.71 %.

6''-Bromo-8'-methoxy-3',3''-(4-(1'''-phenyl-3'''-(pyridin-3''''-yl)-1H-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarin (6f): Yield: 70%., m.p.200-202˚C., IR (KBr, ν_{max}, cm^{-1}): 1728 (C=O stretching of δ-lactone of coumarin), 1620 and 1481 (aromatic C=C and C=N stretchings), 679 and 779 (C-H bending vibrations of mono substituted benzene ring), 2985 (aliphatic C-H stretching), 3047 (aromatic C-H stretching).^{1}H-NMR (400 MHz, $CDCl_3$, δ): 4.00 (3H, singlet, OCH_3), 7.13-8.08 (13H, multiplet, Ar-H except C_5'''-H, C_3-H,C_5-H, C_6''''-H, C_4'-H, C_4''-H and C_2''''-H), 8.34 (2H, multiplet, C_5'''-H and C_3-H), 8.41 (1H, poorly resolved doublet, C_5-H), 8.64 (1H, poorly resolved doublet of doublet, C_6''''-H), 8.76 (1H, singlet, C_4'-H), 8.78 (1H, singlet, C_4''-H), 8.82 (1H, poorly resolved doublet, C_2''''-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ): 56.22(OCH_3), 114.11(CH), 115.79(C), 117.17(CH), 118.06(CH), 119.34(C), 119.99(CH), 120.28(C), 120.53(CH), 120.92(CH), 122.50(CH), 122.80(CH), 123.64(CH), 124.43(CH), 125.17(C), 126.06(C), 127.29(CH), 127.88(CH), 129.66(CH), 131.04(CH), 131.83(C), 134.99(CH), 136.13(CH), 139.48(C), 141.31(CH), 141.70(C), 142.94(CH), 144.76(C), 145.90(C), 146.64(C), 146.93(C), 149.35(C), 150.64(C), 151.52(C), 160.06(CO of coumarin), 161.64(CO of coumarin).Anal.Calcd. for $C_{38}H_{23}BrN_4O_5$: C, 65.62; H, 3.33; N, 8.06 %. Found: C, 65.70; H, 3.37; N, 8.13 %.

6'-Bromo-3',3''-(4-(1'''-phenyl-3'''-(pyridin-3''''-yl)-1H-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarin (6g): Yield: 68%., m.p.145-147˚C., IR (KBr, ν_{max}, cm^{-1}): 1720 (C=O stretching of δ-lactone of coumarin),

1620 and 1496 (aromatic C=C and C=N stretchings), 694 and 771 (C-H bending vibrations of mono substituted benzene ring), 3055 (aromatic C-H stretching).^{1}H-NMR (400 MHz, $CDCl_3$, δ): 7.38-8.08 (14H, multiplet, Ar-H except C_5'''-H, C_3-H,C_5-H, C_6''''-H, C_4'-H, C_4''-H and C_2''''-H), 8.36 (1H, singlet, C_5'''-H), 8.37 (1H, doublet, *J = 1.6 Hz*, C_3-H), 8.41 (1H, doublet, *J = 1.6 Hz*, C_5-H), 8.65 (1H, doublet of doublet, *J = 4.4 Hz* and *1.6 Hz*, C_6''''-H), 8.76 (1H, singlet, C_4'-H), 8.80 (1H, singlet, C_4''-H), 8.83 (1H, doublet, *J = 1.2 Hz*, C_2''''-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ): 109.99(C), 112.95(C), 115.63(C), 115.83(CH), 117.13(CH), 118.08(CH), 118.59(C), 118.71(C) 118.82(CH), 119.31(C), 120.08(CH), 122.25(C), 122.63(CH), 126.92(CH), 128.01(CH), 129.44(CH), 130.15(CH), 130.56(CH), 132.41(CH), 137.06(CH), 138.18(C), 139.29(CH), 140.65(CH), 141.08(CH), 143.62(C), 145.72(C), 146.26(CH), 146.65(CH), 146.76(C), 148.68(CH), 149.67(C), 152.90(C), 154.14(C), 160.77(CO of coumarin), 161.11(CO of coumarin).Anal.Calcd. for $C_{37}H_{21}BrN_4O_4$: C, 66.78; H, 3.18; N, 8.42 %. Found: C, 66.82; H, 3.22; N, 8.49 %.

6'-Bromo-8''-methoxy-3',3''-(4-(1'''-phenyl-3'''-(pyridin-3''''-yl)-1H-pyrazol-4'''-yl) pyridine-2,6-diyl)dicoumarin (6h): Yield: 70%., m.p.200-202˚C., IR (KBr, ν_{max}, cm^{-1}): 1728 (C=O stretching of δ-lactone of coumarin), 1620 and 1481 (aromatic C=C and C=N stretchings), 679 and 779 (C-H bending vibrations of mono substituted benzene ring), 2985 (aliphatic C-H stretching), 3047 (aromatic C-H stretching).^{1}H-NMR (400 MHz, $CDCl_3$, δ): 4.10 (3H, singlet, OCH_3), 7.13-8.08 (13H, multiplet, Ar-H except C_5'''-H, C_3-H,C_5-H, C_6''''-H, C_4'-H, C_4''-H and C_2''''-H), 8.35 (2H, multiplet, C_5'''-H and C_3-H), 8.41 (1H, poorly resolved doublet, C_5-H), 8.63 (1H, poorly resolved doublet of doublet, C_6''''-H), 8.76 (1H, singlet, C_4'-H), 8.78 (1H, singlet, C_4''-H), 8.83 (1H, poorly resolved doublet, C_2''''-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ): 56.28(OCH_3), 114.92(CH), 115.79(C), 117.13(CH), 118.11(CH), 119.31(C), 119.90(CH), 120.22(C), 120.57(CH), 120.89(CH), 122.59(CH), 122.80(CH), 123.64(CH), 124.34(CH), 125.13(C), 126.11(C), 127.29(CH), 127.89(CH), 129.69(CH), 131.11(CH), 131.92(C), 134.90(CH), 136.17(CH), 139.43(C), 141.28(CH), 141.76(C), 142.99(CH), 144.70(C), 145.93(C), 146.69(C), 146.99(C), 149.43(C), 150.69(C), 151.59(C), 160.11(CO of coumarin), 161.66(CO of coumarin).Anal.Calcd. for $C_{38}H_{23}BrN_4O_5$: C, 65.62; H, 3.33; N, 8.06 %. Found: C, 65.70; H, 3.37; N, 8.13 %.

6',6''-Dibromo-3',3''-(4-(1'''-phenyl-3'''-(pyridin-3''''-yl)-1H-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarin (6i): Yield: 65%., m.p.134-136˚C., IR (KBr, ν_{max}, cm^{-1}): 1728 (C=O stretching of δ-lactone of coumarin), 1620 and 1481 (aromatic C=C and C=N stretchings), 678 and 779 (C-H bending vibrations of mono substituted benzene ring), 3070 (aromatic C-H stretching).^{1}H-NMR (400 MHz, $CDCl_3$, δ): 7.35-8.07 (13H, multiplet, Ar-H except C_5'''-H, C_3-H,C_5-H, C_6''''-H, C_4'-H, C_4''-H and C_2''''-H), 8.36 (1H, singlet, C_5'''-H), 8.37 (2H, singlet, C_3-H and C_5-H), 8.65 (1H, doublet of doublet, *J = 4.8 Hz* and *1.6 Hz*, C_6''''-H), 8.82 (2H, singlet, C_4'-H and C_4''-H), 8.84 (1H, poorly resolved doublet, C_2''''-H).^{13}C-APT (100 MHz, $CDCl_3$, δ): 119.30(CH), 119.53(CH), 124.60(CH), 124.90(C), 125.24(C), 125.46(CH), 125.58(CH), 126.91(CH), 127.79(CH), 129.67(CH), 130.77(C), 133.20(CH), 134.19(CH), 136.07(CH), 143.91(C), 145.35(C), 146.79(CH), 147.34(CH), 149.33(C), 151.31(C), 156.06(C), 157.28(C), 158.27(C), 160.31(CO of coumarin)Anal.Calcd. for $C_{37}H_{20}Br_2N_4O_4$: C, 59.70; H, 2.71; N, 7.53 %. Found: C, 59.76; H, 2.77; N, 7.59 %.

3',3''-(4-(1'''-phenyl-3'''-(pyridin-4''''-yl)-1H-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarin (7a): Yield: 78%., m.p.293-295˚C., IR (KBr, ν_{max}, cm^{-1}): 1736 (C=O stretching of δ-lactone of coumarin), 1627 and 1488 (aromatic C=C and C=N stretchings), 679 and 756 (C-H bending vibrations of mono substituted benzene ring), 3062 (aromatic C-H stretching). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 7.28-7.86 (15H, multiplet, Ar-H except C_5'''-H, C_3-H, C_5-H, C_2''''-H, C_6''''-H, C_4'-H and C_4''-H), 8.30 (1H, singlet, C_5'''-H), 8.37 (2H, singlet, C_3-H and C_5-H), 8.64 (2H, doublet, *J = 6.0 Hz*, C_2''''-H and C_6''''-H), 8.82 (2H, singlet, C_4'-H and C_4''-H).^{13}C-APT (100 MHz, $CDCl_3$, δ): 116.50(CH), 119.40(CH), 121.11(C), 122.92(CH), 122.96(CH), 123.68(CH), 124.68(CH), 125.20(C), 127.46(CH), 128.32(CH), 128.91(CH), 129.65(C), 132.43(CH), 139.38(C), 140.68(C), 141.46(C), 142.85(CH), 147.94(C), 149.54(CH), 151.58(C), 154.00(C), 160.13(CO of coumarin). Anal.Calcd. for $C_{37}H_{22}N_4O_4$: C, 75.76; H, 3.78; N, 9.55 %. Found: C, 75.82; H, 3.85; N, 9.62 %.

8''-Methoxy-3',3''-(4-(1'''-phenyl-3'''-(pyridin-4''''-yl)-1H-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarin (7b): Yield: 74%., m.p.282-284˚C., IR (KBr, ν_{max}, cm^{-1}): 1728 (C=O stretching of δ-lactone of coumarin), 1612 and 1473 (aromatic C=C and C=N stretchings), 686 and 756 (C-H bending vibrations of mono substituted benzene ring), 2977 (aliphatic C-H stretching), 3055 (aromatic C-H stretching). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 4.03 (3H, singlet, OCH_3), 7.13-7.86 (14H, multiplet, Ar-H except C_5'''-H, C_3-H, C_5-H, C_2''''-H, C_6''''-H, C_4'-H and C_4''-H), 8.30 (1H, singlet, C_5'''-H), 8.35 (1H, doublet, *J = 1.2 Hz*, C_3-H), 8.39 (1H, doublet, *J = 2.0 Hz*, C_5-H), 8.63 (2H, doublet, *J = 6.0 Hz*, C_2''''-H and C_6''''-H), 8.81 (1H, singlet, C_4'-H), 8.82 (1H, singlet, C_4''-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ): 56.24(OCH_3), 114.06(CH), 116.43(CH), 119.34(C), 119.38(CH), 119.44(C), 120.00(CH), 120.27(C), 121.04(CH), 122.84(CH), 122.96(CH), 124.50(CH), 124.65(CH), 125.09(C), 125.25(C), 127.42(CH), 128.32(CH), 128.94(CH), 129.62(CH), 132.38(CH), 139.37(C), 140.68(C), 141.40(C), 142.85(CH), 143.00(CH), 143.61(C), 146.94(C), 147.90(C), 149.51(CH), 151.45(C), 151.48(C), 153.95(C), 159.58(CO of coumarin), 160.11(CO of coumarin). Anal.Calcd. for $C_{38}H_{24}N_4O_5$: C, 74.02; H, 3.92; N, 9.09 %. Found: C, 74.11; H, 4.09; N, 9.16 %.

6''-Bromo-3',3''-(4-(1'''-phenyl-3'''-(pyridin-4''''-yl)-1H-pyrazol-4'''-yl)pyridine-2,6-diyl) dicoumarin (7c): Yield: 69%., m.p.170-172˚C., IR (KBr, ν_{max}, cm^{-1}): 1712 (C=O stretching of δ-lactone of coumarin), 1627 and 1488 (aromatic C=C and C=N stretchings), 679 and 779 (C-H bending vibrations of mono substituted benzene ring), 3061 (aromatic C-H stretching).^{1}H-NMR (400 MHz, $CDCl_3$, δ): 7.36-7.88 (14H, multiplet, Ar-H except C_5'''-H, C_3-H, C_5-H, C_2''''-H, C_6''''-H, C_4'-H and C_4''-H), 8.31 (1H, singlet, C_5'''-H), 8.37 (1H, doublet, *J = 1.6 Hz*, C_3-H), 8.41 (1H, doublet, *J = 1.6 Hz*, C_5-H), 8.64 (2H, doublet, *J = 6.4 Hz*, C_2''''-H and C_6''''-H), 8.76 (1H, singlet, C_4'-H), 8.81 (1H, singlet, C_4''-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ):112.43(C), 114.44(CH), 115.96(CH), 117.22(CH), 118.03(C), 119.54(CH), 120.48(CH), 121.96(C), 122.99(CH), 123.73(CH), 126.02(CH), 127.16(CH), 127.72(C), 127.86(CH), 127.99(CH), 129.48(CH), 129.72(CH), 130.58(CH), 133.26(CH), 135.23(C), 139.07(C), 139.56(C), 140.08(C), 143.85(C), 146.10(C), 148.61(C), 150.15(CH), 150.66(C), 152.64(C), 154.71(C), 161.79(CO of coumarin), 162.03(CO of coumarin).Anal.Calcd. for $C_{37}H_{21}BrN_4O_4$: C, 66.78; H, 3.18; N, 8.42 %. Found: C, 66.82; H, 3.24; N, 8.50 %.

8'-Methoxy-3',3''-(4-(1'''-phenyl-3'''-(pyridin-4''''-yl)-1H-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarin (7d): Yield: 74%., m.p.282˚C., IR (KBr, ν_{max}, cm^{-1}): 1728 (C=O stretching of δ-lactone of coumarin), 1612 and 1473 (aromatic C=C and C=N stretchings), 686 and 756 (C-H bending vibrations of mono substituted benzene ring), 2977 (aliphatic C-H stretching), 3055 (aromatic C-H stretching).^{1}H-NMR (400 MHz, $CDCl_3$, δ): 4.02 (3H, singlet, OCH_3), 7.15-7.87 (14H, multiplet, Ar-H except C_5'''-H, C_3-H, C_5-H, C_2''''-H, C_6''''-H, C_4'-H and C_4''-H), 8.31 (1H, singlet, C_5'''-H), 8.37 (1H, doublet, *J = 1.2 Hz*, C_3-H), 8.41 (1H, doublet, *J = 1.2 Hz*, C_5-H), 8.64 (2H, doublet, *J = 5.2 Hz*, C_2''''-H and C_6''''-H), 8.81 (1H, singlet, C_4'-H), 8.82 (1H, singlet, C_4''-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ): 56.28(OCH_3), 114.09(CH), 116.50(CH), 119.32(C), 119.34(CH), 119.43(C), 120.04(CH), 120.25(C), 121.00(CH), 122.86(CH), 122.94(CH), 124.48(CH), 124.62(CH), 125.06(C), 125.27(C), 127.43(CH), 128.37(CH), 128.94(CH), 129.65(CH), 132.42(CH), 139.40(C), 140.62(C), 141.37(C), 142.90(CH), 143.00(CH), 143.68(C), 146.96(C), 147.85(C), 149.51(CH), 151.45(C), 151.50(C), 153.96(C), 159.61(CO of coumarin), 160.14(CO of coumarin).Anal.Calcd. for $C_{38}H_{24}N_4O_5$: C, 74.02; H, 3.92; N, 9.09 %. Found: C, 74.11; H, 4.09; N, 9.16 %.

8',8''-Dimethoxy-3',3''-(4-(1'''-phenyl-3'''-(pyridin-4''''-yl)-1H-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarin (7e): Yield: 76%., m.p.296-298˚C., IR (KBr, ν_{max}, cm^{-1}): 1720 (C=O stretching of δ-lactone of coumarin), 1627 and 1496 (aromatic C=C and C=N stretchings), 687 and 779 (C-H bending vibrations of mono substituted benzene ring), 2985 (aliphatic C-H stretching), 3070 (aromatic C-H stretching). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 3.99 (6H, singlet, 2 x OCH_3), 7.13-7.84 (13H, multiplet, Ar-H except C_5'''-H, C_3-H, C_5-H, C_2''''-H, C_6''''-H, C_4'-H and C_4''-H), 8.30 (1H, singlet, C_5'''-H), 8.37 (2H, singlet, C_3-H and C_5-H), 8.60 (2H, poorly resolved doublet, C_2''''-H and C_6''''-H), 8.79 (2H, singlet, C_4'-H and C_4''-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ): 56.61(OCH_3), 110.26(C), 113.13(C), 114.24(C), 114.52(CH), 116.00(C), 118.77(CH), 120.07(CH), 121.73(CH), 122.84(CH), 123.92(CH), 125.34(CH), 126.91(CH), 129.41(CH), 130.06(CH), 138.15(C), 140.11(CH), 143.65(C), 144.30(C), 146.98(C), 148.83(C), 149.94(C), 160.86(CO of coumarin).

Anal.Calcd. for $C_{39}H_{26}N_4O_6$: C, 72.44; H, 4.05; N, 8.66 %. Found: C, 72.59; H, 4.15; N, 8.71 %.

6''-Bromo-8'-methoxy-3',3''-(4-(1'''-phenyl-3'''-(pyridin-4''''-yl)-1H-pyrazol-4'''-yl) pyridine-2,6-diyl)dicoumarin (7f): Yield: 72%., m.p.148-150˚C., IR (KBr, ν_{max}, cm^{-1}): 1735 (C=O stretching of δ-lactone of coumarin), 1628 and 1458 (aromatic C=C and C=N stretchings), 678 and 786 (C-H bending vibrations of mono substituted benzene ring), 2970 (aliphatic C-H stretching), 3063 (aromatic C-H stretching). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 3.99 (3H, singlet, OCH_3), 7.11-7.86 (13H, multiplet, Ar-H except C_5'''-H, C_3-H, C_5-H, C_2''''-H, C_6''''-H, C_4'-H and C_4''-H), 8.30 (1H, singlet, C_5'''-H), 8.34 (1H, poorly resolved doublet, C_3-H), 8.38 (1H, poorly resolved doublet, C_5-H), 8.62 (2H, doublet, *J = 6.0 Hz*, C_2''''-H and C_6''''-H), 8.80 (1H, singlet, C_4'-H), 8.81 (1H, singlet, C_4''-H).^{13}C-APT (100 MHz, $CDCl_3$, δ):56.28(OCH_3), 114.11(CH), 116.46(CH), 119.41(C), 119.46(CH), 120.03(C), 120.27(CH), 121.07(C), 122.92(CH), 124.49(CH), 124.64(CH), 125.19(C), 125.35(C), 127.41(CH), 128.31(CH), 128.92(CH), 129.62(CH), 132.38(CH), 139.41(C), 140.55(C), 141.47(C), 142.81(CH), 142.97(CH), 143.68(C), 147.00(C), 148.00(C), 149.68(CH), 151.50(C), 151.53(C), 153.99(C), 159.56(CO of coumarin), 160.09(CO of coumarin).Anal.Calcd. for $C_{38}H_{23}BrN_4O_5$: C, 65.62; H, 3.33; N, 8.06 %. Found: C, 65.67; H, 3.40; N, 8.13 %.

6'-Bromo-3',3''-(4-(1'''-phenyl-3'''-(pyridin-4''''-yl)-1H-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarin (7g): Yield: 69%., m.p.170-172˚C., IR (KBr, ν_{max}, cm^{-1}):1712 (C=O stretching of δ-lactone of coumarin), 1620 and 1488 (aromatic C=C and C=N stretchings), 679 and 779 (C-H bending vibrations of mono substituted benzene ring), 3061 (aromatic C-H stretching). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 7.36-7.88 (14H, multiplet, Ar-H except C_5'''-H, C_3-H, C_5-H, C_2''''-H, C_6''''-H, C_4'-H and C_4''-H), 8.32 (1H, singlet, C_5'''-H), 8.36 (1H, doublet, *J = 2.0 Hz*, C_3-H), 8.41 (1H, doublet, *J = 2.0 Hz*, C_5-H), 8.64 (2H, doublet, *J = 6.4 Hz*, C_2''''-H and C_6''''-H), 8.76 (1H, singlet, C_4'-H), 8.81 (1H, singlet, C_4''-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ):112.48(C), 114.48(CH), 115.99(CH), 117.22(CH), 118.07(C), 119.58(CH), 120.43(CH), 121.99(C), 122.96(CH), 123.79(CH), 126.08(CH), 127.16(CH), 127.71(C), 127.86(CH), 127.96(CH), 129.43(CH), 129.71(CH), 130.54(CH), 133.26(CH), 135.23(C), 139.03(C), 139.54(C), 140.02(C), 143.86(C), 146.10(C), 148.64(C), 150.15(CH), 150.66(C), 152.61(C), 154.79(C), 161.73(CO of coumarin),162.13(CO of coumarin).Anal.Calcd. for $C_{37}H_{21}BrN_4O_4$: C, 66.78; H, 3.18; N, 8.42 %. Found: C, 66.82; H, 3.24; N, 8.50 %.

6'-Bromo-8''-methoxy-3',3''-(4-(1'''-phenyl-3'''-(pyridin-4''''-yl)-1H-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarin (7h): Yield: 72%., m.p.148-150˚C., IR (KBr, ν_{max}, cm^{-1}): 1735 (C=O stretching of δ-lactone of coumarin), 1628 and 1458 (aromatic C=C and C=N stretchings), 678 and 786 (C-H bending vibrations of mono substituted benzene ring), 2970 (aliphatic C-H stretching), 3063 (aromatic C-H stretching). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 4.00 (3H, singlet, OCH_3), 7.12-7.86 (13H, multiplet, Ar-H except C_5'''-H, C_3-H, C_5-H, C_2''''-H, C_6''''-H, C_4'-H and C_4''-H), 8.30 (1H, singlet, C_5'''-H), 8.34 (1H, poorly resolved doublet, C_3-H), 8.38 (1H, poorly resolved doublet, C_5-H), 8.62 (2H, doublet, *J = 5.6 Hz*, C_2''''-H and C_6''''-H), 8.80 (1H, singlet, C_4'-H), 8.82 (1H, singlet, C_4''-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ):56.32(OCH_3), 114.13(CH), 116.41(CH), 119.41(C), 119.46(CH), 120.03(C), 120.28(CH), 121.03(C), 122.99(CH), 124.50(CH), 124.68(CH), 125.19(C), 125.35(C), 127.41(CH), 128.33(CH), 128.97(CH), 129.62(CH), 132.33(CH), 139.47(C), 140.56(C), 141.41(C), 142.80(CH), 142.92(CH), 143.68(C), 147.04(C), 148.02(C), 149.64(CH), 151.50(C), 151.53(C), 153.92(C), 159.55(CO of coumarin), 160.08(CO of coumarin). Anal.Calcd. for $C_{38}H_{23}BrN_4O_5$: C, 65.62; H, 3.33; N, 8.06 %. Found: C, 65.67; H, 3.40; N, 8.13 %.

6',6''-Dibromo-3',3''-(4-(1'''-phenyl-3'''-(pyridin-4''''-yl)-1H-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarin (7i): Yield: 66%., m.p.142˚C., IR (KBr, ν_{max}, cm^{-1}): 1728 (C=O stretching of δ-lactone of coumarin), 1627 and 1488 (aromatic C=C and C=N stretchings), 687 and 756 (C-H bending vibrations of mono substituted benzene ring), 3070 (aromatic C-H stretching).^{1}H-NMR (400 MHz, $CDCl_3$, δ): 7.34-7.84 (13H, multiplet, Ar-H except C_5'''-H, C_3-H, C_5-H, C_2''''-H, C_6''''-H, C_4'-H and C_4''-H), 8.30 (1H, singlet, C_5'''-H), 8.36 (2H, singlet, C_3-H and C_5-H), 8.62 (2H, doublet, *J = 4.8 Hz*, C_2''''-H and C_6''''-H), 8.81 (2H, singlet, C_4'-H and C_4''-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ):111.79(C), 117.88(C), 119.25(CH), 119.55(CH), 121.43(C), 122.28(CH), 123.05(CH), 123.76(C), 125.60(CH), 127.23(CH), 127.97(CH), 129.74(CH), 130.07(C), 130.77(CH), 133.03(CH), 133.69(CH), 139.05(C), 140.15(C), 150.05(C), 150.72(C), 154.04(C), 161.06(CO of

coumarin).Anal.Calcd. for $C_{37}H_{20}Br_2N_4O_4$: C, 59.70; H, 2.71; N, 7.53 %. Found: C, 59.76; H, 2.77; N, 7.59 %.

RESULT AND DISCUSSION

Chemistry

In the present work the synthesis of various3',3''- (4-(1'''-phenyl-3'''-(pyridin-3''''-yl)-1*H*-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarins**(6a-i)** and 3',3''-(4-(1'''-phenyl-3'''-(pyridin-4''''-yl)-1*H*-pyrazol-4'''-yl)pyridine-2,6-diyl)dicoumarins**(7a-i)** has been carried out by the reaction ofappropriate 3-coumarinoyl methyl pyridinium bromide salt **(3a-c)**withcoumarinchalcones 3-(3-(1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazol-4-yl)acryloyl)coumarins**(4a-c)** and 3-(3-(1-phenyl-3-(pyridin-4-yl)-1*H*-pyrazol-4-yl)acryloyl)coumarins**(5a-c)**respectively in the presence of ammonium acetate in refluxing acetic acid proceeded smoothly and gave the compound **6a** as a yellow solidproduct in 71% yield.The structures of all synthesized compounds **(6a-i)** and **(7a-i)**were established by IR, ^{1}H-NMR, ^{13}C-APT and selected mass spectral data are shown in experimental section.

BIOLOGICAL EVALUATION

Antimicrobial activity

The newly synthesized target compounds **(6a-i)** and **(7a-i)**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 activityagainst *Candida albicans*(MTCC 227) and *Aspergillusniger*(MTCC 282) as fungal strains.Broth dilution method was used for the determination of the antibacterial and antifungal activity as recommended by NCCLS[33]. Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin and Gentamycinwereused asstandard antibacterial drugs, whereas Griseofulvin and Nystatin were used as standard antifungaldrugs. All MTCC cultures were collected from Institute of Microbial Technology, Chandigarh andtested against above mentioned known drugs. Mueller-Hinton broth was used as the nutrientmedium for the test bacteria and Sabouraud Dextrose broth was used for the test fungi. Inoculumsize for the test strains was adjusted to 108 CFU (Colony Forming Unit per millilitre) per millilitre by comparing the turbidity. Each synthesized compound was diluted with DMSO so as to have thestock 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 **(6a-i)** and **(7a-i)**were screened fortheir antibacterial and antifungal activity at the concentration of 1000, 500 and 250 *µg*/mL for theprimary screening. The synthesized compound showing activity against microbes in the primaryscreening were further screened in a second set of dilution at concentrations of 200, 100, 62.5, 50and 25 *µg*/mL. The suspention of 10 *µ*L from each well were further incubated and growth wasnoted at 37°C after 24 hour for bacteria and 48 hour for fungi. The lowest concentration whichshowed no visible growth (turbidity) after spot subculture was considered as the minimuminhibitory concentration (MIC) for each compound.

The investigation of the data summarized in **(Table-1)** reveals that many compounds werefound to be active against Gram-positive bacteria while some of the compounds were found to beactive against Gram-negative bacterial and fungal species as compared to that of the standardantimicrobial drugs.

Antimicrobial results

The compounds **(6a-i)** and **(7a-i)**were screened for their *in vitro* antibacterial and antifungal evaluationagainst various bacterial and fungal pathogens by broth dilution method. Ampicillin,Chloramphe- nicol, Norfloxacin, Ciprofloxacin,Genta- mycin,Griseofulvin and Nystatin were used as standard drugs. The valuesof MIC are summarized in **Table-1**.

Upon evaluating the antimicrobial activity data, it has been observed that compound **7e** and **7f** (MIC=62.5µg/mL) showed excellent activity compared to Ampicillin (MIC=250µg/mL) and Norfloxacin (MIC= 100µg/mL) against gram positive bacteria *Bacillus subtilis*. Compounds **6b**, **6f**, **7a** and **7i** (MIC=100µg/mL) exhibited excellent activity against gram positive bacteria *Bacillus subtilis* compared to Ampicillin (MIC=250µg/mL) and equipotent to Norfloxacin (MIC=100µg/mL). Compounds **6a**, **6c**, **6d** and **7d** (MIC=125µg/mL) exhibited excellent activity compared to Ampicillin (MIC=250µg/mL) against gram positive bacteria *Bacillus subtilis*. Compounds **6g**, **6h**, **7c** and **7h** (MIC=200µg/mL) showed good activity against gram positive bacteria *Bacillus subtilis* compared to Ampicillin (MIC=250µg/mL). Compounds **6e**, **6i**, **7b** and **7g** (MIC=250µg/mL) were found comparable to Ampicillin (MIC=250µg/mL) against gram positive bacteria *Bacillus subtilis*. Compounds **6f**

(MIC=50µg/mL), **7a** (MIC=62.5µg/mL), compounds **6b**, **6d**, **6g**, **7b**, **7d**, **7f** (MIC=100µg/mL) and compounds **6c**, **6e**, **6h**, **7e**, **7h** and **7i** (MIC=125µg/mL) showed excellent activity against gram positive bacteria *Staphylococcus aureus* compared to Ampicillin (MIC= 250µg/mL). Compounds **6i**, **7c** and **7g** (MIC=200µg/mL) showed good activity against gram positive bacteria *Staphylococcus aureus* compared to Ampicillin (MIC=250µg/mL).Compounds **6g**, **7c** and **7i** (MIC=62.5µg/mL) have shown excellent activity against *Escherichia coli* compared to Ampicillin (MIC=100µg/mL). Compounds **6c**, **6d**, **7f** and **7g** (MIC=100µg/mL) showed equipotent activity to Ampicillin (MIC= 100µg/mL) against gram negative bacteria *Escherichia coli*. Compounds **7h** (MIC= 50µg/mL) exhibited excellent activity against *Salmonella typhi* compared to Ampicillin (MIC=100µg/mL) and equipo- tent to Chloramphenicol (MIC=50µg/mL). Compounds **6c** and **6h** (MIC=62.5µg/mL) showed excellent activity against *Salmonella typhi* compared to Ampicillin (MIC=100µg/mL). Compounds **6b**, **6f**, **6g**, **6i**, **7a**, **7b**, **7c**, **7e**, **7f** and **7i** (MIC=100µg/mL) were found equipotent to Ampicillin (MIC = 100µg/mL) against gram negative bacteria *Salmonella typhi*.Compounds **6d** (MIC=200µg/mL), compounds **6a** and **7c** (MIC=250µg/mL) were found to be more active than Griseofulvin (MIC=500µg/mL) against fungal pathogen *Candida albicans* while compounds **6b**, **7b**, **7e** and **7g** were found equipotent to Griseofulvin (MIC= 500µg/mL) against *Candida albicans*. None of the tested compounds showed better activity against *Aspergillusniger*fungi.Upon examining the antimicrobial data it is apparent that almost all the compounds **6a-i** and **7a-i** exhibited excellent activity against gram-positive bacteria *Bacillus subtilis*and *Staphylococcus aureus* as compared to Ampicillin. Examining the antimicrobial data from the table, it has been observed that the derivatization of the parent molecule altered the antimicrobial potency of the synthesized analogs. The observation indicates that varying the substitution on coumarin ring i.e. R, R_2 = OCH_3 and R_1, R_3 = Br affect the antibacterial activity to a remarkable extent. When OCH_3 group present as a substituent on coumarin ring increased the antimicrobial activity against *Bacillus subtilis*and *Staphylococcus aureus*, while introducing of Br group in coumarin nucleus the antibacterial potency enhanced markedly.

Among all the tested compounds, the compounds **6c**, **6g**, **6h**, **7c**, **7f**, **7h** and **7i** were found to be the most proficient members of the series.

Table-1 Antimicrobial activity of compounds (6a-i) and (7a-i)

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.*
6a	**125**	**250**	125	200	500	**250**
6b	**100**	**100**	200	**100**	1000	**500**
6c	**125**	**125**	**100**	**62.5**	500	1000
6d	**125**	**100**	**100**	200	>1000	**200**
6e	**250**	**125**	125	250	200	1000
6f	**100**	**50**	200	**100**	>1000	>1000
6g	**200**	**100**	**62.5**	**100**	>1000	1000
6h	**200**	**125**	250	**62.5**	500	>1000
6i	**250**	**200**	200	**100**	500	>1000
7a	**100**	**62.5**	250	**100**	1000	>1000
7b	**250**	**100**	125	**100**	>1000	**500**
7c	**200**	**200**	**62.5**	**100**	500	**250**
7d	**125**	**100**	200	250	1000	>1000
7e	**62.5**	**125**	250	**100**	1000	**500**
7f	**62.5**	**100**	**100**	**100**	1000	1000
7g	**250**	**200**	**100**	125	1000	**500**
7h	**200**	**125**	125	**50**	>1000	1000
7i	**100**	**125**	**62.5**	**100**	500	1000
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.:Aspergillusniger, C.a.: Candida albicans

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.*
6a	**125**	**250**	125	200	500	**250**
6b	**100**	**100**	200	**100**	1000	**500**
6c	**125**	**125**	**100**	**62.5**	500	1000
6d	**125**	**100**	**100**	200	>1000	**200**
6e	**250**	**125**	125	250	200	1000
6f	**100**	**50**	200	**100**	>1000	>1000
6g	**200**	**100**	**62.5**	**100**	>1000	1000
6h	**200**	**125**	250	**62.5**	500	>1000
6i	**250**	**200**	200	**100**	500	>1000
7a	**100**	**62.5**	250	**100**	1000	>1000
7b	**250**	**100**	125	**100**	>1000	**500**
7c	**200**	**200**	**62.5**	**100**	500	**250**
7d	**125**	**100**	200	250	1000	>1000
7e	**62.5**	**125**	250	**100**	1000	**500**
7f	**62.5**	**100**	**100**	**100**	1000	1000
7g	**250**	**200**	**100**	125	1000	**500**
7h	**200**	**125**	125	**50**	>1000	1000
7i	**100**	**125**	**62.5**	**100**	500	1000
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.:Aspergillusniger, C.a.: Candida albicans

CONCLUSION

A simple and convenient methodology has been developed for synthesis for a series of pyrazolylpyridyl substituted dicoumarins was described and the synthesized compounds were screened fortheir *in vitro* antimicrobial evaluation. The results indicated that all the synthesized compoundsshown good antibacterial activity against bacterial and fungal pathogens as compared to standard drugs and emerged as potential lead compounds for further investigations.

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