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Synthesis, characterization and antimicrobial evaluation of some new styryl bipyridinyl substituted coumarins

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ABSTRACT

*The synthesis of various 3-[4-styryl-(2,2'-bipyridin)-6-yl]coumarins **(7a-f);** 3-[4-styryl-(2,3'-bipyridin)-6-yl] coumarins **(8a-f)** and 3-[4-styryl-(2,4'-bipyridin)-6-yl]coumarins **(9a-f)** have been carried out by the reaction of appropriate 3-(5-arylpenta-2,4-dienoyl)coumarins **(3a-f)** with 2-pyridoyl methyl pyridinium iodide salt **(4),** 3-pyridoyl methyl pyridinium iodide salt **(5)** and 4-pyridoyl methyl pyridinium iodide salt **(6)** respectively in the presence of ammonium acetate in refluxing acetic acid. The synthesized compounds were fully characterized by IR, ^{1}H-NMR, ^{13}C-APT and Mass spectral data. The synthesized compounds were screened for in vitro antimicrobial activity using Broth micro dilution method.*

Keywords: Bipyridine, styryl bipyridine, Krohnke reaction, antimicrobial activity, Broth dilution method.

INTRODUCTION

Coumarins are important oxygen heterocycles and are known for their varied biological properties such as antitumor [1], anti-inflammatory [2] and antibacterial [3] activities. Coumarin derivatives especially pyridyl coumarins have been reported to have important biological activities like CNS depressant [4], antifungal [5], moth proofing activity [6], fish toxicity [7], MAO inhibitor [8], antibacterial agents [9] and antitubercular [10]. During our literature survey we came across some bipyridines derivatives which have been reported to possess wide applications in the field of bioinorganic chemistry [11], supramolecular chemistry [12] and polymeric material [13]. Bipyridine derivatives exhibit wide range of physiological activities such as anticancer [14], cardiotonic [15], DNA binding properties [16] and antibacterial properties [17]. Similarly, certain styryl pyridine derivatives are also reported to have varied biological activities like anthelmentics [18], antimicrobial[19], plant growth regulators[20], inhibitors of choline acetyl transferase[21] (CNS depressant) and have interesting applications like liquid crystals[22], biosensors[23], activators in detection of leukocytes in body fluids[24]. In view of the aforementioned properties of coumarins, bipyridines and styryl pyridine moieties, it was thought worthwhile to synthesize a hybrid molecule incorporating all these moieties in a single scaffold so that one can expect better biological properties. Keeping this objective in mind and in continuation of our work on synthesizing newer heterocyclic substituted coumarins[25], here with we report the synthesis of some styryl bipyridine substituted coumarins using a *Krohnke's* reaction.

MATERIALS AND METHODS

All the melting points are uncorrected. All reactions were performed with commercially available reagents and they were used without further purification. Organic solvents were purified by standard methods and stored over molecular sieves. All the IR spectra (KBr disc) were recorded on Shimadzu FT-IR 8400-S spectrometer. ^{1}H-NMR and ^{13}C APT spectra were recorded on Bruker Advance 400 spectrometer operating at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-APT. The chemical shift (δ) is reported in ppm using chloroform-d as a solvent and calibrated standard solvent signal. Mass spectra were recorded on Shimadzu QP 2010 spectrometer. Elemental analysis was carried out on Perkin- Elmer 2400 C-H-N-S-O Analyzer Series-II. Column chromatography was performed with

silica gel 60–120 mesh (Merck, Mumbai, India.). All the compounds were routinely checked for completion of the reaction on silica gel 60 F254 TLC plates and their spots were visualized by exposure to a UV lamp, iodine vapour or $KMnO_4$ reagents.

Starting precursors 3-acetyl coumarins **1(a-c)** [27] and pyridoyl methyl pyridinium iodide salts **4**, **5** and **6** [28] were prepared using the reported procedures.

3. 1. General procedure for the synthesis of 3-(5-arylpenta-2,4-dienoyl)coumarins (3a-f)

In a 100 mL round bottom flask, an appropriate 3-acetyl coumarins (0.01 mol) and appropriate cinnamaldehyde (0.01 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 reaction mixture was then refluxed on water bath for 4 hours. It was then allowed to cool to room temperature. A solid product separated out was filtered off, washed with cold ethanol and dried. It was recrystallized from ethanol.

Compound 3a: R=R_1=R_2=H, Yield: 52%; Mp: 182-184°C (lit.[29] mp 184˚C)
Compound 3b: R = OCH_3, R_1 = R2 = H, Yield = 85%; mp 208-210°C (lit.[30] mp 184˚C)
Compound 3c: R=R_2=H, R_1=Br Yield 54%; mp: 235-236°C (lit.[29] mp 237˚C)
Compound 3d: R=R_1=H, R_2= OCH_3 Yield = 82%; mp 198-201°C (lit.[30] mp 199-201˚C)
Compound 3e: R=R_2= OCH_3, R_1= H Yield = 86%; mp 220°C (lit.[30] mp 217-219˚C)

Compound 3f: R = H, R_1 = Br, R_2 = OCH_3;		
Yield = 82% mp 199-201°C		Molecular Formula: $C_{21}H_{15}BrO_4$
Analysis	% C	% H
Found	61.29	3.65
Calculated	61.33	3.68
IR (cm^{-1})	ν_{max} 1720 (C=O stretching of δ-lactone of coumarin), 1627 (α,β unsaturated carbonyl group), 1595 (aromatic C=C), 740 (C-H bending vibrations of *o*-disubstituted benzene ring), 2933 (aliphatic C-H stretching), 3064 (aromatic C-H stretching),	
^{1}H-NMR (δ, ppm) ($CDCl_3$)	3.94 (3H, singlet, OCH_3), 7.40-8.16 (12H, multiplet, eight Ar-H + four olefinic protons), 8.56 (1H, singlet, C_4-H of coumarin)	

2. 2.General procedure for the synthesis of 3-[4-styryl-(2,2'-bipyridin)-6-yl]coumarins (7a-f), 3-[4-styryl-(2,3'-bipyridin)-6-yl]coumarins (8a-f) and 3-[4-styryl-(2,4'-bipyridin)-6-yl]coumarins (9a-f).

In a 100 mL round bottom flask equipped with a condenser, guard tube and magnetic needle, an appropriate pyridoyl methyl pyridinium iodide salt **4** or **5** or **6** (0.003 mole) in glacial acetic acid (15mL) was taken. To this ammonium acetate (0.03 mole) was added with stirring at room temperature. Then a solution of an appropriate 3-(5-arylpenta-2,4-dienoyl)coumarin **3a-f** (0.003 mole) in glacial acetic acid (15 mL) was added with stirring at room temperature and 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 sulphate. The removal of chloroform under reduced pressure gave crude material which was subjected to column chromatography using silica gel and chloroform-petroleum ether (60-80) (1:4) as an eluent to give compounds **7a-f, 8a-f and 9a-f**. The compounds were recrystallized from chloroform-hexane.

The structure of all the eighteen synthesized **(7a-f)**, **(8a-f), (9a-f)**compounds were confirmed by their ^{1}H-NMR, ^{13}C-NMR, IR, elemental analysis and representative mass spectral data given below.

3-[4-Styryl-(2,2'-bipyridin)-6-yl]coumarin (7a): White solid; yield = 68% ; mp 205-209°C; Anal. Calcd. For $C_{27}H_{18}N_2O_2$: C, 80.58; H, 4.51; N, 6.96%. Found: C, 80.56; H, 4.50; N, 6.95%. IR (KBr, ν_{max}, cm^{-1}); 1715 (C=O stretching of δ-lactone of coumarin), 1581 (aromatic C=C stretching), 1477 (aromatic C=N stretching), 3023 (aromatic C-H stretching), 690 and 735 (C-H out of plane bending vibrations for mono substituted benzene ring), ^{1}H NMR (400MHz, $CDCl_3$, δ): 7.23-8.61 (16H, multiplet, aromatic protons except $C_{6''}$-H and C_4-H), 8.78 (1H, doublet of doublet, J= 0.8 Hz and J= 4.8 Hz, $C_{6''}$-H), 8.97 (1H, singlet, C_4-H). ^{13}C NMR (100MHz, $CDCl_3$, δ) : 114.53(CH), 116.44(CH), 117.42(CH), 118.91(C), 120.43(CH), 121.27(C), 123.61(CH), 124.73(CH), 126.04(CH), 126.44(C), 127.20(CH), 128.92(CH), 129.07(CH), 132.44(CH), 133.91(CH), 134.41(CH), 134.67(C), 136.16(C), 142.97(CH), 146.79(C), 148.51(CH), 150.07(CH), 151.71(C), 154.67(C), 159.94 (CO of coumarin). The mass spectrum of compound showed M^+ peak at 402(18%) (m/z%) along with some other fragments peaks at 257(23%), 77(12%), 57(11%), 44(100%) etc. The appearance of molecular ion peak at 402 mass unit supports the structure of compound 7a.

8-Methoxy-3-[4-styryl-(2,2'-bipyridin)-6-yl]coumarin (7b): White solid; yield = 75% ; mp 193-196°C; Anal. Calcd. For $C_{28}H_{20}N2O_3$: C, 77.76; H, 4.66; N, 6.48%. Found: C, 77.75; H, 4.65; N, 6.46%. IR (KBr, ν_{max}, cm^{-1});

1719 (C=O stretching of δ-lactone of coumarin), 1586 and 1470 (aromatic C=C and C=N stretchings), 3028 (aromatic C-H stretching), 742 and 693 (C-H bending vibrations of mono substituted benzene ring), 2932 (aliphatic C-H stretching). ^{1}H NMR (400MHz, $CDCl_3$, δ): 4.04 (3H, singlet, OCH_3), 7.15-8.63 (15H, multiplet, aromatic protons except $C_{6''}$-H and C_4-H), 8.77(1H, poorly resolved doublet of doublet, $C_{6''}$-H), 8.97 (1H, singlet, C_4-H). ^{13}C NMR (100MHz, $CDCl_3$, δ) : 56.28(OCH_3), 113.82(CH), 117.46(CH), 120.24(C), 120.30(CH), 121.29(CH), 121.75(CH), 123.90(CH), 124.39(CH), 125.45(C), 126.44(CH), 127.14(CH), 128.63(CH), 128.83(CH), 133.51(CH), 136.40(C), 136.90(CH), 142.68(CH), 146.52(C), 146.94(C), 149.16(CH), 150.89(C), 155.79(C), 156.79(C), 159.81 (CO of coumarin).

6-bromo-3-[4-styryl-(2,2'-bipyridin)-6-yl]coumarin (7c): White solid; yield = 65% ; mp 196-199°C; Anal. Calcd. For $C_{27}H_{17}BrN_2O_2$: C, 67.37; H, 3.56; N, 5.82%. Found: C, 67.37; H, 3.55; N, 5.80%. IR (KBr, ν_{max}, cm^{-1}); 1723 (C=O stretching of δ-lactone of coumarin), 1579 and 1468 (aromatic C=C and C=N stretchings), 3060 (aromatic C-H stretching), 750 and 688 (C-H bending vibrations of mono substituted benzene ring). ^{1}H NMR (400MHz, $CDCl_3$, δ): 6.94-8.59 (15H, multiplet, aromatic protons except $C_{6''}$-H and C_4-H), 8.77 (1H, doublet of doublet, J= 0.8 Hz and J= 4.8 Hz, $C_{6''}$-H), 8.93 (1H, singlet, C_4-H).^{13}C NMR (100MHz, $CDCl_3$, δ) : 113.81(CH), 117.46(CH), 120.24(C), 120.30(CH), 121.29(CH), 121.75(CH), 123.90(CH), 124.39(CH), 125.45(C), 126.45(CH), 127.14(CH), 128.63(CH), 128.83(CH), 133.51(CH), 136.40(C), 136.90(CH), 142.68(CH), 146.51(C), 146.94(C), 149.16(CH), 150.89(C), 155.79(C), 156.06(C), 157.46(C),159.82(CO of coumarin).

3-[4-(2-Methoxystyryl)-(2,2'-bipyridin)-6-yl]coumarin (7d): White solid; yield = 67% ; mp 209-212°C; Anal. Calcd. For $C_{28}H_{20}N_2O_3$: C, 77.76; H, 4.66; N, 6.48%. Found: C, 77.74; H, 4.65; N, 6.46%. IR (KBr, ν_{max}, cm^{-1}); 1727 (C=O stretching of δ-lactone of coumarin), 1592 and 1472 (aromatic C=C and C=N stretchings), 3068 (aromatic C-H stretching), 2928 (aliphatic C-H stretching). ^{1}H NMR (400MHz, $CDCl_3$, δ): 3.95 (3H, singlet, OCH_3), 6.95-8.61 (15H, multiplet, aromatic protons except $C_{6''}$-H and C_4-H), 8.78(1H, poorly resolved doublet of doublet, $C_{6''}$-H), 8.97 (1H, singlet, C_4-H). ^{13}C NMR (100MHz, $CDCl_3$, δ) : 56.31(OCH_3), 113.93(CH), 114.01(CH), 117.27(CH), 119.41(C), 120.34(CH), 120.57(CH), 123.44(CH), 123.56(CH), 124.51(CH), 125.14(C), 126.10(CH), 127.90(CH), 128.66(CH), 128.75(CH), 133.86(CH), 134.37(CH), 134.84(C), 136.13(C), 142.80(C), 143.06(CH), 146.69(C), 146.95(C), 148.53(CH), 150.04(CH), 151.67(C), 154.60(C), 159.82 (CO of coumarin).

8-Methoxy-3-[4-(2-methoxystyryl)-(2,2'-bipyridin)-6-yl]coumarin (7e): White solid; yield = 74% ; mp 122-125°C; Anal. Calcd. For $C_{29}H_{22}N_2O_4$: C, 75.31; H, 4.79; N, 6.06%. Found: C, 75.30; H, 4.77; N, 6.04%. IR (KBr, ν_{max}, cm^{-1}); 1736 (C=O stretching of δ-lactone of coumarin), 1582 and 1470 (aromatic C=C and C=N stretchings), 3062 (aromatic C-H stretching), 2930 (aliphatic C-H stretching). ^{1}H NMR (400MHz, $CDCl_3$, δ): 3.95(3H, singlet, OCH_3), 4.03 (3H, singlet, OCH_3), 6.94-8.59 (14H, multiplet, aromatic protons except $C_{6''}$-H and C_4-H), 8.77(1H, poorly resolved doublet of doublet, $C_{6''}$-H), 8.93 (1H, singlet, C_4-H).^{13}C NMR (100MHz, $CDCl_3$, δ) : 55.64(OCH_3), 56.47(OCH_3), 111.49(CH), 116.47(CH), 119.62(CH), 120.71(CH), 121.22(CH), 121.53(C), 122.92(CH), 123.79(CH), 124.62(CH), 125.61(C), 127.31(CH), 128.12(CH), 128.57(C), 128.87(CH), 129.58(CH), 132.18(CH), 136.86(CH), 139.49(C), 141.96(C), 142.65(CH), 149.31(CH), 151.03(C), 154.03(C), 154.87(C), 155.92(C), 156.02(C), 160.20(CO of coumarin).

6-Bromo-3-[4-(2-methoxystyryl)-(2,2'-bipyridin)-6-yl]coumarin (7f): White solid; yield = 70% ; mp 229-232°C; Anal. Calcd. For $C_{28}H_{19}BrN_2O_3$: C, 65.76; H, 3.75; N, 5.48%. Found: C, 65.74; H, 3.73; N, 5.47%. IR (KBr, ν_{max}, cm^{-1}); 1733 (C=O stretching of δ-lactone of coumarin), 1595 and 1479 (aromatic C=C and C=N stretchings), 3058 (aromatic C-H stretching), 2934 (aliphatic C-H stretching). ^{1}H NMR (400MHz, $CDCl_3$, δ): 3.95 (3H, singlet, OCH_3), 6.95-8.59 (14H, multiplet, aromatic protons except $C_{6''}$-H and C_4-H), 8.77(1H, doublet of doublet J= 0.8Hz and J= 4.8Hz, $C_{6''}$-H), 8.89 (1H, singlet, C_4-H). ^{13}C NMR (100MHz, $CDCl_3$, δ) : 56.32(OCH_3), 111.45(CH), 114.10(CH), 119.66(CH), 120.14(C), 120.43(CH), 121.31(C), 122.60(CH), 123.20(CH), 123.56(CH), 124.54(CH), 124.92(CH), 125.21(C), 127.48(CH), 128.06(CH), 128.41(C), 129.62(CH), 134.24(CH), 134.60(C), 139.40(C), 142.02(C), 143.17(CH), 146.99(C), 148.55(CH), 150.12(CH), 151.73(C), 154.35(C), 159.73 (CO of coumarin).

3-[4-Styryl-(2,3'-bipyridin)-6-yl]coumarin (8a): White solid; yield = 72% ; mp 152-155°C; Anal. Calcd. For $C_{27}H_{18}N_2O_2$: C, 80.58; H, 4.51; N, 6.96%. Found: C, 80.57; H, 4.50; N, 6.94%. IR (KBr, ν_{max}, cm^{-1}); 1730 (C=O stretching of δ-lactone of coumarin), 1591 and 1475 (aromatic C=C and C=N stretchings), 3056 (aromatic C-H stretching), 745 and 683 (C-H bending vibrations of mono substituted benzene ring). ^{1}H NMR (400MHz, $CDCl_3$, δ): 7.20-8.47 (14H, multiplet, aromatic protons except C_5'-H, C_6''-H, C_4-H, C_2''-H), 8.65 (1H, doublet, J=0.8Hz, C_5'-H), 8.74 (1H, poorly resolved doublet of doublet, C_6''-H), 9.02 (1H, singlet, C_4-H), 9.41 (1H, poorly resolved doublet, C_2''-H).^{13}C NMR (100MHz, $CDCl_3$, δ) : 114.50(CH), 116.44(CH), 117.42(CH), 118.91(C), 120.43(CH), 121.27(C), 123.61(CH), 124.73(CH), 126.07(CH), 126.44(C), 127.20(CH), 128.92(CH), 129.04(CH),

132.36(CH), 133.91(CH), 134.41(CH), 134.67(C), 136.16(C) 142.97(CH), 146.71(C), 148.53(CH), 150.07(CH), 151.71(C), 154.67(C), 159.84(CO of coumarin).

8-Methoxy-3-[4-styryl-(2,3'-bipyridin)-6-yl]coumarin (8b): White solid; yield = 74% ; mp 196-199°C; Anal. Calcd. For $C_{28}H_{20}N_2O_3$: C, 77.76; H, 4.66; N, 6.48%. Found: C, 77.73; H, 4.65; N, 6.46%. IR (KBr, ν_{max}, cm^{-1}); 1717 (C=O stretching of δ-lactone of coumarin), 1592 and 1463 (aromatic C=C and C=N stretchings), 3027 (aromatic C-H stretching), 738 and 694 (C-H bending vibrations of mono substituted benzene ring), 2945 (aliphatic C-H stretching). ^{1}H NMR (400MHz, $CDCl_3$, δ) : 4.04(3H, singlet, OCH_3), 7.15-8.46 (13H, multiplet, aromatic protons except $C_{5'}$-H, $C_{6''}$-H, C_4-H and $C_{2''}$-H), 8.66(1H, doublet, J= 1.2Hz, $C_{5'}$-H), 8.73(1H, doublet of doublet J= 1.6Hz and J= 4.8Hz, $C_{6''}$-H), 9.00 (1H, singlet, C_4-H), 9.40 (1H, poorly resolved doublet, $C_{2''}$-H).^{13}C NMR (100MHz, $CDCl_3$, δ) : 56.30(OCH_3), 113.95(CH), 117.27(CH), 120.17(C), 120.40(CH), 120.58(CH), 123.59(CH), 124.53(CH), 125.12(C), 126.08(CH), 127.20(CH), 128.76(CH), 128.90(CH), 133.84(CH), 134.39(CH), 134.83(C), 136.11(C), 143.09(CH), 146.67(C), 146.92(C), 148.52(CH), 150.05(CH), 151.65(C), 154.58(C), 159.84(CO of coumarin).

6-Bromo-3-[4-styryl-(2,3'-bipyridin)-6-yl]coumarin (8c): White solid; yield = 64% ; mp 204-206°C; Anal. Calcd. For $C_{27}H_{17}BrN_2O_2$: C, 67.37; H, 3.56; N, 5.82%. Found: C, 67.35; H, 3.55; N, 5.81%. IR (KBr, ν_{max}, cm^{-1}); 1726 (C=O stretching of δ-lactone of coumarin), 1589 and 1424 (aromatic C=C and C=N stretchings), 3027 (aromatic C-H stretching), 749 and 690 (C-H bending vibrations of mono substituted benzene ring). ^{1}H NMR (400MHz, $CDCl_3$, δ): 7.20-8.46 (13H, multiplet, aromatic protons except $C_{5'}$-H, $C_{6''}$-H, C_4-H and $C_{2''}$-H), 8.63(1H, doublet, J= 1.2Hz, $C_{5'}$-H), 8.74(1H, doublet of doublet J= 1.6Hz and J= 4.8Hz, $C_{6''}$-H), 8.94 (1H, singlet, C_4-H), 9.38 (1H, doublet, J= 2.0Hz, $C_{2''}$-H).^{13}C NMR (100MHz, $CDCl_3$, δ) : 117.24(C), 117.66(CH), 118.12(CH), 120.47(CH), 121.06(C), 123.59(CH), 125.92(CH), 126.08(C), 127.21(CH), 128.91(CH), 128.97(CH), 131.13(CH), 134.04(CH), 134.37(CH), 134.67(C), 134.97(CH), 136.04(C), 141.37(CH), 146.80(C), 148.44(CH), 150.18(CH), 151.12(C), 152.75(C), 154.76(C), 159.71(CO of coumarin).

3-[4-(2-Methoxystyryl)-(2,3'-bipyridin)-6-yl]coumarin (8d): White solid; yield = 68% ; mp 192-196°C; Anal. Calcd. For $C_{28}H_{20}N_2O_3$: C, 77.76; H, 4.66; N, 6.48%. Found: C, 77.73; H, 4.64; N, 6.48%. IR (KBr, ν_{max}, cm^{-1}); 1714 (C=O stretching of δ-lactone of coumarin), 1596 and 1468 (aromatic C=C and C=N stretchings), 3062 (aromatic C-H stretching), 2934 (aliphatic C-H stretching). ^{1}H NMR (400MHz, $CDCl_3$, δ): 3.96(3H, singlet, OCH_3), 6.96-8.46 (13H, multiplet, aromatic protons except $C_{5'}$-H, $C_{6''}$-H, C_4-H and $C_{2''}$-H), 8.61(1H, poorly resolved doublet, $C_{5'}$-H), 8.73(1H, poorly resolved doublet of doublet, $C_{6''}$-H), 9.00 (1H, singlet, C_4-H), 9.41 (1H, poorly resolved doublet, $C_{2''}$-H). ^{13}C NMR (100MHz, $CDCl_3$, δ) : 55.55(OCH_3), 111.04(CH), 116.42(CH), 117.17(CH), 119.00(C), 120.85(CH), 120.89(CH), 122.26(C), 123.59(CH), 124.69(CH), 125.45(C), 126.53(CH), 127.32(CH), 128.94(CH), 129.00(CH), 130.04(CH), 132.28(CH), 134.46(CH), 134.83(C), 136.16(C), 142.87(CH), 146.74(C), 148.55(CH), 149.97(CH), 150.50(C), 151.36(C), 154.36(C), 159.72 (CO of coumarin).

8-Methoxy-3-[4-(2-methoxystyryl)-(2,3'-bipyridin)-6-yl]coumarin (8e): White solid; yield = 76% ; mp 199-201°C; Anal. Calcd. For $C_{29}H_{22}N_2O_4$: C, 75.31; H, 4.79; N, 6.06%. Found: C, 75.30; H, 4.78; N, 6.04%. IR (KBr, ν_{max}, cm^{-1}); 1718 (C=O stretching of δ-lactone of coumarin), 1590 and 1470 (aromatic C=C and C=N stretchings), 3058 (aromatic C-H stretching), 2936 (aliphatic C-H stretching). ^{1}H NMR (400MHz, $CDCl_3$, δ): 3.96(3H, singlet, OCH_3), 4.04 (3H, singlet, OCH_3), 6.96-8.47 (12H, multiplet, aromatic protons except $C_{5'}$-H, $C_{6''}$-H, C_4-H and $C_{2''}$-H), 8.61(1H, poorly resolved doublet, $C_{5'}$-H), 8.73(1H, poorly resolved doublet of doublet, $C_{6''}$-H), 8.98 (1H, singlet, C_4-H), 9.41 (1H, doublet J= 1.6Hz, $C_{2''}$-H).^{13}C NMR (100MHz, $CDCl_3$, δ) : 55.54(OCH_3), 56.31(OCH_3), 111.03(CH), 113.92(CH), 117.02(CH), 120.21(C), 120.38(CH), 120.85(CH), 121.06(CH), 123.56(CH), 124.49(CH), 125.14(C), 125.34(C), 126.55(CH), 127.31(CH), 128.88(CH), 130.00(CH), 134.42(CH), 134.99(C), 142.99(CH), 146.93(C), 147.38(C), 148.57(CH), 149.98(CH), 151.64(C), 154.48(C), 157.45(C), 159.82(CO of coumarin).

6-Bromo-3-[4-(2-methoxystyryl)-(2,3'-bipyridin)-6-yl]coumarin (8f): White solid; yield = 72% ; mp 162-167°C; Anal. Calcd. For $C_{28}H_{19}BrN_2O_3$: C, 65.76; H, 3.75; N, 5.48%. Found: C, 65.75; H, 3.73 N, 5.46%.IR (KBr, ν_{max}, cm^{-1}); 1722 (C=O stretching of δ-lactone of coumarin), 1597 and 1474 (aromatic C=C and C=N stretchings), 3053 (aromatic C-H stretching), 2925 (aliphatic C-H stretching). ^{1}H NMR (400MHz, $CDCl_3$, δ) : 3.96(3H, singlet, OCH_3), 7.23-8.50 (12H, multiplet, aromatic protons except $C_{5'}$-H, $C_{6''}$-H, C_4-H and $C_{2''}$-H), 8.59(1H, doublet, J=0.8Hz, $C_{5'}$-H), 8.74(1H, poorly resolved doublet of doublet, $C_{6''}$-H), 8.91 (1H, singlet, C_4-H), 9.39 (1H, poorly resolved doublet, $C_{2''}$-H).^{13}C NMR (100MHz, $CDCl_3$, δ) : 55.53(OCH_3), 111.64(CH), 116.47(CH), 119.62(CH), 120.71(CH), 121.22(CH), 121.53(C), 122.92(CH), 123.79(CH), 124.62(CH), 125.61(C), 127.31(CH), 128.12(CH), 128.57(C), 128.87(CH), 129.58(CH), 132.18(CH), 136.86(CH), 139.49(C), 141.96(C), 142.62(CH), 149.31(CH), 151.03(C), 154.03(C), 154.87(C), 155.92(C), 156.02(C), 160.20 (CO of coumarin).

3-[4-Styryl-(2,4'-bipyridin)-6-yl]coumarin (9a): White solid; yield = 62% ; mp 208-211°C; Anal. Calcd. For $C_{27}H_{18}N_2O_2$: C, 80.58; H, 4.51; N, 6.96%. Found: C, 80.57; H, 4.50; N, 6.94%. IR (KBr, ν_{max}, cm^{-1}); 1729 (C=O stretching of δ-lactone of coumarin), 1587 and 1452 (aromatic C=C and C=N stretchings), 3029 (aromatic C-H stretching), 757 and 688 (C-H bending vibrations of mono substituted benzene ring).^{1}H NMR (400MHz, $CDCl_3$, δ): 7.13-8.06 (14H, multiplet, aromatic protons except $C_{5'}$-H, $C_{2''}$-H, $C_{6''}$-H and C_4-H), 8.66(1H, poorly resolved doublet, $C_{5'}$-H), 8.81(2H, poorly resolved doublet, $C_{2''}$-H and $C_{6''}$-H), 9.04 (1H, singlet, C_4-H).^{13}C NMR (100MHz, $CDCl_3$, δ) : 116.43(CH), 117.55(CH), 119.47(C), 121.32(CH), 124.72(CH), 124.82(C), 125.86(CH), 127.20(CH), 128.71(CH), 128.92(CH), 128.98(CH), 129.05(CH), 132.42(CH), 133.99(CH), 136.00(C), 142.97(CH), 146.38(C), 146.74(C), 150.43(CH), 151.73(C), 153.97(C), 154.36(C), 160.37 (CO of coumarin).

8-Methoxy-3-[4-styryl-(2,4'-bipyridin)-6-yl]coumarin (9b): White solid; yield = 76% ; mp 191-193°C; Anal. Calcd. For $C_{28}H_{20}N_2O_3$: C, 77.76; H, 4.66; N, 6.48%. Found: C, 77.74; H, 4.65; N, 6.46%. IR (KBr, ν_{max}, cm^{-1}); 1724 (C=O stretching of δ-lactone of coumarin), 1594 and 1456 (aromatic C=C and C=N stretchings), 3046 (aromatic C-H stretching), 748 and 687 (C-H bending vibrations of mono substituted benzene ring), 2932 (aliphatic C-H stretching). ^{1}H NMR (400MHz, $CDCl_3$, δ): 4.05(3H, singlet, OCH_3), 7.16-8.08 (13H, multiplet, aromatic protons except $C_{5'}$-H, $C_{2''}$-H, $C_{6''}$-H and C_4-H), 8.69(1H, poorly resolved doublet, $C_{5'}$-H), 8.82(2H, doublet, J=4.8Hz, $C_{2''}$-H and $C_{6''}$-H), 8.99 (1H, singlet, C_4-H).^{13}C NMR (100MHz, $CDCl_3$, δ): 56.36(OCH_3), 114.09(CH), 117.47(CH), 120.41(C), 120.99(CH), 121.27(C), 121.54(CH), 122.08(CH), 124.61(CH), 125.10(C), 126.01(CH), 127.27(CH), 128.72(CH), 129.03(CH), 134.04(CH), 136.16(C), 143.21(CH), 146.50(C), 146.82(C), 147.04(C), 150.50(CH), 151.81(C), 154.44(C), 159.84 (CO of coumarin).

6-Bromo-3-[4-styryl-(2,4'-bipyridin)-6-yl]coumarin (9c): White solid; yield = 70% ; mp 263-266°C; Anal. Calcd. For $C_{27}H_{17}BrN_2O_2$: C, 67.37; H, 3.56; N, 5.82%. Found: C, 67.35; H, 3.55; N, 5.80%. IR (KBr, ν_{max}, cm^{-1}); 1718 (C=O stretching of δ-lactone of coumarin), 1589 and 1460 (aromatic C=C and C=N stretchings), 3058 (aromatic C-H stretching), 748 and 698 (C-H bending vibrations of mono substituted benzene ring). ^{1}H NMR (400MHz, $CDCl_3$, δ): 7.20-8.05 (13H, multiplet, aromatic protons except $C_{5'}$-H, $C_{2''}$-H, $C_{6''}$-H and C_4-H), 8.67(1H, doublet, J= 1.2Hz, $C_{5'}$-H), 8.82(2H, doublet, J=6.0Hz, $C_{2''}$-H and $C_{6''}$-H), 8.94 (1H, singlet, C_4-H).^{13}C NMR (100MHz, $CDCl_3$, δ) : 114.50(CH), 116.97(CH), 120.44(C), 120.98(CH), 121.29(C), 121.50(CH), 122.10(CH), 124.61(CH), 125.04(C), 126.01(CH), 127.24(CH), 128.73(CH), 129.04(CH), 134.04(CH), 136.16(C), 142.89(CH), 146.52(C), 146.82(C), 147.04(C), 150.50(CH), 151.98(C), 154.44(C), 159.89 (CO of coumarin).

3-[4-(2-Methoxystyryl)-(2,4'-bipyridin)-6-yl]coumarin (9d): White solid; yield = 65% ; mp 181-183°C; Anal. Calcd. For $C_{28}H_{20}N_2O_3$: C, 77.76; H, 4.66; N, 6.48%. Found: C, 77.75; H, 4.65; N, 6.46%. IR (KBr, ν_{max}, cm^{-1}); 1723 (C=O stretching of δ-lactone of coumarin), 1597 and 1458 (aromatic C=C and C=N stretchings), 3045 (aromatic C-H stretching), 2936 (aliphatic C-H stretching). ^{1}H NMR (400MHz, $CDCl_3$, δ): 3.97(3H, singlet, OCH_3), 6.96-8.08 (13H, multiplet, aromatic protons except $C_{5'}$-H, $C_{2''}$-H, $C_{6''}$-H and C_4-H), 8.63(1H, poorly resolved doublet, $C_{5'}$-H), 8.82(2H, doublet, J=6.0Hz, $C_{2''}$-H and $C_{6''}$-H), 8.98 (1H, singlet, C_4-H).^{13}C NMR (100MHz, $CDCl_3$, δ) : 55.53(OCH_3), 111.04(CH), 116.42(CH), 117.36(CH), 119.52(C), 120.85(CH), 121.24(CH), 121.73(CH), 124.68(CH), 125.04(C), 125.07(C), 126.38(CH), 127.34(CH), 129.01(CH), 129.05(CH), 130.10(CH), 132.33(CH), 142.89(CH), 146.54(C), 147.45(C), 150.43(CH), 151.73(C), 153.97(C), 154.29(C), 157.46(C), 160.38 (CO of coumarin).

8-Methoxy-3-[4-(2-methoxystyryl)-(2,4'-bipyridin)-6-yl]coumarin (9e): White solid; yield = 78% ; mp 239-241°C; Anal. Calcd. For $C_{29}H_{22}N_2O_4$: C, 75.31; H, 4.79; N, 6.06%. Found: C, 75.29; H, 4.77; N, 6.05%. IR (KBr, ν_{max}, cm^{-1}); 1716 (C=O stretching of δ-lactone of coumarin), 1592 and 1476 (aromatic C=C and C=N stretchings), 3023 (aromatic C-H stretching), 2934 (aliphatic C-H stretching). ^{1}H NMR (400MHz, $CDCl_3$, δ): 3.96(3H, singlet, OCH_3), 4.04(3H, singlet, OCH_3), 6.96-8.07 (12H, multiplet, aromatic protons except $C_{5'}$-H, $C_{2''}$-H, $C_{6''}$-H and C_4-H), 8.64(1H, poorly resolved doublet, $C_{5'}$-H), 8.82(2H, doublet, J=6.0Hz, $C_{2''}$-H and $C_{6''}$-H), 8.96 (1H, singlet, C_4-H). ^{13}C NMR (100MHz, $CDCl_3$, δ) : 55.54(OCH_3), 56.30(OCH_3), 111.04(CH), 113.96(CH), 117.26(CH), 120.16(C), 120.38(CH), 120.86(CH), 121.26(CH), 121.93(CH), 124.51(CH), 125.07(C), 125.27(C), 126.42(CH), 127.35(CH), 129.04(CH), 130.07(CH), 143.05(CH), 146.58(C), 146.94(C), 147.48(C), 150.44(CH), 151.74(C), 154.29(C), 157.46(C), 159.82 (CO of coumarin).

6-Bromo-3-[4-(2-methoxystyryl)-(2,4'-bipyridin)-6-yl]coumarin (9f): White solid; yield = 68% ; mp 236-238°C; Anal. Calcd. For $C_{28}H_{19}BrN_2O_3$: C, 65.76; H, 3.75; N, 5.48%. Found: C, 65.74; H, 3.73; N, 5.46%. IR (KBr, ν_{max}, cm^{-1}); 1720 (C=O stretching of δ-lactone of coumarin), 1590 and 1460 (aromatic C=C and C=N stretchings), 3058 (aromatic C-H stretching), 2925 (aliphatic C-H stretching). ^{1}H NMR (400MHz, $CDCl_3$, δ): 3.97(3H, singlet, OCH_3), 6.96-8.06 (12H, multiplet, aromatic protons except $C_{5'}$-H, $C_{2''}$-H, $C_{6''}$-H and C_4-H), 8.62(1H, poorly resolved doublet, $C_{5'}$-H), 8.82(2H, doublet, J=6.0Hz, $C_{2''}$-H and $C_{6''}$-H), 8.91 (1H, singlet, C_4-H).^{13}C NMR (100MHz, $CDCl_3$, δ) : 55.54(OCH_3), 111.06(CH), 117.24(C), 117.65(CH), 118.14(CH), 120.86(CH), 121.04(CH), 121.20(C),

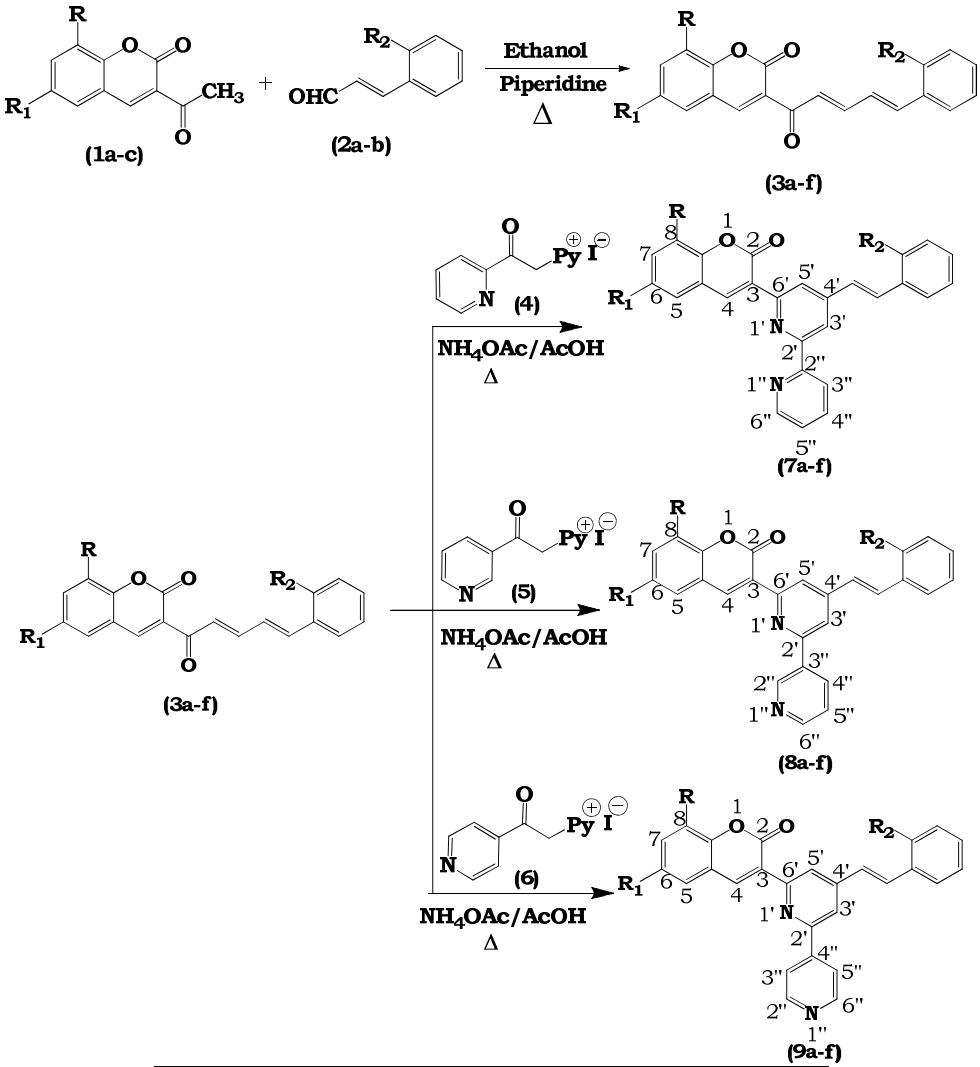
121.78(CH), 124.97(C), 126.10(C), 126.25(CH), 127.37(CH), 129.25(CH), 130.14(CH), 131.12(CH), 134.98(CH), 141.39(CH), 146.38(C), 147.60(C), 150.46(CH), 151.17(C), 152.75(C), 154.41(C), 157.48(C), 159.72 (CO of coumarin).

In case of the compounds **7b, 8b, 8e** and **9e**, the number of non-equivalent carbon signals observed is one less than expected. This may be due to identical chemical shifts of two carbons which may appear at same position.

RESULTS AND DISCUSSION

2.1. CHEMISTRY:

In the present work, various 3-[4-styryl-(2,2'-bipyridin)-6-yl]coumarins **(7a-f);** 3-[4-styryl-(2,3'-bipyridin)-6-yl]coumarins **(8a-f)** and 3-[4-styryl-(2,4'-bipyridin)-6-yl]coumarins **(9a-f)** have been synthesized by reacting appropriate 3-(5-arylpenta-2,4-dienoyl)coumarins (coumarin chalcones) **(3a-f)** with 2-pyridoyl methyl pyridinium iodide salt **(4),** 3-pyridoyl methyl pyridinium iodide salt **(5)** and 4-pyridoyl methyl pyridinium iodide salt **(6)** respectively under *Krohnke's* reaction condition. The starting material 3-(5-arylpenta-2,4-dienoyl)coumarins (coumarin chalcones) **(3a-f)** were prepared by the reaction of 3-acetyl coumarins **(1a-c)** with appropriate cinnamaldehydes **(2a-b)** in the presence of piperidine in ethanol. **(Scheme-1).**



Compounds	R	R_1	R_2	Compounds	R	R_1	R_2
5a, 6a, 7a	H	H	H	**5d, 6d, 7d**	H	H	OCH_3
5b, 6b, 7b	OCH_3	H	H	**5e, 6e, 7e**	OCH_3	H	OCH_3
5c, 6c, 7c	H	Br	H	**5f, 6f, 7f**	H	Br	OCH_3

Scheme-1: Synthetic scheme for the compounds (7a-f), (8a-f) and (9a-f)

2.2. BIOLOGICAL RESULTS:

2.2.1 ANTIMICROBIAL ACTIVITY

The newly synthesized target compounds **(7a-f)**, **(8a-f) and (9a-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 [26]. Ampicillin, Chloramphenicol and Norfloxacin were used as standard antibacterial drugs, whereas Griseofulvin and Nystatin were used as standard antifungal drugs. All MTCC cultures were collected from Institute of Microbial Technology, Chandigarh and tested against above mentioned known drugs. Mueller-Hinton broth was used as the nutrient medium for the test bacteria and Sabouraud Dextrose broth was used for the test fungi. Inoculum size for the test strains was adjusted to 10^8 CFU (Colony Forming Unit per milliliter) per milliliter by comparing the turbidity. Each synthesized compound was diluted with DMSO so as to have the stock solution of 2000 $\mu g/$mL concentration as a stock solution. The results were recorded in the form of primary and secondary screening. The synthesized compounds **(7a-f)**, **(8a-f) and (9a-f)** were screened for their antibacterial and antifungal activity at the concentration of 1000, 500 and 250 $\mu g/$mL for the primary screening. The synthesized compound showing activity against microbes in the primary screening were further screened in a second set of dilution at concentrations of 200, 100, 62.5, 50 and 25 $\mu g/$mL. The suspention of 10 μL from each well were further incubated and growth was noted at 37°C after 24 hour for bacteria and 48 hour for fungi. The lowest concentration which showed no visible growth (turbidity) after spot subculture was considered as the minimum inhibitory concentration (MIC) for each compound.

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

2.2.2. ANTIMICROBIAL EVALUTION

The compounds **(7a-f)**, **(8a-f) and (9a-f)** were screened for their *in vitro* antibacterial and antifungal evaluation against various bacterial and fungal pathogens by broth dilution method. Ampicillin, Chloramphenicol, Norfloxacin, Griseofulvin and Nystatin were used as standard drugs. The values of MIC are summarized in **Table-1**.

Table-1 : In *vitro* Antimicrobial activity of compounds (7a-f), (8a-f) and (9a-f)

Compound	Minimum Inhibitory Concentration (MIC, $\mu g mL^{-1}$)							
	Gram +ve bacteria			Gram –ve bacteria			Fungi	
	B.s.	*S.a.*		*E.c.*	*S.t.*		*A.n.*	*C.a.*
	MTCC441	MTCC96		MTCC443	MTCC98		MTCC282	MTCC227
7a	**250**	**250**		250	200		250	>1000
7b	**100**	**250**		**100**	250		250	**500**
7c	**250**	**200**		250	**62.5**		1000	**500**
7d	**100**	**125**		200	125		1000	1000
7e	**125**	**100**		125	200		>1000	>1000
7f	**250**	**250**		200	250		>1000	**500**
8a	**250**	**250**		200	250		500	1000
8b	**125**	**200**		125	125		>1000	>1000
8c	**100**	**250**		125	250		250	500
8d	**250**	**250**		200	**100**		1000	>1000
8e	**250**	**100**		250	200		>1000	**250**
8f	**200**	**200**		200	500		500	>1000
9a	**200**	**200**		200	250		>1000	**500**
9b	**200**	**125**		250	200		500	>1000
9c	**100**	**250**		200	200		1000	**500**
9d	**250**	**200**		250	125		>1000	1000
9e	**200**	**250**		125	**62.5**		>1000	>1000
9f	**200**	**125**		125	200		>1000	**500**
Ampicillin	250	250		100	100		-	-
Chloramphenicol	50	50		50	50		-	-
Norfloxacin	100	10		10	10		-	-
Griseofulvin	-	-		-	-		100	500
Nystatin	-	-		-	-		100	100

The assessment of antimicrobial screening data reveals that all the compounds exerted significant inhibitory activity against gram positive and gram negative bacteria. Compounds **7b, 7d, 8c** and **9c** (MIC=100, *µg/*mL) exhibited excellent activity toward Gram-positive bacteria *Bacillus subtilis* as compared to Ampicillin (MIC=250, *µg/*mL) and showed equipotent activity to Norfloxacin (MIC=100, *µg/*mL). Against Gram-positive bacteria *Bacillus subtilis*, compounds **7e** and **8b** (MIC=125, *µg/*mL) showed activity higher than that of Ampicillin (MIC=250, *µg/*mL). Compounds **8f, 9a, 9b, 9e** and **9f** (MIC=200, *µg/*mL) displayed better activity than Ampicillin (MIC=250, *µg/*mL) toward Gram-positive bacteria *Bacillus subtilis.* Compounds **7a, 7c, 7f, 8a, 8d, 8e** and **9d** (MIC=250, *µg/*mL) showed equipotent activity to Ampicillin (MIC=250, *µg/*mL) toward Gram-positive bacteria *Bacillus subtilis.* Compounds **7e** and **8e** (MIC=100, *µg/*mL) were found to be more effective against Gram-positive bacteria *Staphylococcus aureus* than Ampicillin (MIC=250, *µg/*mL). Against Gram-positive bacteria *Staphylococcus aureus*, compounds **7d, 9b** and **9f** (MIC=125, *µg/*mL) showed activity higher than that of Ampicillin (MIC=250, *µg/*mL). Compounds **7c, 8b, 8f, 9a** and **9d** (MIC=200, *µg/*mL) showed good activity against Gram-positive bacteria *Staphylococcus aureus* as compared to Ampicillin (MIC=250, *µg/*mL). Against Gram-positive bacteria *Staphylococcus aureus*, compound **7a, 7b, 7f, 8a, 8c, 8d, 9c,** and **9e** (MIC=250, *µg/*mL) showed equipotent activity to that of Ampicillin (MIC=250, *µg/*mL).

Moreover, Against Gram-negative bacteria *Escherichia coli*, compounds **7b** (MIC=100, *µg/*mL) showed activity comparable to Ampicillin (MIC=100, *µg/*mL). Against Gram-negative bacteria *Salmonella typhi*, compounds **7c** and **9e** (MIC=62.5, *µg/*mL) showed excellent activity as compared to Ampicillin (MIC=100, *µg/*mL). Whereas compounds **8d** (MIC=100, *µg/*mL) showed equipotent to Ampicillin (MIC=100, *µg/*mL) toward Gram-negative bacteria *Salmonella typhi.*

Furthermore, against *Candida albicans* fungal pathogen, however compound **8e** (MIC=250, *µg/*mL) showed better inhibition action as compare to the standard drug Griseofulvin (MIC=500, *µg/*mL). Whereas compounds **7b, 7c, 7f, 8c, 9a, 9c** and **9f** (MIC=500, *µg/*mL) showed activity comparable to Griseofulvin (MIC=500, *µg/*mL) against fungal pathogen *Candida albicans*. None of the tested compounds showed better activity against *Aspergillus niger* than standard drugs.

Majority of the synthesized compounds were active against Gram-positive bacteria *viz*. *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 96), Gram-negative bacteria *viz*. *Escherichia coli* (MTCC 443) and *Salmonella typhi* (MTCC 98). Some of the synthesized compounds were found sufficiently potent to inhibit fungal pathogen *viz*. *Candida albicans* (MTCC 227).

CONCLUSION

Present study described successful hybridization strategy of three bioactive moieties, pyridyl substituted coumarin, bipyridine and styryl 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*. Antimicrobial screening results revealed that compounds **7b, 7c, 7d, 7e, 8c, 9c** and **9e** 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 styryl entities as substitution serve as promising lead scaffolds for further generation of new antimicrobial agents.

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