A convergent approach for the synthesis of new pyrazolyl bipyridinyl substituted coumarin derivatives as antimicrobials

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ABSTRACT

Some new 3-[3-(1-phenyl-3-aryl-1H-pyrazol-4-yl)acryloyl]coumarins 3a-f were synthesized (coumarin chalcones) by the condensation of various 3-acetyl coumarins 1 and appropriate 1-phenyl-3-aryl-1H-pyrazole-4-carbaldehyde 2. These coumarin chalcones 3a-f were then employed for the synthesis of pyrazolyl bipyridinyl substituted coumarins 7a-f, 8a-f, and 9a-f under Krohnke’s reaction condition. The characterization of all the synthesized compounds was carried out by elemental analysis, IR, \textsuperscript{1}H-NMR, \textsuperscript{13}C-NMR, DEPT-135 and mass spectral analysis. In addition to that, in vitro antimicrobial competency of the title compounds was assessed against selected pathogens. Compounds 3b, 3e, 7b, 8b, 8c and 9b exhibited excellent antimicrobial activity and said to be the most proficient members of the series.

Keywords: Coumarin; Bipyridine; Pyrazole; Kröhnke’s reaction; Antimicrobial activity

1. INTRODUCTION

During past few decades, there has been an alarming increase in bacterial resistance to antibiotic drugs. The evolution and spread of these multidrug resistant bacteria have become a major threat to global health care [1]. Consequently, the discovery and design of new efficient antimicrobial agents is of crucial need to counteract the resistant pathogens. Therefore numerous research groups have directed their efforts towards designing the new anti-bacterial drugs with new targets, distinctive modes of action, low toxicity and low probability of inducing resistance to multi resistant bacteria [2-4].

Coumarin is extensively investigated as class of naturally occurring compounds and its derivatives have tremendous contribution in therapeutic field. Many coumarin derivatives used as effective drug in marketplace such as Warfarin, Novobiocin, Dicoumarol, Imperatorin, Calophyllolide and Neo-tarnshinlactone.

Such striking information from literature survey directed us to detail study of various heterocyclic substituted coumarin derivatives.

Among the coumarin derivatives, pyridyl substituted coumarins have been gained considerable interest among medicinal chemists owing to their therapeutic importance as CNS...
depressant [5], antifungal [6], moth proofing activity [7], fish toxicity [8], MAO inhibitor [9], antibacterial agents [10] and antitubercular [11].

During our literature survey we came across some bipyridines derivatives which have been reported to possess wide applications in the field of bioinorganic chemistry [12], supramolecular chemistry [13] and polymeric material [14]. Bipyridine derivatives exhibit wide range of physiological activities such as anticancer [15], cardiotonic [16], DNA binding properties [17] and antibacterial properties [18]. In addition to that, we came across some pyrazolyl substituted pyridines which have been reported to possess insecticidal [19], antiviral [20] and cardiotinic activities [21].

More efficacious antibacterial compounds can be designed by joining two or more biologically active heterocyclic systems together in a single molecular framework [22]. Such structural hybridizations show synergistic influence on the anticipated activity, hoping to discover new entities that would have astonishing antimicrobial activity.

In view of this background and medicinal significance of pyridyl substituted coumarins, bipyridines and pyrazolyl substituted pyridines encouraged us to hybrid these three bioactive moieties in single scaffold.

Hence, in continuation our efforts to synthesize such biologically active pyridyl substituted coumarin derivatives [10,11], we herein, report the synthesis and antimicrobial activity of pyrazolyl bipyridinyl coumarin derivatives 7a-f, 8a-f, and 9a-f.

2. RESULTS AND DISCUSSION

2.1. Chemistry

The synthetic strategies adopted for the preparation of key precursor 3-[3-(1-phenyl-3-aryl-1H-pyrazol-4-yl)acryloyl]coumarins 3a-f and title compounds 7a-f, 8a-f and 9a-f were depicted in Scheme 1.

The title compounds 7a-f, 8a-f and 9a-f were synthesized by reacting appropriate 3-[3-(1-phenyl-3-aryl-1H-pyrazol-4-yl)acryloyl]coumarins 3a-f with pyridoyl methyl pyridinium salts 4, 5 and 6 under Krohnke’s reaction condition [23]. The required precursors 3a-f were prepared by the reaction of various 3-acetyl coumarins 1 and pyrazole aldehydes 2 in presence of catalytical amount of piperidine in ethanol.

The plausible mechanism for the synthesis of target compounds 7a-f, 8a-f and 9a-f is demonstrated in Scheme 2.

The reaction proceeded via Michael addition by the nucleophilic attack of active methylene group of pyridoyl methyl pyridinium salts (4, 5 and 6) on the α,β-unsaturated carbonyl functionality of 3-[3-(1-phenyl-3-aryl-1H-pyrazol-4-yl)acryloyl]- coumarins 3a-f to form in situ 1,5-dicarbonyl intermediate.

The corresponding intermediate underwent cyclization in presence of ammonium acetate which upon subsequent loss of two water molecules afforded the target compounds in good yields.

All the compounds were characterized by IR, $^1$H NMR, $^{13}$C NMR, DEPT-90 spectral and elemental analysis. A mass spectrum was also recorded for representative compound 7a.
Scheme 1. Synthetic strategies adopted for the preparation of key precursors 3a-f and title compounds 7a-f, 8a-f and 9a-f.

7a, 8a, 9a: R = H, R1=H  
7b, 8b, 9b: R = H, R1=CH3  
7c, 8c, 9c: R = H, R1=OCH3  
7d, 8d, 9d: R = OCH3, R1=H  
7e, 8e, 9e: R = OCH3, R1=CH3  
7f, 8f, 9f: R = OCH3, R1=OCH3
2. 2. Biological result

The MICs of synthesized compounds were determined out by broth microdilution method according to National Committee for Clinical Laboratory Standards (NCCLS, 2002) [24]. Upon reviewing antimicrobial data (Table 1) it has been observed that compounds 7b and 9b (MIC = 50 µg/mL) showed excellent activity against gram positive bacteria *Staphylococcus aureus* as compared to ampicillin (MIC = 250 µg/mL). Similarly compounds 3e (MIC = 50 µg/mL) and 8e (MIC = 62.5 µg/mL) showed excellent activity against gram positive bacteria *Bacillus subtilis* as compared to ampicillin (MIC = 250 µg/mL). Compounds 3b, 7e, 8b, 9e (MIC = 100 µg/mL) and 3a, 3e, 7a, 7d, 8a, 8e, 9a, 9c (MIC = 200 µg/mL) were found to be more potent compared to ampicillin (MIC = 250 µg/mL) against *Staphylococcus aureus* while 3b, 3f, 7c, 8c, 8d, 9d, 9f were found to be equipotent compared to ampicillin (MIC = 250 µg/mL) against *Staphylococcus aureus*. Compounds 7b, 9b (MIC = 100 µg/mL) and 3b, 3d, 7a, 7c, 8b, 8d, 9c (MIC = 200 µg/mL) were found to be more active while compounds 3a, 7f, 8f, 9e (MIC = 250 µg/mL) were found to be equally active as compared to ampicillin (MIC = 250 µg/mL) against *Bacillus subtilis*.
Table 1. *In vitro* antimicrobial activity of various synthesized coumarin derivatives 3a-f, 7a-f, 8a-f and 9a-f (MICs, µg/mL).

| Compounds | Gram positive bacteria | Gram negative bacteria | Fungi |
|-----------|------------------------|------------------------|-------|
|           | Sa. MTCC | Bs. MTCC | Ec. MTCC | St. MTCC | Ca. MTCC | An. MTCC |
| 3a | H | H | 200 | 250 | 200 | 200 | 500 | >1000 |
| 3b | H | CH3 | 250 | 200 | 50 | 200 | 100 | 500 |
| 3c | H | OCH3 | 500 | 500 | 250 | 250 | >1000 | 500 |
| 3d | OCH3 | H | 100 | 200 | 250 | 200 | 250 | 250 |
| 3e | OCH3 | CH3 | 200 | 50 | 100 | 200 | 500 | 250 |
| 3f | OCH3 | OCH3 | 250 | 500 | 500 | 500 | 1000 | 1000 |
| 7a | H | H | 200 | 200 | 250 | 200 | 500 | 500 |
| 7b | H | CH3 | 50 | 100 | 100 | 62.5 | 1000 | 100 |
| 7c | H | OCH3 | 250 | 200 | 200 | 250 | 250 | 500 |
| 7d | OCH3 | H | 200 | 500 | 250 | 500 | 500 | 500 |
| 7e | OCH3 | CH3 | 100 | 500 | 250 | 100 | 200 | 250 |
| 7f | OCH3 | OCH3 | 500 | 250 | 500 | 250 | >1000 | 500 |
| 8a | H | H | 200 | 500 | 200 | 250 | 200 | 1000 |
| 8b | H | CH3 | 100 | 200 | 100 | 50 | 250 | 500 |
| 8c | H | OCH3 | 250 | 62.5 | 200 | 200 | 200 | 250 |
| 8d | OCH3 | H | 250 | 200 | 100 | 250 | 500 | >1000 |
| 8e | OCH3 | CH3 | 200 | 500 | 500 | 250 | 500 | >1000 |
| 8f | OCH3 | OCH3 | 500 | 250 | 250 | 500 | 1000 | 500 |
| 9a | H | H | 200 | 500 | 200 | 250 | >1000 | 500 |
| 9b | H | CH3 | 50 | 100 | 250 | 500 | 500 | 250 |
| 9c | H | OCH3 | 200 | 200 | 200 | 250 | 500 | 1000 |
| 9d | OCH3 | H | 250 | 500 | 250 | 200 | 250 | 250 |
| 9e | OCH3 | CH3 | 100 | 250 | 200 | 500 | 250 | 500 |
| 9f | OCH3 | OCH3 | 250 | 500 | 500 | 500 | 500 | 250 |
| Ampicillin | 250 | 250 | 100 | 100 | - | - |
| Nystatin | - | - | - | - | 100 | 100 |
| Griseofulvin | - | - | - | - | 500 | 100 |

Compounds 3e, 7b, 8b, 8d (MIC = 100 µg/mL) were found to be equipotent against gram negative bacteria *Escherichia coli* as compared to ampicillin (MIC = 100 µg/mL) while on the other hand only one compound 7e (MIC = 100 µg/mL) showed equipotent activity against gram negative bacteria *Salmonella typhi* as compared to ampicillin (MIC = 100 µg/mL).

Compounds 3b and 7b (MIC = 100 µg/mL) were the only candidates which showed equal activity against anti fungal strain *Candida albicans* and *Aspergillus niger* respectively as compared to nystatin (MIC = 100 µg/mL). Compounds 8a, 8c (MIC = 200 µg/mL) and 3d, 7c, 8b, 9d, 9e (MIC = 250 µg/mL) showed more activity as compared to griseofulvin (MIC = 500 µg/mL) against *Candida albicans*.
Interestingly, pyrazolyl acrolyl coumarin 3b (MIC = 250 and 200 µg/mL) after bipyridyl ring formation in 7b and 9b (MIC = 50 and 100 µg/mL) found to possess increased potency against *Staphylococcus aureus* and *Bacillus subtilis* respectively.

A general observation was made from the activity data (Table 1) that compounds 7a-c, 8a-c and 9a-c having no substitution in coumarin ring were more potent than their analogous having methoxyl substitution in most of the cases against all the bacterial strains. Compounds 9a-f bearing 4-pyridyl ring showed poor activity compared to standard drugs against gram negative bacterial strains. Compounds 7b and 8b, with R1 = CH3 showed better activity than their analogous against all the bacterial strains while 9b with R1 = CH3 showed better activity against gram positive bacterial strains only.

3. EXPERIMENTAL

3.1. Chemistry

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 reactions were monitored by thin-layer chromatography (TLC, on aluminium plates coated with silica gel 60 F254, 0.25 mm thickness, Merck) and detection of the components was made by exposure to UV light. Melting points were determined in open capillaries and are uncorrected. Infrared spectra were recorded on Shimadzu FTIR 8401 spectrophotometer using potassium bromide pellets in the range 4000-400 cm⁻¹ and frequencies of only characteristic peaks are expressed in cm⁻¹. ¹H and ¹³C NMR spectra were recorded in CDCl₃ on a Bruker Avance 400 (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using TMS signal as an internal standard at 400 MHz and 100 MHz respectively. Chemical shifts are reported in parts per million (ppm). The coupling constants (J) are given in Hertz (Hz). Mass spectrum of one representative compound was scanned on a Shimadzu QP 2010 spectrometer (Shimadzu, Tokyo, Japan). The compounds were purified by column chromatography using silica gel (60-120 mesh). Reference drugs ampicillin, griseofulvin, nystatin were of commercial grade.

Starting precursors 3-acetyl coumarins 1 [25], pyrazole aldehydes 2 [26], pyridoyl methyl pyridinium iodide salts 4, 5 and 6 [27] were prepared using the reported procedures.

3.1.1. General procedure for the synthesis of 3-[3-(1-phenyl-3-aryl-1H-pyrazol-4-yl)acryloyl]coumarins 3a-f.

In a 100 mL round bottom flask fitted with a reflux condenser, a solution of appropriate 3-acetyl coumarin, 0.01 mol (1a and 1b: 0.5g), 1-phenyl-3-aryl-1H-pyrazole-4-carbaldehyde (pyrazole aldehyde), 0.01 mol (2a:0.65g, 2b:0.69g, 2c:0.73g, 2d:0.56g, 2e:0.6g, 2f:0.63g) and catalytic amount of piperidine were taken in ethanol (30 mL). The reaction mixture was refluxed in water bath for 3 hours. The pyrazolyl acrolyl coumarin was formed, which was filtered out and washed with cold ethanol and dried. It was recrystallized from chloroform.

3-[3-(1,3-diphenyl-1H-pyrazol-4-yl)acryloyl]coumarin (3a):

Yield: 86%; m.p. 234’C; IR (KBr, νmax, cm⁻¹): 1720 (C=O δ-lactone stretching), 1659 (C=O ketone stretching), 1605 and 1535 (aromatic C=C and C=N stretchings), 756 & 687 (C-H bending of mono substituted benzene), 3063 (aromatic C-H stretching); ¹H NMR (400 MHz, CDCl₃, δ): 7.35-8.01 (16H, m, 14 Ar-H and C₂’-H, C₃’-H), 8.47 (1H, s, C₅’-H), 8.60 (1H, s, C₄- H); ¹³C NMR (100 MHz, CDCl₃, δ): 119.69(C₂’), 118.41(C₁₀), 118.63(C₄), 119.49(C₈),
3-[3-(1-phenyl-3-p-toly1-1H-pyrazol-4-yl)acryloyl]coumarin (3b):

Yield: 81%; m.p. 208°C; IR (KBr, νmax, cm⁻¹): 1705 (C=O δ-lactone stretching), 1659 (C=O ketone stretching), 1605 and 1528 (aromatic C=C and C=N stretchings), 764 & 679 (C-H bending of mono substituted benzene), 3024 (aromatic C-H stretching); ¹H NMR (400MHz, CDCl₃, δ): 2.45 (3H, s, C₆-CH₃), 7.32-8.01 (15H, m, 13 Ar-H and C₂'-H, C₃'-H), 8.46 (1H, s, C₅'-H), 8.60 (1H, s, C₄-H); ¹³C NMR (100MHz, CDCl₃, δ): 21.38(C₆'-CH₃), 116.68(C₂'), 118.35(C₁₀), 118.65(C₃'), 119.46(C₅'), 123.01(C₇', Cₕ'), 124.97(C₄'), 125.34(C₃), 126.94(C₆), 127.25(C₉), 128.74(C₄, C₅), 129.24(C₈), 129.50(C₃'), 129.56(C₇, C₉), 130.02(C₄, C₅), 134.15(C₆), 136.09(C₇), 138.62(C₈'), 139.44(C₉), 148.05(C₃'), 154.46(C₄), 155.20(C₅), 159.47(C₇'), 185.15(C₈'); Anal. Calcd. for C₂₇H₁₈N₂O₃ C, 77.70; H, 4.34; N, 6.69%. Found: C, 77.43; H, 4.39; N, 6.61%.

3-[3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)acryloyl]coumarin (3c):

Yield: 84%; m.p. 180°C; IR (KBr, νmax, cm⁻¹): 1736 (C=O δ-lactone stretching), 1666 (C=O ketone stretching), 1612 and 1528 (aromatic C=C and C=N stretchings), 787 & 679 (C-H bending of mono substituted benzene), 3040 (aromatic C-H stretching); ¹H NMR (400MHz, CDCl₃, δ): 3.90 (3H, s, C₆-CH(OCH₃)), 7.04-8.00 (15H, m, 13 Ar-H and C₂'-H, C₃'-H), 8.45 (1H, s, C₅'-H), 8.60 (1H, s, C₄-H); ¹³C NMR (100MHz, CDCl₃, δ): 55.39(C₆'-OCH₃), 114.19(C₂'), 114.29(C₆, C₅), 116.65(C₄), 118.21(C₁₀), 118.63(C₃'), 119.68(C₇', Cₕ'), 122.95(C₈), 124.65(C₉), 124.96(C₆), 125.31(C₃), 126.94(C₄'), 127.21(C₇', Cₕ'), 127.89(C₄), 129.54(C₇, C₉), 130.27(C₆), 134.14(C₅), 136.08(C₇'), 139.40(C₈), 140.02(C₄), 154.17(C₇), 155.17(C₅), 159.44(C₈'), 160.11(C₉') 185.05(C₈'); Anal. Calcd. for C₂₈H₂₀N₂O₄ C, 74.99; H, 4.50; N, 6.25%. Found: C, 74.90; H, 4.41; N, 6.32%.

8-methoxy-3-[3-(1,3-diphenyl-1H-pyrazol-4-yl)acryloyl]coumarin (3d):

Yield: 89%; m.p. 220°C; IR (KBr, νmax, cm⁻¹): 1720 (C=O δ-lactone stretching), 1661 (C=O ketone stretching), 1610 and 1535 (aromatic C=C and C=N stretchings), 780 & 687 (C-H bending of mono substituted benzene), 3046 (aromatic C-H stretching); ¹H NMR (400MHz, CDCl₃, δ): 4.02 (3H, s, C₈-CH(OCH₃)), 7.19-8.01 (15H, m, 13 Ar-H and C₂'-H, C₃'-H), 8.48 (1H, s, C₅'-H), 8.58 (1H, s, C₄-H); ¹³C NMR (100MHz, CDCl₃, δ): 55.39(C₆'-OCH₃), 116.00(C₇'), 118.40(C₁₀), 118.40(C₁₀), 122.10(C₇'), 122.97(C₉), 125.00(C₈'), 125.45(C₅'), 125.96(C₅), 126.87(C₅), 127.29(C₆, C₅), 128.65(C₆), 129.00(C₆, C₅), 129.89(C₄), 135.45(C₇', Cₕ'), 138.50(C₉), 140.65(C₇), 146.56(C₈), 147.68(C₃'), 148.52(C₈), 155.42(C₇), 158.17(C₂'), 185.09 (C₁'); Anal. Calcd. for C₃₈H₂₆N₂O₄ C, 74.99; H, 4.50; N, 6.25%. Found: C, 74.89; H, 4.44; N, 6.30%.

8-methoxy-3-[3-(1-phenyl-3-p-toly1-1H-pyrazol-4-yl)acryloyl]coumarin (3e):

Yield: 91%; m.p. 160°C; IR (KBr, νmax, cm⁻¹): 1728 (C=O δ-lactone stretching), 1651 (C=O ketone stretching), 1605 and 1574 (aromatic C=C and C=N stretchings), 779 & 680 (C-H bending of mono substituted benzene), 3056 (aromatic C-H stretching); ¹H NMR (400MHz, CDCl₃, δ): 2.45 (3H, s, C₆-CH₃), 4.02 (3H, s, C₆-CH(OCH₃)), 7.19-8.01 (14H, m, 12 Ar-H and C₂'-H, C₃'-H), 8.46 (1H, s, Py-H), 8.58 (1H, s, C₄-H); ¹³C NMR (100MHz, CDCl₃, δ): 21.37(C₆'-CH₃), 56.37(C₈-CH(OCH₃)), 115.89(C₂'), 118.35(C₄'), 119.27(C₃), 121.20(C₇), 121.35(C₉', Cₕ').
8-methoxy-3-[3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl]acryloyl]coumarin (3f):
Yield: 90%; m.p. 188°C; IR (KBr, νmax, cm⁻¹): 1720 (C=O δ-lactone stretching), 1666 (C=O ketone stretching), 1605 and 1528 (aromatic C=C and C=N stretchings), 787 & 687 (C-H bending of mono substituted benzene), 3060 (aromatic C-H stretching); ¹H NMR (400MHz, CDCl₃, δ): 3.90 (3H, s, CD₉-OCH₃), 4.02 (3H, s, C₈-OCH₃), 7.04-8.00 (14H, m, 12 Ar-H and C₂'-H, C₃'-H), 8.45 (1H, s, C₅'-H), 8.58 (1H, s, C₄-H); ¹³C NMR (100MHz, CDCl₃, δ): 55.39(C₆'′-OCH₃), 56.57(C₈-OCH₃), 114.29(C₈), 115.66(C₇), 118.22(C₁₀), 119.43(C₉), 119.71(C₅, C₆), 121.19(C₅′, C₆′), 122.97(C₆), 124.67(C₅′), 125.49(C₉), 127.00(C₉), 127.20(C₅′), 129.67(C₇′), 130.27(C₆, C₇), 136.12(C₈′), 139.44(C₅), 144.88(C₈), 147.06(C₉), 148.27(C₄), 154.21(C₆), 158.92(C₂), 160.10(C₉), 185.55(C₄'); Anal. Calcd. for C₂⁰H₂₂N₂O₃ C, 72.79; H, 4.63; N, 5.85%. Found: C, 72.72; H, 4.55; N, 5.79%.

3. 3. General procedure for the synthesis of bipyridinyl substituted coumarins (7a-f, 8a-f, and 9a-f).

In a 100 mL round bottom flask equipped with a condenser, guard tube and magnetic needle, an appropriate pyridyl methyl pyridinium iodide salt 4, 5 and 6, 0.003 mol (4, 5 and 6: 1g) in glacial acetic acid (15mL) was taken. To this ammonium acetate, 0.03 mol (2.36g) was added with stirring at room temperature. Then a solution of an appropriate 3-[3-(1,3-diaryl-1H-pyrazol-4-yl)acryloyl]coumarin 3a-f, 0.003 mol (3a:1.33g, 3b:1.37g, 3c:1.42g, 3d:1.42g, 3f:1.5g) in glacial acetic acid (15 mL) was added with stirring at room temperature and the reaction mixture was further stirred for 1 hour at room temperature 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.

3-[4-(1,3-Diphenyl-1H-pyrazol-4-yl)-2,2′-bipyridinyl-6-yl]coumarin (7a):
Yield: 68%; m.p. 210°C; IR (KBr, νmax, cm⁻¹): 1722 (C=O δ-lactone stretching), 1596 and 1474 (aromatic C=C and C=N stretchings), 3058 (aromatic C-H stretching); ¹H NMR (400MHz, CDCl₃, δ): 7.34-8.47 (19H, m, Ar-H except C₆′′-H and C₆′-H), 8.55 (1H, poorly resolved dd, C₅′′-H), 8.70 (1H, d, J = 4.0 Hz, C₅′-H), 8.89 (1H, s, C₄-H); ¹³C NMR (100MHz, CDCl₃, δ): 116.45(C₂), 119.22(C₈), 119.59(C₁₀), 119.85(C₆, C₇), 120.60(C₄′′), 121.26(C₅′), 123.23(C₉), 123.88(C₆), 124.53(C₅), 125.61(C₇), 126.85(C₅′′), 127.58(C₈′), 128.39(C₉′), 128.59(C₉), 128.72(C₆′), 128.83(C₅′′, C₆′), 129.52(C₅, C₆), 132.10(C₅), 132.58(C₉), 136.87(C₄′′), 139.75(C₆), 142.46(C₈), 142.69(C₅′′), 149.25(C₆′′), 150.95(C₅), 151.28(C₂′), 153.99(C₇′), 155.96(C₇′′), 156.02(C₆′) and 160.04(C₂); Anal. Calcd. for C₃₄H₂₄N₂O₂ C, 78.75; H, 4.28; N, 10.80%. Found: C, 78.70; H, 4.34; N, 10.86%.

3-[4-(1-Phenyl-3-p-tolyl-1H-pyrazol-4-yl)-2,2′-bipyridinyl-6-yl]coumarin (7b):
Yield: 75%; m.p. 182°C; IR (KBr, νmax, cm⁻¹): 1722 (C=O δ-lactone stretching), 1596 and 1474 (aromatic C=C and C=N stretchings), 3058 (aromatic C-H stretching); ¹H NMR (400MHz, CDCl₃, δ): 2.39 (3H, s, CH₃), 7.19-8.46 (18H, m, Ar-H except C₅′-H and C₆′′-H), 8.54 (1H, poorly resolved dd, C₆′′-H), 8.69 (1H, d, J = 4.0 Hz, C₅′-H), 8.88 (1H, s, C₄-H); ¹³C
3-[4-(3-(4-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-2,2'-bipyridin-6-yl]coumarin (7c): Yield: 65%; m.p. 204°C; IR (KBr, νmax cm⁻¹): 1720 (C=O δ-lactone stretching), 1597 and 1458 (aromatic C=C and C=N stretches), 3060 (aromatic C-H stretching); 1H NMR (400MHz, CDCl₃, δ): 3.84 (3H, s, OCH₃), 6.92-8.45 (18H, m, Ar-H except C₅′-H and C₆′′-H), 8.52 (1H, poorly resolved dd, C₆′′-H), 8.70 (1H, d, J = 4.0 Hz, C₅′-H), 8.91 (1H, s, C₄-H), 13C NMR (100MHz, CDCl₃, δ): 55.32(OCH₃), 114.08(C₃′), 116.41(C₃′′, C₄′), 119.14(C₆), 119.23(C₄′′), 119.58(C₅), 119.90(C₇, C₈), 120.22(C₆′′), 121.29(C₅′′), 123.23(C₉), 123.95(C₈), 124.56(C₅′), 125.10(C₈), 127.52(C₉′′), 127.62(C₈′), 128.91(C₅′′), 129.50(C₇, C₉), 129.90(C₅′′, C₆′′), 131.27(C₇), 137.03(C₈′′), 139.75(C₅′′′), 142.56(C₈), 143.10(C₈′′), 149.16(C₆′′), 150.92(C₇′′), 150.99(C₇), 153.99(C₄′), 159.79(C₆′), 160.07(C₂); Anal. Calcd. for C₃₅H₂₈N₄O₂ C, 76.73; H, 4.41; N, 10.21%. Found: C, 76.70; H, 4.35; N, 10.15%.

8-Methoxy-3-[4-(1,3-phenyl-1H-pyrazol-4-yl)-2,2'-bipyridin-6-yl]coumarin (7d): Yield: 67%; m.p. 188°C; IR (KBr, νmax cm⁻¹): 1725 (C=O δ-lactone stretching), 1592 and 1470 (aromatic C=C and C=N stretches), 3064 (aromatic C-H stretching); 1H NMR (400MHz, CDCl₃, δ): 4.00 (3H, s, OCH₃), 7.11-8.44 (18H, m, Ar-H except C₅′-H and C₆′′-H), 8.51 (1H, poorly resolved dd, C₆′′-H), 8.69 (1H, d, J = 4.4 Hz, C₅′-H), 8.88 (1H, s, C₄-H), 13C NMR (100MHz, CDCl₃, δ): 56.28(OCH₃), 113.89(C₃′), 119.21(C₇), 119.88(C₆, C₉), 120.20(C₉′), 120.29(C₆′′), 120.55(C₄′′), 121.27(C₈′), 123.26(C₈), 123.89(C₉), 124.33(C₇), 125.62(C₅), 126.81(C₆′′), 127.63(C₈′), 128.36(C₅′, C₇′), 128.56(C₅′′), 128.68(C₅′′′), 129.51(C₆, C₉), 132.56(C₅′), 136.98(C₇′), 139.73(C₉), 142.67(C₅′′), 142.73(C₄′), 143.67(C₉), 146.96(C₈), 149.14(C₆′), 150.92(C₉′′), 151.26(C₈), 155.66(C₄), 155.83(C₉′), 159.45(C₂); Anal. Calcd. for C₃₆H₂₉N₄O₃ C, 76.63; H, 4.41; N, 10.21%. Found: C, 76.68; H, 4.48; N, 10.17%.

8-Methoxy-3-[4-(1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)-2,2'-bipyridin-6-yl]coumarin (7e): Yield: 74%; m.p. 226°C; IR (KBr, νmax cm⁻¹): 1728 (C=O δ-lactone stretching), 1582 and 1474 (aromatic C=C and C=N stretches), 3060 (aromatic C-H stretching); 1H NMR (400MHz, CDCl₃, δ): 2.39 (3H, s, CH₃), 4.04 (3H, s, OCH₃), 7.12-8.45 (17H, m, Ar-H except C₅′-H and C₆′′-H), 8.53 (1H, poorly resolved dd, C₆′′-H), 8.69 (1H, d, J = 4.4 Hz, C₅′-H), 8.86 (1H, s, C₄-H); 13C NMR (100MHz, CDCl₃, δ): 21.48(CH₃), 56.44(OCH₃), 113.86(C₅′), 114.10(C₇), 114.62(C₁₀), 115.65(C₆′′), 119.18(C₉, C₁), 119.94(C₅′′), 120.01(C₃), 120.22(C₅), 120.49(C₆), 120.73(C₇), 121.24(C₈), 123.34(C₉), 123.81(C₆′′), 124.32(C₃), 124.38(C₅′′), 124.63(C₅′), 126.70(C₉), 127.58(C₃′′), 128.45(C₆′, C₇′), 129.24(C₉, C₁), 129.48(C₈), 136.81(C₆′′), 138.05(C₈), 142.54(C₉), 144.77(C₂′′), 146.97(C₂′), 149.23(C₆′′), 151.26(C₄′), 155.83(C₁′), 159.45(C₂); Anal. Calcd. for C₃₆H₂₈N₄O₃ C, 76.85; H, 4.66; N, 9.96%. Found: C, 76.78; H, 4.72; N, 9.91%.
8-Methoxy-3-[4-[(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl]-2,2′-bipyridin-6-yl] coumarin (7f):
Yield: 70%; m.p. 238°C; IR (KBr, ν max, cm⁻¹): 1733 (C=O δ-lactone stretching), 1597 and 1477 (aromatic C=C and C=N stretchings), 3058 (aromatic C-H stretching); ¹H NMR (400MHz, CDCl₃, δ): 3.84 (3H, s, OCH₃), 4.02 (3H, s, OCH₃), 6.91-8.45 (17H, m, Ar-H except C₅'-H and C₆'-H), 8.53 (1H, poorly resolved dd, C₆''-H), 8.70 (1H, d, J = 4.0 Hz, C₅'-H), 8.89 (1H, s, C₄-H); ¹³C NMR (100MHz, CDCl₃, δ): 55.27 (OCH₃), 56.29 (OCH₃), 114.06 (C₅'), 119.15 (C₇), 119.27 (C₁₀), 119.96 (C₅'', C₆''), 120.22 (C₆, C₇), 120.33 (C₆'''), 121.31 (C₅''), 123.34 (C₆), 123.90 (C₅), 124.36 (C₈), 125.08 (C₅), 126.70 (C₃), 127.60 (C₅'''), 129.50 (C₃''), 129.89 (C₅, C₆), 137.08 (C₆', C₇'), 139.75 (C₆'), 142.76 (C₆''), 143.05 (C₅''''), 143.72 (C₉), 146.96 (C₈), 149.02 (C₄), 149.14 (C₆''), 150.92 (C₂'''), 150.99 (C₂'), 151.43 (C₃), 153.83 (C₄), 159.48 (C₆'), 159.78 (C₂); Anal. Calcd. for C₃₆H₂₆N₄O₄ C, 74.73; H, 4.53; N, 9.68%. Found: C, 74.78; H, 4.60; N, 9.75%.

3-[4-(1,3-Diphenyl-1H-pyrazol-4-yl)-2,3′-bipyridin-6-yl]coumarin (8a):
Yield: 72%; m.p. 184°C; IR (KBr, ν max, cm⁻¹): 1720 (C=O δ-lactone stretching), 1590 and 1460 (aromatic C=C and C=N stretchings), 3060 (aromatic C-H stretching); ¹H NMR (400MHz, CDCl₃, δ): 2.44 (3H, singlet, CH₃), 7.24-8.20 (16H, m, Ar-H), 8.34 (1H, s, C₅''''-H), 8.60 (1H, poorly resolved d, C₅'-H), 8.66 (1H, poorly resolved dd, C₆''-H), 8.98 (1H, s, C₄-H), 9.14 (1H, d, J = 2.0 Hz, C₅''-H); ¹³C NMR (100MHz, CDCl₃, δ): 116.41 (C₅'), 119.29 (C₆), 119.53 (C₁₀), 120.19 (C₆''), 121.71 (C₅'), 123.50 (C₅'''), 124.68 (C₃'), 125.02 (C₂'), 127.03 (C₄), 127.41 (C₆), 128.69 (C₆''), 128.71 (C₆'', C₇''), 129.01 (C₁), 129.56 (C₆'', C₆'''), 132.33 (C₆, C₇), 132.67 (C₆''), 134.21 (C₇), 134.64 (C₆'), 139.62 (C₆), 140.42 (C₄), 142.50 (C₅'''''), 142.86 (C₄'), 148.41 (C₆'''), 149.95 (C₅'''''), 151.32 (C₅), 151.74 (C₂'), 154.01 (C₄''), 154.14 (C₆'), 160.27 (C₂); Anal. Calcd. for C₃₄H₂₂N₄O₂ C, 78.75; H, 4.28; N, 10.80%. Found: C, 78.69; H, 4.36; N, 10.76%.

3-[4-(1-Phenyl-3-p-tolyl-1H-pyrazol-4-yl)-2,3′-bipyridin-6-yl]coumarin (8b):
Yield: 74%; m.p. 220°C; IR (KBr, ν max, cm⁻¹): 1720 (C=O δ-lactone stretching), 1590 and 1460 (aromatic C=C and C=N stretchings), 3060 (aromatic C-H stretching); ¹H NMR (400MHz, CDCl₃, δ): 2.44 (3H, singlet, CH₃), 7.24-8.20 (16H, m, Ar-H), 8.34 (1H, s, C₅''''-H), 8.60 (1H, poorly resolved d, C₅'-H), 8.66 (1H, poorly resolved dd, C₆''-H), 8.98 (1H, s, C₄-H), 9.14 (1H, d, J = 2.0 Hz, C₅''-H); ¹³C NMR (100MHz, CDCl₃, δ): 21.40 (CH₃), 116.41 (C₅'), 119.24 (C₆), 119.34 (C₆', C₇), 119.53 (C₁₀), 120.06 (C₆''), 121.79 (C₅'''), 123.54 (C₃'), 124.68 (C₄), 125.05 (C₂'), 126.93 (C₅'), 127.41 (C₆''), 128.66 (C₆'), 129.01 (C₅'', C₆'''), 129.54 (C₆, C₇), 129.71 (C₆''), 132.32 (C₂), 134.72 (C₄), 138.50 (C₆''), 139.64 (C₆), 142.72 (C₆'''), 142.85 (C₄'), 146.83 (C₆'''), 143.85 (C₇'), 151.26 (C₆), 151.72 (C₂'), 154.00 (C₄'), 154.04 (C₆'), 160.27 (C₂); Anal. Calcd. for C₃₅H₂₄N₄O₂ C, 78.93; H, 4.54; N, 10.52%. Found: C, 78.86; H, 4.59; N, 10.47%.

3-[4-[3-(4-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl]-2,3′-bipyridin-6-yl]coumarin (8c):
Yield: 64%; m.p. 216°C; IR (KBr, ν max, cm⁻¹): 1721 (C=O δ-lactone stretching), 1596 and 1456 (aromatic C=C and C=N stretchings), 3065 (aromatic C-H stretching); ¹H NMR (400MHz, CDCl₃, δ): 3.88 (3H, s, OCH₃), 6.97-8.23 (16H, m, Ar-H), 8.34 (1H, s, C₅''''-H), 8.61 (1H, d, J = 1.2 Hz, C₅'-H), 8.67 (1H, poorly resolved dd, C₆''-H), 8.96 (1H, s, C₄-H), 9.19 (1H, poorly resolved d, C₂''-H); ¹³C NMR (100MHz, CDCl₃, δ): 55.41 (OCH₃), 114.17 (C₅', C₆'), 116.45 (C₅'''), 119.27 (C₈), 119.38 (C₆, C₇), 119.95 (C₆''), 121.90 (C₃'), 123.60 (C₈), 124.71 (C₆), 125.05 (C₁₀), 125.13 (C₄'), 126.12 (C₃), 126.92 (C₅'''''), 127.43 (C₅')}
8-Methoxy-3-[4-(1,3-phenyl-1H-pyrazol-4-yl)-2,3'-bipyridin-6-yl]coumarin (8d):

Yield: 68%; m.p. 240°C; IR (KBr, ν_max, cm⁻¹): 1713 (C=O δ-lactone stretching), 1597 and 1474 (aromatic C=C and C=N stretchings), 3062 (aromatic C-H stretching); ¹H NMR (400MHz, CDCl₃, δ): 4.02 (3H, s, OCH₃), 7.14-8.18 (16H, m, Ar-H), 8.39 (1H, s, C₅'''-H), 8.62 (1H, poorly resolved d, C₅'-H), 8.65 (1H, poorly resolved dd, C₆'''-H), 8.96 (1H, s, C₄-H), 9.11 (1H, d, J = 1.8 Hz, C₃'''-H); ¹³C NMR (100MHz, CDCl₃, δ): 56.32(OCH₃), 114.08(C₅'), 119.29(C₅), 119.34(C₆, C₇), 120.15(C₄'''), 120.42(C₄'), 121.81(C₅'), 123.59(C₆), 124.51(C₅), 125.15(C₃), 127.02(C₂), 127.49(C₅''), 128.67(C₄'), 128.7(C₆', C₇'), 129.56(C₆6, C₇6), 132.67(C₅''), 134.46(C₄'), 134.79(C₆), 136.44(C₇), 139.63(C₅'''), 142.56(C₅), 143.03(C₄), 143.68(C₈), 148.17(C₆''), 149.66(C₄'), 151.32(C₂), 151.77(C₇), 153.97(C₆), 159.71(C₅); Anal. Calcd. for C₃₅H₃₄N₄O₃ C, 76.63; H, 4.41; N, 10.21%. Found: C, 76.58; H, 4.36; N, 10.29%.

8-Methoxy-3-[4-(1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)-2,3'-bipyridin-6-yl]coumarin (8e):

Yield: 76%; m.p. 248°C; IR (KBr, ν_max, cm⁻¹): 1718 (C=O δ-lactone stretching), 1590 and 1470 (aromatic C=C and C=N stretchings), 3058 (aromatic C-H stretching); ¹H NMR (400MHz, CDCl₃, δ): 2.43 (3H, s, CH₃), 4.02 (3H, s, OCH₃), 7.14-8.19 (15H, m, Ar-H), 8.34 (1H, s, C₅'''-H), 8.61 (1H, poorly resolved d, C₅'-H), 8.67 (1H, poorly resolved dd, C₆'''-H), 8.96 (1H, s, C₄-H), 9.12 (1H, d, J = 1.2 Hz, C₃'''-H); ¹³C NMR (100MHz, CDCl₃, δ): 21.40(CH₃), 56.30(OCH₃), 114.02(C₇), 119.25(C₅'), 119.40(C₆, C₇), 120.07(C₁₀), 120.17(C₄''), 120.38(C₅'), 121.82(C₅'), 123.48(C₆), 124.49(C₇), 125.23(C₈), 126.90(C₉), 127.46(C₅''), 128.65(C₅', C₆'), 129.35(C₆', C₇'), 129.53(C₂, C₉), 129.72(C₆', C₇'), 134.24(C₄'), 134.68(C₅'), 138.47(C₃'), 139.66(C₆), 142.71(C₃''), 142.98(C₄), 143.67(C₉), 146.95(C₆), 148.45(C₅'), 149.94(C₆'), 151.25(C₂), 151.69(C₇'), 154.05(C₆'), 159.70(C₅); Anal. Calcd. for C₃₆H₃₅N₄O₃ C, 76.85; H, 4.66; N, 9.96%. Found: C, 76.77; H, 4.58; N, 9.88%.

8-Methoxy-3-[4-[3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl]-2,3'-bipyridin-6-yl]coumarin (8f):

Yield: 72%; m.p. 250°C; IR (KBr, ν_max, cm⁻¹): 1720 (C=O δ-lactone stretching), 1597 and 1474 (aromatic C=C and C=N stretchings), 3057 (aromatic C-H stretching); ¹H NMR (400MHz, CDCl₃, δ): 3.87 (3H, s, CH₃), 4.03 (3H, s, OCH₃), 6.96-8.25 (15H, m, Ar-H), 8.34 (1H, s, C₅'''-H), 8.62 (1H, poorly resolved d, C₅'-H), 8.68 (1H, poorly resolved dd, C₆'''-H), 8.96 (1H, s, C₄-H), 9.21 (1H, poorly resolved d, C₃'''-H); ¹³C NMR (100MHz, CDCl₃, δ): 55.38(OCH₃), 56.32(OCH₃), 114.09(C₇), 114.15(C₆', C₇'), 119.23(C₅'), 119.34(C₆, C₇), 119.85(C₁₀), 120.16(C₄''), 120.43(C₅'''), 122.01(C₅'), 123.78(C₆), 124.52(C₇), 125.01(C₈), 125.15(C₉), 126.90(C₆), 127.49(C₅''), 129.53(C₆, C₇), 130.03(C₆', C₇'), 134.85(C₄'), 135.00(C₃'), 139.64(C₆), 142.90(C₇''), 143.08(C₈), 143.69(C₉), 146.96(C₆), 147.81(C₄''), 149.19(C₃'), 150.98(C₅'), 151.81(C₂'), 153.72(C₆'), 159.69(C₆'), 159.99(C₅); Anal. Calcd. for C₃₆H₃₆N₄O₃ C, 74.73; H, 4.53; N, 9.68%. Found: C, 74.79; H, 4.60; N, 9.60%

3-[4-(1,3-Diphenyl-1H-pyrazol-4-yl)-2,4'-bipyridin-6-yl]coumarin (9a):

Yield: 62%; m.p. 212°C; IR (KBr, ν_max, cm⁻¹): 1720 (C=O δ-lactone stretching), 1597 and 1458 (aromatic C=C and C=N stretchings), 3052 (aromatic C-H stretching); ¹H NMR
3-[4-(1-Phenyl-3-p-toly1-1H-pyrazol-4-yl)-2,4'-bipyridin-6-yl]coumarin (9b):
Yield: 76%; m.p. 224°C; IR (KBr, v_{max}, cm^{-1}): 1720 (C=O &-lactone stretching), 1597 and 1458 (aromatic C=C and C=N stretches); 3052 (aromatic C-H stretching); ^1H NMR (400MHz, CDCl3, δ): 2.45 (3H, s, CH3), 7.26-7.87 (16H, m, Ar-H), 8.35 (1H, s, C5′′′-H), 8.63 (1H, poorly resolved d, Cγ′′′-H), 8.72 (2H, poorly resolved dd, C2''-H and C2''''-H), 8.97 (1H, s, C4-H); ^13C NMR (100MHz, CDCl3, δ): 116.45(C'), 119.29(C6), 119.48(C10), 119.56(Cb, Cc), 120.02(C3), 121.05(Cd), 122.60(Ce), 124.70(Cf), 124.96(C4'''), 127.08(C3''), 127.40(C5''), 128.67(Cd), 128.73(Cf, Cg), 128.88(Cc), 129.02(Cf, Ce), 129.58(Cb, Ce), 132.42(Cf), 132.67(Ca), 139.58(Cb), 142.62(Cc'''), 142.94(Cc), 146.35(Cc''''), 150.25(C2'', C6'''), 151.30(Ca), 151.88(Cf), 153.91(Cf'), 154.03(Ca'), 160.24 (C2); Anal. Calcd. for C34H22N4O2C, 78.75; H, 4.28; N, 10.80%. Found: C, 78.40; H, 4.21; N, 10.86%.

3-[4-[3-(4-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl]-2,4'-bipyridin-6-yl]coumarin (9c):
Yield: 70%; m.p. 210°C; IR (KBr, v_{max}, cm^{-1}): 1718 (C=O &-lactone stretching), 1595 and 1460 (aromatic C=C and C=N stretches); 3060 (aromatic C-H stretching); ^1H NMR (400MHz, CDCl3, δ): 4.04 (3H, s, OCH3), 7.15-7.88 (16H, m, Ar-H), 8.37 (1H, s, C5′′′-H), 8.63 (1H, poorly resolved d, Cγ′′′-H), 8.71 (2H, poorly resolved dd, C2''-H and C2''''-H), 8.98 (1H, s, C4-H); ^13C NMR (100MHz, CDCl3, δ): 56.40(OCH3), 116.43(C'), 119.32(C', C'), 119.52(C10), 120.17(C4''''), 121.73(C4), 123.53(Cb, Cc), 124.65(Cd), 125.07(C3), 127.06(C6), 127.47(Ca), 128.66(C5'''), 128.57(C5''), 129.04(C5), 129.56(Ca), 132.33(Cc, Cc), 132.87(Cc), 134.21(Cb, Cc), 134.64(C4''), 139.42(C2'''), 142.30(Cc), 142.76(Cc), 148.41(C4''), 149.45(C6''), 151.12(Cc), 151.34(Cc'), 154.04(C4''), 160.22(C2); Anal. Calcd. for C35H24N4O3C, 76.63; H, 4.41; N, 10.21%. Found: C, 76.58; H, 4.35; N, 10.28%.

8-Methoxy-3-[4-(1,3-phenyl-1H-pyrazol-4-yl)-2,4'-bipyridin-6-yl]coumarin (9d):
Yield: 65%; m.p. 232°C; IR (KBr, v_{max}, cm^{-1}): 1720 (C=O &-lactone stretching), 1597 and 1458 (aromatic C=C and N stretches); 3045 (aromatic C-H stretching); ^1H NMR (400MHz, CDCl3, δ): 4.03 (3H, s, OCH3), 7.15-7.88 (16H, m, Ar-H), 8.38 (1H, s, C5′′′-H), 8.65 (1H, poorly resolved d, Cγ′′′-H), 8.71 (2H, poorly resolved dd, C2''-H and C2''''-H), 8.96 (1H, s, C4-H); ^13C NMR (100MHz, CDCl3, δ): 56.32(OCH3), 114.13(C'), 119.31(Cc), 119.63(Cc, Cc), 120.01(C10), 120.12(C4''''), 120.39(Cb), 121.07(Cc), 122.67(Cd), 124.53(C5'''), 125.14(Cc), 127.06(C4''), 128.65(Cd), 128.71(Cb, Cc), 128.87(Cc', Cc'), 129.57(Cc, Cc), 132.68(Cc'), 139.59(Ca), 142.65(C4''), 143.10(C4), 143.72(C5'''), 146.44(Cc), 147.00(Cc), 150.14(C6''), 151.29(Cc), 151.89(Cc), 153.82(Cc', Cc''), 159.59(Cc); Anal. Calcd. for C35H24N4O3C, 76.63; H, 4.41; N, 10.21%. Found: C, 76.55; H, 4.50; N, 10.27%.

8-Methoxy-3-[4-(1-phenyl-3-p-toly1-1H-pyrazol-4-yl)-2,4'-bipyridin-6-yl]coumarin (9e):
Yield: 78%; m.p. 184°C; IR (KBr, v_{max}, cm^{-1}): 1716 (C=O &-lactone stretching), 1592 and 1476 (aromatic C=C and C=N stretches); 3023 (aromatic C-H stretching); ^1H NMR
(400MHz, CDCl$_3$, $\delta$): 2.44 (3H, s, CH$_3$), 4.02 (3H, s, OCH$_3$), 7.14-7.86 (15H, m, Ar -H), 8.70 (2H, poorly resolved dd, C$_2$'-H and C$_6$''-H), 8.93 (1H, s, C$_5$'-H); $^{13}$C NMR (100MHz, CDCl$_3$, $\delta$): 21.37(CH$_3$), 56.30(OCH$_3$), 114.08(C$_5$'), 119.25(C$_7$), 119.62(C$_b$, C$_5$), 120.13(C$_4'$'), 120.38(C$_6$), 121.03(C$_4$), 122.64(C$_d$), 124.51(C$_3$), 125.18(C$_8$), 126.95(C$_5''$), 127.39(C$_5'''$), 128.70(C$_5'$, C$_6'$), 129.37(C$_b'$, C$_7'$), 129.53(C$_c$, C$_6$), 129.75(C$_d'$), 138.45(C$_a'$), 139.62(C$_a$), 142.78(C$_4''$'), 143.04(C$_4$), 143.69(C$_3'$'), 146.26(C$_8$), 146.98(C$_6'$), 150.35(C$_6''$), 151.28(C$_6'''$), 151.78(C$_4$'), 153.84(C$_5$'), 159.70(C$_2$ of coumarin); Anal. Calcd. for C$_{36}$H$_{26}$N$_4$O$_3$ C, 76.85; H, 4.66; N, 9.96%. Found: C, 76.78; H, 4.72; N, 9.89%.

8-Methoxy-3-{4-[3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl]-2,4'-bipyridin-6-yl} coumarin (9f):

Yield: 68%; m.p. 244°C; IR (KBr, $v_{max}$, cm$^{-1}$): 1720 (C=O $\delta$-lactone stretching), 1590 and 1460 (aromatic C=C and C=N stretchings), 3058 (aromatic C-H stretching); $^1$H NMR (400MHz, CDCl$_3$, $\delta$): 3.88 (3H, s, OCH$_3$), 4.04 (3H, s, OCH$_3$), 6.97-8.35 (15H, m, Ar-H), 8.34 (1H, s, C$_6$''-H), 8.65 (1H, poorly resolved d, C$_5'$-H), 8.71 (2H, poorly resolved dd, C$_2$'-H and C$_6$''-H), 8.95 (1H, s, C$_4$-H); $^{13}$C NMR (100MHz, CDCl$_3$, $\delta$): 55.40(OCH$_3$), 56.33(OCH$_3$), 114.17(C$_5$'), 119.25(C$_7$), 119.66(C$_b'$, C$_6'$), 119.76(C$_b$, C$_5$), 120.14(C$_4'$'), 120.39(C$_6$), 121.16(C$_5$), 122.81(C$_d$), 124.54(C$_3$), 125.05(C$_5$), 125.21(C$_a'$), 126.95(C$_5''$), 127.42(C$_5'''$), 129.55(C$_c$, C$_6$), 130.07(C$_b'$, C$_7'$), 139.64(C$_a$), 142.94(C$_4''$'), 143.10(C$_4$), 143.74(C$_3'$'), 146.65(C$_9$), 147.01(C$_6$), 149.98(C$_6''$'), 151.01(C$_2'$), 151.94(C$_4$'), 153.77(C$_5$'), 159.68(C$_5$'), 160.01(C$_2$); Anal. Calcd. for C$_{36}$H$_{26}$N$_4$O$_3$ C, 74.73; H, 4.53; N, 9.68%. Found: C, 74.79; H, 4.60; N, 9.74%.

3. 2. Biological assay

All the newly synthesized target compounds were evaluated for their in vitro antibacterial activity against Staphylococcus aureus (MTCC 96) and Bacillus subtilis (MTCC 441) as examples of Gram positive bacteria and Escherichia coli (MTCC 443) and Salmonella typhi (MTCC 98) as examples of Gram negative bacteria. They were also evaluated for their in vitro antifungal activity against Candida albicans (MTCC 227) and Aspergillus niger (MTCC 282) as fungal strains. For comparison, the standard drug used for antibacterial potency of the compounds was ampicillin, a broad spectrum antibiotic, while the drugs used for antifungal potency of the compounds were griseofulvin and nystatin. The screening results (Table 1) indicated that all the tested compounds exhibited different inhibitory effects against different test organisms. All MTCC cultures were collected from Institute of Microbial Technology, Chandigarh and tested against above mentioned known drugs. Mueller Hinton broth was used as nutrient medium to grow and dilute the drug suspension for the test. Inoculum’s size for test strain was adjusted to $10^8$ CFU (Colony Forming Unit) per milliliter by comparing the turbidity. The newly prepared compounds were screened for their MICs by broth microdilution method. DMSO was used as a diluent to get desired concentration of compounds to test upon standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic was immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37°C overnight. The tubes were then incubated overnight. The MIC of the control organism was read to check the accuracy of the compound concentrations. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). All the tubes showing no visible growth (same as control
tube) were sub cultured and incubated overnight at 37°C. The amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Subcultures might show (i) similar number of colonies indicating bacteriostatic (ii) a reduced number of colonies indicating a partial or slow bactericidal activity (iii) no growth if the whole inoculum has been killed. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity. Each synthesized compound was diluted obtaining 2000 µg/mL concentration as a stock solution. In primary screening 500, 250 and 200 µg/mL concentrations of the synthesized compounds were taken. The compounds which found active in this primary screening were further tested in a second set of dilution using 100, 62.5, 50 and 25 µg/mL concentrations against all microorganisms. The highest dilution showing at least 99% inhibition is taken as MIC.

4. CONCLUSION

Present study described successful hybridization strategy of three bioactive moieties, pyridyl substituted coumarin, bipyridine and pyrazolyl substituted pyridine in single scaffold. The target compounds were synthesized in good yield by adopting Krohnke’s protocol. Majority of the compounds were found to be active against Staphylococcus aureus and Bacillus subtilis. In antifungal activity, majority of the compounds showed excellent activity against Candida albicans as compared to griseofulvin. Antimicrobial screening results revealed that compounds 3b, 3e, 7b, 8b, 8c and 9b were found to be the most proficient members of the series. Reviewing the antimicrobial data, it is worth mentioning here that coumarins bearing bipyridine and pyrazole entities as substitution serve as promising lead scaffolds for further generation of new antimicrobial agents.

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