Synthesis, characterization and biological evaluation of some new pyrazolyl bipyridyl substituted dicoumarins

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The present study describes the synthesis of pyrazolyl bipyridyl substituted dicoumarin derivatives carried out by the reaction of various 3-[3-{3-(coumarin-3-yl)-1-phenyl-1H-pyrazol-4-yl}acryloyl]coumarins (coumarin chalcones) (4a-f) with appropriate pyridoyl methyl pyridinium iodide salts (5-7) in the presence of ammonium acetate in refluxing acetic acid. All synthesized target compounds (8a-f), (9a-f) and (10a-f) were characterized by IR, $^1$H-NMR, $^{13}$C-APT and representative mass spectral data. The compounds were subjected to in vitro antimicrobial screening against a representative panel of bacteria (Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhi) and fungi (Aspergillus niger, Candida albicans).

Keywords: coumarins, coumarin chalcones, Krohnke reaction, antimicrobial screening.

INTRODUCTION

Coumarins are the best known aromatic lactones isolated from various natural sources. Over the past decades coumarins have attracted strong scientific interest stemming from their broad spectrum of pharmacological properties such as antimicrobial [1], anti-inflammatory [2], CNS depressant [3], antioxidant [4], antitumor [5], antiviral [6], antiasthmatic [7], antitubercular [8], cytotoxic properties [9], etc. The actual trend in the field of synthetic chemistry of coumarins is the introduction of a heterocyclic moiety as a substituent either in the lactone or benzene ring in a coumarin core structure possessing various biological activities [10-14] in which pyrazolyl-substituted coumarins are well documented in the literature and known for their various biological properties like antimicrobial [15], anticonvulsant [16], antioxidant, antihyperglycemic [17], etc. They also show fluorescent and absorption emission characteristics [18].

Bipyridines are compounds formed by coupling of two pyridine rings. Bipyridines are widely used in coordination and supramolecular chemistry [19]. They also possess effective biological properties like antibacterial [20], antifungal [21], antimycoplasmal [22], antimalarial [23], antitumor activity [24], etc. Moreover, a large number of bipyridines are used in photocatalysis [25], as chemosensors [26] and luminescent probes for biomolecular systems [27].

Thus, considering the importance of pyrazolyl-substituted coumarins and bipyridines, it was thought worthwhile to synthesize some new coumarin derivatives having both of these structural features in a single molecule. Therefore, in the present work, synthesis of various pyrazolyl-bipyridyl-substituted dicoumarins was carried out and all synthesized compounds were screened for their antimicrobial activity.

EXPERIMENTAL

All melting points are uncorrected. All reactions were performed with commercially available reagents used without further purification. Organic solvents were purified by standard methods and stored over molecular sieves. All IR spectra (KBr disc) were recorded on a Shimadzu FTIR 8400-S spectrometer. $^1$H-NMR and $^{13}$C-APT spectra were recorded on a 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 a Shimadzu QP 2010 spectrometer. All 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, various 3-[4'-[3'''-(coumarin-3''''-yl)-1'''''-phenyl-1H-pyrazol-4'''''-yl]-2',3'''''-bipyridin-6'''''-yl]coumarins (8a-f), 3-[4''''-[3'''-(coumarin-3''''-yl)-1'''''-phenyl-1H-pyrazol-4'''''-yl]2''''-bipyridin-6''''-yl]coumarins (9a-f) and 3-[4''''-[3'''-(coumarin-3''''-yl)-1'''''-phenyl-1H-pyrazol-4'''''-yl]2''''-bipyridin-6''''-yl]coumarins (10a-f) were synthesized by the reaction of various 3-[3-(coumarin-3-yl)-1-phenyl-1H-pyrazol-4-yl] acryloyl]coumarins (coumarin chalcones) (4a-f) with appropriate pyridoyl methyl pyridinium iodide salts (5-7) under Krohnke’s reaction conditions.

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Compounds (5-7) were prepared according to the procedure given in [28].

General procedure for the preparation of 3-[3-{3-(coumarin-3-yl)-1-phenyl-1H-pyrazol-4-y1}acryloyl]coumarins (4a-f) (coumarin chalcones).

In a 100-mL round bottom flask, an appropriate 3-{(1-phenyl-4-formyl)pyrazol-3-yl}coumarin (0.01 mole) and an appropriate 3-acetyl coumarin (0.01 mole) were taken in 50 mL of ethanol. Catalytic amount of piperidine was added and the reaction mixture was stirred for 10 min at room temperature. The mixture was then refluxed on a water bath for 4 h. It was allowed to cool to room temperature. The solid product separated out was filtered off, washed with cold ethanol and dried. It was recrystallized from ethanol. Coumarin chalcones 4a, 4b, 4d and 4e were prepared according to procedure [29].

5,6-Benz-3-[3-{3-(coumarin-3-yl)-1-phenyl-1H-pyrazol-4-yl}acryloyl]coumarin (4c): IR(KBr, \(v_{\text{max}}\) cm\(^{-1}\)): 1736(C=O stretching of \(\alpha,\beta\) unsaturated carbonyl group), 1605 and 1535(aromatic C=C and C=N stretchings), 748 and 687(C-H bending vibrations of mono substituted benzene ring), 3055(aromatic carbonyl group), 1605 and 1478(aromatic C=C and C=N stretchings), 748 and 687(C-H bending vibrations of mono substituted benzene ring), 3063(aromatic C-H stretching).

The mixture was then refluxed on a water bath for 4 h. Then a solution of an appropriate 3-[3-(coumarin-3-yl)-1-phenyl-1H-pyrazol-4-yl]acryloyl]coumarin (coumarin chalcone) (4a-f) (0.003 mole) in glacial acetic acid (15 mL) was added under stirring at room temperature and was poured into ice-cold water (75 mL). The crude solid obtained was extracted with chloroform (3 × 30 mL). The organic layer was washed with 5% sodium bicarbonate solution (3 × 20 mL) and dried over anhydrous sodium sulphate. The removal of chloroform under reduced pressure gave a crude material which was subjected to column chromatography using silica gel and chloroform-ethyl acetate (9:1) as an eluent to give compounds (8a-f) (9a-f) and (10a-f). The compounds were recrystallized from chloroform-hexane.

Yield: 65%, m.p.184-185˚C, IR(KBr, \(v_{\text{max}}\) cm\(^{-1}\)): 1729(C=O stretching of \(\delta\)-lactone of coumarin), 1605 and 1478(aromatic C=C and C=N stretchings), 758 and 691(C-H bending vibrations of mono substituted benzene ring), 3062(aromatic C-H stretching). \(^1\)H NMR(400 MHz, CDCl\(_3\), \(\delta\)): 7.36-8.49 (18H, multiplet, fifteen aromatic protons + two olefinic protons + C\(_3\) proton of pyrazole ring), 9.36 and 9.41(2H, two singlets, C\(_4\) and C\(_4\) protons of coumarin).

5,6-Benz-8''-methoxy-3-[3-{3-(coumarin-3-yl)-1-phenyl-1H-pyrazol-4-yl}acryloyl]coumarin (4f): IR(KBr, \(v_{\text{max}}\) cm\(^{-1}\)): 1705(C=O stretching of \(\delta\)-lactone of coumarin), 1682(\(\alpha,\beta\) unsaturated carbonyl group), 1605 and 1535(aromatic C=C and C=N stretchings), 748(C-H bending vibrations of mono substituted benzene ring), 3063(aromatic C-H stretching). \(^1\)H NMR(400MHz, CDCl\(_3\), \(\delta\)): 4.03(3H, singlet, OCH\(_3\)_), 7.18-8.48(17H, multiplet, fourteen aromatic protons + two olefinic protons + C\(_3\) proton of pyrazole ring), 9.35 and 9.38(2H, two singlets, C\(_4\) and C\(_4\) protons of coumarin).

General procedure for the synthesis of 3-[4'-[3''''-(coumarin-3''''-yl)-1'''-phenyl-1H-pyrazol-4'''-yl]-2',2'''-bipyridin-6'''-yl]-1'''-phenyl-1H-pyrazol-4'''-yl]coumarins (8a-f) (coumarin bipyridyls).}

In a 100-mL round bottom flask equipped with a condenser, guard tube and magnetic needle, an appropriate pyridoyl methyl pyridinium iodide salt (5) or (6) or (7) (0.003 mole) in glacial acetic acid (15 mL) was taken. This ammonium acetate (0.03 mole) was added under stirring at room temperature. Then a solution of an appropriate 3-[3-{3-(coumarin-3-yl)-1-phenyl-1H-pyrazol-4-yl}acryloyl]coumarin (coumarin chalcone) (4a-f) (0.003 mole) in glacial acetic acid (15 mL) was added under stirring at room temperature and the reaction mixture was further stirred for 1 h at room temperature and then refluxed for 8 h at 140˚C. It was then allowed to cool to room temperature and was poured into ice-cold water (75 mL). The crude solid obtained was extracted with chloroform (3 × 30 mL). Water (2 × 20 mL) and dried over anhydrous sodium sulphate. The removal of chloroform under reduced pressure gave a crude material which was subjected to column chromatography using silica gel and chloroform-ethyl acetate (9:1) as an eluent to give compounds (8a-f), (9a-f) and (10a-f). The compounds were recrystallized from chloroform-hexane.
lactone of coumarin), 1612 and 1497 (aromatic C=C and C=N stretchings), 756 and 694 (C-H bending vibrations of mono substituted benzene ring), 2839 (aliphatic C-H stretching), 3063 (aromatic C-H stretching). ¹H-NMR (400 MHz, CDCl₃, δ): 4.01 (3H, singlet, OCH₃), 7.09-7.89 (14H, multiplet, aromatic protons except C_3′′′-H, C_5′'′-H, C_7′′-H, C_8′′′-H, C_9′′′-H, C_9′′′-H, C_10′′′-H), 8.27 (1H, singlet, C_1′′′-H), 8.49-8.65 (4H, multiplet, protons at C_2′′′, C_3′, C_4′′′ and C_5′′′), 8.63 (1H, poorly resolved doublet of doublet, C_6′′′-H), 8.94 (1H, singlet, C_2′-H). ¹³C-NMR (100 MHz, CDCl₃, δ): 56.25 (OCH₃), 113.80 (CH), 116.05 (CH), 117.68 (CH), 118.60 (CH), 119.25 (CH), 119.60 (CH), 120.16 (CH), 120.33 (CH), 121.33 (CH), 121.55 (CH), 122.07 (CH), 122.41 (CH), 123.88 (CH), 124.32 (CH), 124.58 (CH), 125.27 (CH), 127.42 (CH), 128.68 (CH), 129.67 (CH), 131.93 (CH), 133.83 (CH), 136.99 (CH), 139.61 (CH), 140.15 (CH), 142.32 (CH), 142.95 (CH), 143.85 (CH), 146.02 (CH), 146.92 (CH), 150.80 (CH), 154.33 (CH), 155.86 (CH), 158.57 (CO of coumarin), 159.56 (CO of coumarin). Anal. Calcd. for C₉H₈N₂O₃: C, 74.02; H, 3.92; N, 9.09 %. Found: C, 74.10; H, 4.00; N, 9.13 %.

5.6-Benzox-4′-{3′′-(coumarin-3′′′-yl)-1′′-phenyl-1H-pyrazol-4′′-yl[2′′′,2′′′′-bipyridin-6′′′-yl]coumarin (8c): Yield: 72%, m.p.207-209°C, IR (KBr, ν max, cm⁻¹): 1712 (C=O stretching of δ-lactone of coumarin), 1604 and 1474 (aromatic C=C and C=N stretchings), 763 and 686 (C-H bending vibrations of mono substituted benzene ring), 3055 (aromatic C-H stretching). ¹H-NMR (400 MHz, CDCl₃, δ): 7.33-8.01 (17H, multiplet, aromatic protons except C_3′′′-H, C_4′′′'-H, C_5′′′'-H, C_6′-H, C_7′-H, C_8′-H, C_9′-H, C_10′-H), 8.26 (1H, singlet, C_2′-H), 8.47-8.65 (5H, multiplet, protons at C_1′′′, C_1′, C_2′′′, C_3′′′′ and C_4′′′′), 9.75 (1H, singlet, C_2′-H). ¹³C-NMR (100 MHz, CDCl₃, δ): 113.82 (C), 116.27 (CH), 116.59 (CH), 116.70 (CH), 117.15 (CH), 117.77 (CH), 118.45 (CH), 119.31 (CH), 119.51 (CH), 120.78 (CH), 121.24 (CH), 121.73 (C), 121.89 (CH), 121.99 (C), 122.48 (C), 123.82 (CH), 123.97 (C), 124.47 (CH), 126.11 (CH), 127.25 (CH), 127.34 (C), 128.37 (CH), 128.48 (CH), 129.11 (CH), 129.71 (CH), 130.40 (C), 131.96 (CH), 133.48 (CH), 136.93 (CH), 138.13 (CH), 139.60 (CH), 140.09 (C), 143.56 (CH), 149.13 (CH), 151.01 (C), 155.89 (CH), 156.10 (C), 159.69 (CO of coumarin), 160.16 (CO of coumarin). Anal. Calcd. for C₁₉H₁₄N₂O₅: C, 77.08; H, 3.80; N, 8.80 %. Found: C, 77.13; H, 3.89; N, 8.96 %.

8''-Methoxy-3′-4′-3′′-(coumarin-3′′′-yl)-1''-phenyl-1H-pyrazol-4′′-yl[2′′′,2′′′′-bipyridin-6′′′-yl]coumarin (8d): Yield: 67%, m.p.230-231°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), 748 and 678 (C-H bending vibrations of mono substituted benzene ring), 2847 (aliphatic C-H stretching), 3070 (aromatic C-H stretching). ¹H-NMR (400 MHz, CDCl₃, δ): 3.96 (3H, singlet, OCH₃), 7.12-7.89 (14H, multiplet, aromatic protons except C_2′′′-H, C_3′′′′-H, C_5′′′-H, C_7′′-H, C_8′′′-H, C_9′′′-H, C_10′′′-H), 8.26 (1H, singlet, C_1′′′-H), 8.47-8.56 (4H, multiplet, protons at C_2′′′, C_3′, C_5′ and C_6′), 8.63 (1H, poorly resolved doublet of doublet, C_6′′′-H), 8.94 (1H, singlet, C_2′-H). ¹³C-NMR (100 MHz, CDCl₃, δ): 56.34 (OCH₃), 114.22 (CH), 114.82 (C), 116.23 (CH), 118.70 (CH), 119.52 (CH), 120.09 (CH), 121.50 (CH), 122.09 (CH), 123.88 (CH), 124.32 (CH), 124.54 (CH), 126.99 (CH), 127.24 (CH), 127.46 (CH), 129.09 (CH), 129.72 (CH), 130.31 (C), 132.01 (CH), 135.77 (C), 137.12 (C), 138.25 (C), 139.50 (C), 141.07 (CH), 142.30 (CH), 142.70 (CH), 143.71 (C), 145.75 (CH), 146.03 (CH), 148.98 (CH), 150.67 (C), 151.86 (C), 153.87 (C), 154.53 (C), 159.11 (CO of coumarin), 160.11 (CO of coumarin). Anal. Calcd. for C₁₃H₁₅N₂O₅: C, 74.02; H, 3.92; N, 9.09 %. Found: C, 74.16; H, 4.02; N, 9.19 %.
Yield: 68%, m.p.168-170°C, IR(KBr, v_max, cm⁻¹): 1718(C=O stretching of 2,3'-bipyridin-6'-yl)coumarin (9b): Yield: 68%, m.p.168-170°C, IR(KBr, v_max, cm⁻¹): 1718(C=O stretching of 2,3'-bipyridin-6'-yl)coumarin (9b): Yield: 68%, m.p.168-170°C, IR(KBr, v_max, cm⁻¹): 1718(C=O stretching of 2,3'-bipyridin-6'-yl)coumarin (9b): Yield: 68%, m.p.168-170°C, IR(KBr, v_max, cm⁻¹): 1718(C=O stretching of 2,3'-bipyridin-6'-yl)coumarin (9b): Yield: 68%, m.p.168-170°C, IR(KBr, v_max, cm⁻¹): 1718(C=O stretching of 2,3'-bipyridin-6'-yl)coumarin (9b):
N. N. Gohil, D. I. Brahmbhatt: Synthesis, characterization and biological evaluation of some new pyrazolyl bipyridyl... 8'-''-Methoxy-3''-4''-''-(coumarin-3''''-yl)-1''-phenyl-1''-pyrazol-4''-''yl)-2',3''-bipyridin-6''-yl)
coumarin (9d): Yield: 72%, m.p.230-231°C, IR(KBr, ν max, cm⁻¹): 1713(C=O stretching of δ-lactone of coumarin), 1605 and 1458(aromatic C=C and C=N stretchings), 756 and 694(C-H bending vibrations of mono substituted benzene ring), 2977(aliphatic C-H stretching), 3055(aromatic C-H stretching). Anal.Calcd. for C₃₈H₃₈N₄O₆: C, 74.02; H, 3.92; N, 9.09 %. Found: C, 74.16; H, 4.02; N, 9.19 %. 8,8''-Dimethoxy-3''-4''-''-(coumarin-3''''-yl)-1''-phenyl-1''-pyrazol-4''-''yl)-2',3''-bipyridin-6''-yl) coumarin (9e): Yield: 64%, m.p.198-200°C, IR(KBr, ν max, cm⁻¹): 1720(C=O stretching of δ-lactone of coumarin), 1504 and 1478(aromatic C=C and C=N stretchings), 771 and 694(C-H bending vibrations of mono substituted benzene ring), 2962(aliphatic C-H stretching), 3063(aromatic C-H stretching). 1H-NMR(400MHz, CDCl₃, δ): 3.95 and 3.97(6H, two singlets, 2 x OCH₃), 7.09-8.57(18H, multiplet, aromatic protons + C₆''-H except C₆''-H, C₆''-H, C₆''-H and C₆''''-H). Anal.Calcd. for C₃₈H₃₈N₄O₆: C, 75.67; H, 3.93; N, 8.40 %. Found: C, 75.82; H, 4.03; N, 8.52 %. 5,6-Benzo-8'''-methyl-3'''-4'''-''-(coumarin-3''''-yl)-1''-phenyl-1''-pyrazol-4''-''yl)-2',3''- bipyridin-6''-yl)coumarin (9f): Yield: 68%, m.p.255-256°C, IR(KBr, ν max, cm⁻¹): 1728(C=O stretching of δ-lactone of coumarin), 1605 and 1474(aromatic C=C and C=N stretchings), 799 and 686(C-H bending vibrations of mono substituted benzene ring), 2932(aliphatic C-H stretching), 3062(aromatic C-H stretching). 1H-NMR(400MHz, CDCl₃, δ): 3.97(3H, singlet, OCH), 7.13-8.52 (19H, multiplet, aromatic protons + C₆''-H except C₆''-H, C₆''-H, C₆''-H and C₆''''-H). 8.65(1H, poorly resolved doublet, C₆''''-H), 8.71(1H, doublet, poorly resolved doublet of doublet, C₆''''-H), 9.41(1H, poorly resolved doublet, C₆''''-H), 9.83(1H, singlet, C₆''''-H). 13C-APT(100MHz, CDCl₃, δ): 56.32(OCH₃), 115.61(C), 115.98(CH), 116.04(CH), 117.59(C), 118.44(C), 119.06(C), 119.48(C), 120.76(CH), 121.01(CH), 121.31(CH), 122.34(CH), 122.22(CH), 126.51(CH), 128.09(CH), 128.57(CH), 129.84(CH), 129.70(CH), 129.80(CH), 130.09(CH), 130.51(CH), 130.87(C), 131.69(CH), 131.74(C), 137.87(CH), 140.04(CH), 141.77(CH), 143.13(C), 143.90(CH), 144.22(C), 144.53(CH), 145.24(CH), 145.88(CH), 146.70(CH), 148.13(CH), 148.59(C), 152.39(C), 155.63(CH), 161.09(CO of coumarin), 161.52(CO of coumarin). Anal.Calcd. for C₃₈H₃₈N₄O₆: C, 75.67; H, 3.93; N, 8.40 %. Found: C, 75.82; H, 4.03; N, 8.52 %.

3'''-4''''-(coumarin-3''''-yl)-1''-phenyl-1''-pyrazol-4''''-''yl)-2',4''''-bipyridin-6''''-yl) coumarin (10a): Yield: 63%, m.p.184-185°C, IR(KBr, ν max, cm⁻¹): 1713(C=O stretching of δ-lactone of coumarin), 1604 and 1466(aromatic C=C and C=N stretchings), 748 and 686(C-H bending vibrations of mono substituted benzene ring), 3063(aromatic C-H stretching). 1H-NMR(400MHz, CDCl₃, δ): 7.34-7.96(16H, multiplet, aromatic protons except C₆''''-H, C₆''''-H, C₆''''-H, C₆''''-H and C₆''''''''-H). 8.27(1H, singlet, C₆''''''''-H), 8.40(1H, singlet, C₆''''''''-H). 8.59(1H, poorly resolved doublet, C₆''''''''-H), 8.74(2H, doublet, J=6.0Hz, C₆''''''''-H and C₆''''''''-H), 9.89(1H, singlet, C₆''''''''-H). 13C-APT(100MHz, CDCl₃, δ): 107.79(C), 110.30(C), 113.13(C), 115.97(CH), 116.89(CH), 117.11(CH), 118.08(CH), 118.40(CH), 119.53(CH), 120.70(CH), 126.07(CH), 126.30(CH), 126.77(CH), 129.18(CH), 129.42(CH), 130.13(CH), 134.15(CH), 136.45(CH), 138.18(CH), 143.14(CH), 145.28(CH), 147.62(CH), 149.45(CH), 149.94(C), 150.41(C), 153.71(C), 154.08(C), 154.39(C), 159.80(CO of coumarin), 160.03(CO of coumarin). Anal.Calcd. for C₃₈H₃₈N₄O₆: C,
C=C and C=N stretchings), 756 and 694 (C-H bending vibrations of mono substituted benzene ring), 2970 (aliphatic C-H stretching), 153.71 (C), 159.83 (CO of coumarin), 160.28 (CO), 134.04 (CH), 136.07 (C), 138.24 (C), 142.64 (CH), 125.85 (CH), 126.00 (CH), 126.19 (CH), 126.48 (C), 124.88 (CH), 124.99 (CH), 125.36 (CH), 122.35 (CH), 123.43 (CH), 123.98 (CH), 124.13 (CH), 124.88 (CH), 124.99 (CH), 125.36 (CH), 125.85 (CH), 126.00 (CH), 126.19 (CH), 126.48 (CH), 129.20 (CH), 129.94 (CH), 130.39 (CH), 134.04 (CH), 136.07 (C), 138.24 (CH), 142.64 (CH), 153.71 (C), 159.83 (CO of coumarin), 160.28 (CO of coumarin). Anal. Calcd. for C_{38}H_{36}N_{2}O_{6}: C, 74.02; H, 3.92; N, 9.09 %. Found: C, 74.14; H, 4.02; N, 9.18 %.

5,6-Benzodipyrimido[4',3',4,3''-yl]-1''-phenyl-1'H-pyrazol-4''-yl)-2',4''-bipyridin-6'-yl cofumarin (10b): Yield: 66%, m.p. 170-172°C, IR(KBr, v_{max}, cm^{-1}): 1728 (C=O stretching of δ-lactone of coumarin), 1605 and 1504 (aromatic ring), 2970 (aliphatic C-H stretching), 153.71 (C), 159.83 (CO of coumarin), 160.28 (CO), 134.04 (CH), 136.07 (C), 138.24 (C), 142.64 (CH), 125.85 (CH), 126.00 (CH), 126.19 (CH), 126.48 (CH), 129.20 (CH), 129.94 (CH), 130.39 (CH), 134.04 (CH), 136.07 (C), 138.24 (CH), 142.64 (CH), 153.71 (C), 159.83 (CO of coumarin), 160.28 (CO of coumarin). Anal. Calcd. for C_{41}H_{32}N_{8}O_{6}: C, 77.08; H, 3.80; N, 8.80 %. Found: C, 77.13; H, 3.89; N, 8.96 %.

8''-Methoxy-3-[4''-{(coumarin-3''''-yl)-1''''-phenyl-1'H-pyrazol-4'''''-yl]-2',4'''''-bipyridin-6'''''-yl} cofumarin (10d): Yield: 67%, m.p. 230-231°C, IR(KBr, v_{max}, cm^{-1}): 1720 (C=O stretching of δ-lactone of coumarin), 1597 and 1458 (aromatic C=C and C=N stretchings), 748 and 694 (C-H bending vibrations of mono substituted benzene ring), 2970 (aliphatic C-H stretching), 3063 (aromatic C-H stretching).
N. N. Gohil, D. I. Brahmbhatt: Synthesis, characterization and biological evaluation of some new pyrazolyl bipyridyl... of coumarin). Anal.Calcd. for C_{39}H_{26}N_{4}O_{6}: C, 72.44; H, 4.05; N, 8.66 %. Found: C, 72.63; H, 4.19; N, 8.73 %.

5,6-Benzo-8''''-methoxy-3-{4'-[3'''-(coumarin-3''''-yl)-1'''-phenyl-1H-pyrazol-4''''-yl]-2',4''''. bipyridin-6'''-yl}coumarin (10f): Yield: 70%, m.p.255-256˚C, IR(KBr, υ_{max}, cm^{-1}): 1728(C=O stretching of δ-lactone of coumarin), 1604 and 1488 (aromatic C=C and C=N stretchings), 779 and 663(C-H bending vibrations of mono substituted benzene ring), 2977(aliphatic C-H stretching), 3062(aromatic C-H stretching). \textsuperscript{1}H-NMR(400MHz, CDCl\textsubscript{3}, δ): 3.98(3H, singlet, OCH\textsubscript{3}), 7.14-7.96(17H, multiplet, aromatic protons except C\textsubscript{5}'''-H, C\textsubscript{4}'''-H, C\textsubscript{3}''''-H, C\textsubscript{2}'''-H, C\textsubscript{6}'''-H, C\textsubscript{4}'-H), 8.24(1H, singlet, C\textsubscript{5}''''-H), 8.39(1H, singlet, C\textsubscript{3}''''-H), 8.55(1H, doublet, J=1.2 Hz, C\textsubscript{3}'-H), 8.72(2H, doublet, J=5.2 Hz, C\textsubscript{2}''-H and C\textsubscript{6}''-H), 9.60(1H, singlet, C\textsubscript{4}'-H). \textsuperscript{13}C-APT(100MHz, CDCl\textsubscript{3}, δ): 56.38(OCH\textsubscript{3}), 111.93(C), 116.12(C), 117.58(CH), 117.73(C), 119.85(C), 121.09(CH), 121.19(CH), 122.21(C), 123.31(C), 123.06(CH), 123.16(CH), 123.21(CH), 123.26(C), 123.42(CH), 124.00(CH), 124.09(CH), 125.11(CH), 126.25(CH), 126.37(CH), 127.38(CH), 127.44(CH), 129.11(CH), 129.31(CH), 130.40(CH), 131.98(CH), 133.96(CH), 137.03(C), 138.13(C), 139.63(CH), 142.09(C), 143.56(CH), 147.11(C), 149.00(C), 152.89(C), 153.10(C), 159.71(CO of coumarin), 160.19(CO of coumarin). Anal.Calcd. for C_{42}H_{26}N_{4}O_{5}: C, 75.67; H, 3.93; N, 8.40 %. Found: C, 75.82; H, 4.03; N, 8.52 %.

RESULTS AND DISCUSSION

CHEMISTRY

The synthetic pathway adopted to obtain target compounds (8a-f), (9a-f) and (10a-f) which were synthesized by reacting various 3-[3-(3-(coumarin-3-yl)-1-phenyl-1H-pyrazol-4-yl)acyl]coumarins (coumarin chalcones) (4a-f) with appropriate pyridyl methyl pyridinium iodide salt (5-7) in the presence of ammonium acetate in refluxing acetic acid, is shown in Scheme 1. The structures of all synthesized compounds, supported by IR, \textsuperscript{1}H-NMR, \textsuperscript{13}C-APT and representative mass spectral data are shown in the experimental section.
BIOLOGICAL RESULTS

Antimicrobial Activity

The synthesized target compounds (8a-f), (9a-f) and (10a-f) were evaluated for their in vitro antibacterial activity against two Gram positive bacteria Staphylococcus aureus (MTCC 96) and Bacillus subtilis (MTCC 441) and two Gram negative bacteria Escherichia coli (MTCC 443) and Salmonella typhi (MTCC 98). They were also evaluated for their in vitro antifungal activity against Candida albicans (MTCC 227) and Aspergillus niger (MTCC 282) as fungal strains. Broth dilution method was used for the determination of the antibacterial and antifungal activity as recommended by NCCLS [30]. Ampicillin, chloramphenicol, ciprofloxacin, norfloxacin and gentamycin were used as standard antibacterial drugs whereas griseofulvin and nystatin were used as standard antifungal drugs. All MTCC cultures were collected from the Institute of Microbial Technology, Chandigarh and tested against the above mentioned 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 Units) per millilitre by comparing the turbidity. Each synthesized compound was diluted with DMSO so as to have a stock solution of 2000 μg/mL concentration. The results were recorded in the form of primary and secondary screening. The synthesized compounds (8a-f), (9a-f) and (10a-f) 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
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primary screening were further screened in a second set of dilutions at concentrations of 200, 100, 62.5, 50 and 25 µg/mL. The suspension of 10 µL from each well was further incubated and growth was noted at 37°C after 24 h for bacteria and 48 h 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.

**Antimicrobial Results**

The final compounds (8a-f), (9a-f) and (10a-f) were screened for their in vitro antibacterial and antifungal evaluation against various bacterial and fungal pathogens by the broth dilution method. Ampicillin, chloramphenicol, norfloxacin, ciprofloxacin, gentamycin, griseofulvin and nystatin were used as standard drugs. The values of MIC are summarized in Table 1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Minimum Inhibitory Concentration (MIC, µg/mL)</th>
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<tbody>
<tr>
<td></td>
<td>Gram positive bacteria</td>
</tr>
<tr>
<td>8a</td>
<td>500</td>
</tr>
<tr>
<td>8b</td>
<td>500</td>
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<tr>
<td>8c</td>
<td>250</td>
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<tr>
<td>8f</td>
<td>100</td>
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<tr>
<td>9a</td>
<td>200</td>
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<tr>
<td>9b</td>
<td>500</td>
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<tr>
<td>9c</td>
<td>100</td>
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<tr>
<td>9d</td>
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<td>100</td>
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<tr>
<td>10b</td>
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<td>10c</td>
<td>250</td>
</tr>
<tr>
<td>10d</td>
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<tr>
<td>Chloramphenicol</td>
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<td>Ciprofloxacin</td>
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<td>Norfloxacin</td>
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<tr>
<td>Griseofulvin</td>
<td>-</td>
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<tr>
<td>Nystatin</td>
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</table>

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

The assessment of antimicrobial screening data reveals that compounds 8f, 9c, 9f, 10b and 10e (MIC=100 µg/mL) exerted excellent inhibitory activity against Gram positive bacteria Bacillus subtilis as compared to standard drug ampicillin (MIC=250 µg/mL) and equal activity to norfloxacin (MIC=100 µg/mL). Compound 9e (MIC=125
Fellowship in the form of UGC BSR Research.

Negative bacteria (MIC=100µg/mL) were found to be equipotent to pathogens as compared to standard drugs. These compounds were screened for their activity against several bacterial and fungal pathogens, and compounds 8f (MIC=500µg/mL) were found comparable to griseofulvin (MIC=500µg/mL) against Staphylococcus aureus. Compounds 9a, 9f, 10a, 10b and 10f (MIC=250µg/mL) were found to be equipotent to ampicillin (MIC=250µg/mL) against Staphylococcus aureus. Compounds 8f (MIC=62.5µg/mL) showed excellent activity compared to ampicillin (MIC=250µg/mL) against Gram negative bacteria. None of the tested compounds showed better activity against Salmonella typhi. Compounds 8e, 8f, 9a, 9d, 9f and 10e (MIC=250µg/mL) were found to be more active than griseofulvin (MIC=500µg/mL) whereas, compounds 9b, 10a, 10b and 10c (MIC=100µg/mL) were found to be equipotent to ampicillin (MIC=100µg/mL) against Gram negative bacteria Salmonella typhi. Compounds 8e, 8f, 9a, 9d, 9f and 10e (MIC=250µg/mL) were found to be more active than griseofulvin (MIC=500µg/mL) against Candida albicans. None of the tested compounds showed better activity against Aspergillus Niger than standard drugs. Upon examining the antimicrobial data, it is apparent that some of the compounds exhibit good or equal potency to standard drugs against Gram positive bacterial strains.

CONCLUSION

In summary, we have synthesized pyrazolyl-bipyridyl-substituted dicoumarin derivatives and screened for their in vitro antimicrobial evaluation. The present synthetic compounds have the potential to exhibit antimicrobial activity. In particular, they have shown promising antibacterial and antifungal activity against several bacterial and fungal pathogens as compared to standard drugs. These compounds can be considered as lead molecules for future investigations.

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