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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME PYRIDO[3,4-*C*]COUMARINS

PARIN V. SHAIKH, DINKAR I. BRAHMBHATT

Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar-388120, Gujarat, India.

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Abstract: The synthesis of some 2-(benzofuran-2-yl)-4-phenyl pyrido[3,4-*c*]coumarins and 2-(furan/thiophen-2-yl)-4-phenyl pyrido[3,4-c]coumarins has been carried out. The compounds have been synthesized by reacting various 3- benzoyl coumarins with an appropriate 2-acetylbenzofuran and 2-acetyl furan/thiophene under Kroenhke's reaction condition. The structures of all the synthesized compounds were supported by analytical and spectral data like IR, ^{1}H-NMR, ^{13}C-NMR and Mass. All the synthesized compounds have been screened for their antimicrobial activities. The antibacterial activities were carried out against *Escherichia coli* and *Candida albicans* (Gram -ve bacteria) and *Bacillus subtillis* and *Staphylococcus aureus* (Gram +ve bacteria), while antifungal activities were carried out against fungi *Candida albican and Aspergillis niger*.

Keywords: 3-Benzoyl -coumarins, pyrido[3,4-*c*]coumarin, Krohnke's pyridine synthesis

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Corresponding Author: MS. PARIN V. SHAIKH

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INTRODUCTION

Coumarins form an important group of naturally occurring compounds. Because of their various physiological and biochemical properties, the study of coumarin and its derivatives has remain a subject of an active interest. A large number of coumarins have been isolated from a variety of plant sources and underwent extensive investigations aimed to assess their potential therapeutic applications [1]. Many coumarin derivatives are known for their varied biological activities such as: anti-HIV [2-3], anticancer [4], anticoagulant [5], antitumor [6], anti-inflammatory [7], antianxiety [8] etc. This inherent biological relevance has attracted much interest in the drug discovery field, and coumarin nucleus has emerged as a valuable molecular template for the design of a variety of analogues.

A number of heterocyclic fused coumarins derivatives possess variety of chemical and physiological properties. Among these heterocyclic fused coumarins, pyrido coumarins form a distinct class of coumounds. Coumarins fused with pyridine have also been reported to possess antiallergic [9], anticoagulant [10], antimicrobial [11], antidiabetic activities [12] and even analgesic properties [13]. The survey of the literature methods for the synthesis of pyrido fused coumarins reveals that majority of the methods are reported for pyrido[3,2-*c*]coumarins and hardly a few literature reports are there for the synthesis of pyrido[3,4-*c*]coumarins. Moreover the methods used for the preparation of pyrido[3,4-*c*]coumarins do not have general applicability and preparation of the starting material or condensing agent is quite difficult. Therefore with a view to developing the synthesis of pyrido[3,4-*c*]coumarins utilizing a simple convenient method, which has general applicability also, the present work was carried out and some pyrido[3,4-*c*]coumarins are synthesized.

2. MATERIALS AND METHODS:

2.1 Chemistry

All the melting points were determined in open capillaries and are uncorrected. All solvents and reagents were obtained from commercial sources and used without any additional purification. All the IR spectra (KBr disc) were recorded on Shimadzu FT-IR 8400-S spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR. 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. Thin layer chromatography was performed on precoated silica gel on aluminum sheets (Kieselgel 60, F254, Merck) and spots were visualized with UV light (254 nm) and/or in an iodine chamber.

Starting precursors 3- benzoyl coumarins (**1a-c)** [14-15] and acetylbenzofuran (2a-c) [16] were prepared according to reported procedures.

2.2 General procedure for the synthesis of 2-(benzofuran-2-yl)-4-phenyl pyrido[3,4-*c*]coumarins (4a-i) and 2-(furan/thiophen-2-yl)-4-phenyl pyrido[3,4-*c*]coumarins (5a-f).

In a 100 mL round bottom flask, a solution of appropriate 2-acetylbenzofuran (2a-c) or 2-acetyl furan/thiophene (3) (0.03 mole) was taken in glacial acetic acid (15 ml). To this, ammonium acetate (0.03) was added with stirring at room temperature. Then a solution of appropriate 3-benzoyl coumarins (1a-c) (0.03 mole) in acetic acid (15 ml.) was added with stirring at room temperature. The reaction mixture was further stirred for 45 minutes at room temperature and then refluxed in an oil bath at 140°C for 6 hours. It was then allowed to come to room temperature and poured into ice cold water. The crude solid mass obtained was extracted with chloroform. The combined chloroform extract was washed with 10% sodium bicarbonate solution and then with water. It was then dried over anhydrous sodium sulfate. The removal of chloroform under vacuum gave a solid product was recrystallized from ehanol.

The reaction proceeded smoothly and gave the expected products (**4a-i) and (5a-f)** in good yield (65-80%). The structures of the compounds (**4a-i)** and (**5a-f)** were established on the basis of IR, ^{1}H-NMR, ^{13}C-NMR spectral data and elemental analysis and representative mass spectral data.

2-(Benzofuran-2-yl)-4-phenyl pyrido[3,4-c]coumarin (4a): White solid; yield = 70%; mp 260-261°C, Anal. Calcd. for $C_{26}H_{15}NO_3$:C, 80.19; H, 3.88; N, 3.60%. Found: C, 80.11; H, 3.80; N, 3.57%. IR (KBr, ν_{max}, cm^{-1}):1726 (C=O stretching of δ-lactone of coumarin), 1608 and 1545 (aromatic C=C and C=N stretchings), 750 and 698 (C-H bending vibrations of mono substituted benzene ring), 3050 (aromatic C-H stretching), ^{1}H-NMR (400MHz, $CDCl_3$,δ): 7.10-8.53 (15 H, multiplet, aromatic protons), ^{13}C-NMR (100MHz, $CDCl_3$, δ): 108.72(CH), 111.71(CH), 116.72(CH), 11744(C), 122.19(CH), 122.27(CH), 123.63(CH), 124.41(CH), 125.60(CH), 126.42(CH), 127.21(C), 127.82(CH), 128.08(C), 128.55(CH), 129.89(C), 130.18(CH), 132.34(CH), 132.40(C), 136.25(C), 138.58(C), 152.62(C), 152.90(C), 153.43(C), 159.32 δ (CO of the coumarin). The mass spectrum of compound showed M+1peak at 390(m/z%) mass unit supports the structure of compound **4a**.

2-(Benzofuran-2-yl)-9-bromo-4-phenyl pyrido[3,4-*c*]coumarin (4b): White solid; yield = 72%; mp 294-296 ˚C, Anal. Calcd. for $C_{26}H_{14}BrNO_3$: C, 66.68; H, 3.01; N, 2.99%, Found: C, 66.62; H, 3.08; N, 2.92%. IR (KBr, ν_{max}, cm^{-1}): ν_{max} 1752 (C=O stretching of δ-lactone of coumarin), 1600 and 1512 (aromatic C=C and C=N stretchings), 702 and 754 (C-H bending vibrations of mono substituted benzene ring), 3063 (aromatic C-H stretching), ^{1}H-NMR (400MHz,$CDCl_3$,δ):7.28-84(14H, multiplet, aromatic protons). ^{13}C-NMR (100MHz,$CDCl_3$,δ): 108.20(CH), 112.16(CH),

113.88(CH), 116.14(C), 118.59(CH), 120.81(C), 122.41(CH), 123.95(C), 125.51(CH), 127.08(CH), 128.18(CH), 129.15(CH), 129.79(CH), 132.05(CH), 133.96(CH), 135.13(C), 136.20(CH), 137.27(C), 138.45(C), 139.09(C), 140.85(C), 142.08(C), 155.86(C) and 159.01(CO of coumarin).

2-(Benzofuran-2-yl)-7-methoxy-4-phenyl pyrido[3,4-c]coumarin (4c): White solid; yield = 75%; mp 288˚C, Anal. Calcd. for $C_{27}H_{17}NO_4$: C, 77.32; H, 4.09; N, 3.34%, Found: C, 77.38; H, 4.02; N, 3.26%. IR (KBr, ν_{max}, cm^{-1}): ν_{max} 1725 (C=O stretching of δ-lactone of coumarin), 1598 and 1543 (aromatic C=C and C=N stretchings), 702 and 756 (C-H bending vibrations of mono substituted benzene ring), 2928 (aliphatic C-H stretching), 3060 (aromatic C-H stretching).,^{1}H-NMR (400MHz, $CDCl_3$,δ): 4.106 (3H, singlet, OCH_3), 7.17-8.48 (14H, multiplet, aromatic protons ^{13}C-NMR (100MHz,$CDCl_3$,δ): 52.26(OCH_3), 104.81(CH), 112.51(CH), 117.88(C), 119.24(CH), 120.15(C), 123.69(CH), 124.41(C), *124*.89(CH), 126.16(CH), 126.46(CH), 126.65(CH), 126.75(CH), 127.10(CH), 127.51(C), 127.59(C), 127.98(C), 128.86(CH), 129.60(CH), 13054(C), 142.29(C), 149.79(C), 153.17(C), 154.50(C) and 160.10(CO of coumarin).

2-(5-Bromobenzofuran-2-yl)-4-phenyl pyrido[3,4-c]coumarin (4d): White solid; yield = 78%; mp 198-200˚C, Anal. Calcd. for $C_{26}H_{14}BrNO_3$: C, 66.68; H, 3.01; N, 2.99%, Found: C, 66.62; H, 3.09; N, 3.05%. IR (KBr, ν_{max}, cm^{-1}): ν_{max} 1724 (C=O stretching of δ-lactone of coumarin), 1597 and 1546 (aromatic C=C and C=N stretchings), 699 and 751 (C-H bending vibrations of mono substituted benzene ring), 3054 (aromatic C-H stretching)., ^{1}H-NMR (400MHz, $CDCl_3$,δ): 7.28-8.44 (14H, multiplet, aromatic protons), ^{13}C-NMR (100MHz,$CDCl_3$,δ): 107.35(CH), 109.90(C), 112.12(CH), 112.72(C), 115.54(C), 116.16(C), 116.48(CH), 117.36(CH), 118.37(C), 12136(CH), 124.21(CH), 125.82(CH), 128.10(CH), 130.71(CH), 132.15(CH), 134.15(CH), 136.15(C), 138.10(C), 140.98(C), 152.23(C), 153.30(C) and161.51(CO of coumarin).

2-(5-Bromobenzofuran-2-yl)-9-bromo-4-phenyl pyrido[3,4-c]coumarin (4e): White solid; yield = 78%; mp 215-216˚C, Anal. Calcd. for $C_{26}H_{13}Br_2NO_3$:C, 57.07.19; H, 2.39; N, 2.56%, Found: C, 57.01; H, 2.28; N, 2.59%. IR (KBr, ν_{max}, cm^{-1}): 1725 (C=O stretching of δ-lactone of coumarin), 1600 and 1545 (aromatic C=C and C=N stretchings), 695 and 761 (C-H bending vibrations of mono substituted benzene ring), 3061 (aromatic C-H stretching).,^{1}H-NMR (400MHz, $CDCl_3$,δ): 6.93-8.55 (13H, multiplet, aromatic protons), ^{13}C-NMR (100MHz, $CDCl_3$, δ): 108.78(CH), 111.75(CH), 116.85(CH), 119.44(C), 122.18(CH), 122.21(CH), 123.62(CH), 124.43(CH), 125.60(CH), 126.45(C), 127.24(CH), 127.72(C), 128.08(CH), 128.56(C), 128.85(C), 130.18(CH), 132.3(C), 132.40(C), 136.27(C), 138.59(C), 145.52(C), 152.95(C), 153.42(C), 159.32(CO of coumarin).

2-(5-Bromobenzofuran-2-yl)-7-methoxy-4-phenyl pyrido[3,4-*c*]coumarin (4f): White solid; yield = 70%; mp 225-227˚C, Anal. Calcd. for $C_{27}H_{16}BrNO_4$: C, 65.08; H, 3.24; N, 2.81%, Found: C, 65.01; H, 3.18; N, 2.75%. IR (KBr, ν_{max}, cm^{-1}): ν_{max} 1728 (C=O stretching of δ-lactone of

coumarin), 1605 and 1554 (aromatic C=C and C=N stretchings), 701 and 749 (C-H bending vibrations of mono substituted benzene ring), 2924 (aliphatic C-H stretching), 3051 (aromatic C-H stretching). ^{1}H-NMR (400MHz, $CDCl_3$,δ): 4.01(3H, singlet, OCH_3), 7.17-8.48(13H, multiplet, aromatic protons, ^{13}C-NMR (100MHz,$CDCl_3$,δ): 55.54(OCH_3), 109.13(CH), 114.41(CH), 118.47(C), 118.74(C), 120.80(CH), 122.36(CH), 122.70(CH), 123.76(CH), 125.16(CH), 126.81(CH), 128.13(CH), 128.62(C), 129.03(CH), 130.03(C), 132.24(C), 134.9(C), 135.95(C), 138.83(C), 140.07(C), 142.62(C), 152.67(C), 153.74(C), 156.13(C), 158.72(CO of coumarin).

2-(7-Methoxybenzofuran-2-yl)-4-phenyl pyrido[3,4-c]coumarin (4g): White solid; yield = 75%; mp 215-216˚C, Anal. Calcd. for $C_{27}H_{17}NO_4$: C, 77.32; H, 4.09; N, 3.34%, Found: C, 77.25; H, 4.01; N, 3.39%. IR (KBr, ν_{max}, cm^{-1}): 1735 (C=O stretching of δ-lactone of coumarin), 1604 and 1545 (aromatic C=C and C=N stretchings), 694 and 755 (C-H bending vibrations of mono substituted benzene ring), 2933 (aliphatic C-H stretching), 3042 (aromatic C-H stretching)., ^{1}H-NMR (400MHz, $CDCl_3$,δ): 4.12(3H, singlet, OCH_3),693-8.55(14H,multiplet, aromatic protons), ^{13}C-NMR(100MHz,$CDCl_3$,δ): 55.75(OCH_3), 112.66(C), 114.68(CH), 115.79(C), 117.78(CH), 118.33(C), 118.44(CH), 122.95(CH), 123.43(C), 124.51(CH), 126.98(CH), 127.42(C), 127.83(CH), 128.89(CH), 129.13(CH), 129.30(CH), 129.54(CH), 129.99(CH), 130.51(C), 130.94(C), 132.03(C), 134.16(C), 137.46(C), 153.19(C), 161.40(CO of coumarin).

2-(9-Bromobenzofuran-2-yl)-7-methoxy-4-phenyl pyrido[3,4-c]coumarin (4h): White solid; yield = 80%; mp 289 ˚C Anal. Calcd. for $C_{27}H_{16}BrNO_4$: C, 65.08; H, 3.24; N, 2.81%, Found: C, 65.01; H, 3.30; N, 2.89%. IR (KBr, ν_{max}, cm^{-1}): 1735 (C=O stretching of δ-lactone of coumarin), 1601 and 1545 (aromatic C=C and C=N stretchings), 698 and 755 (C-H bending vibrations of mono substituted benzene ring), 2925 (aliphatic C-H stretching), 3061 (aromatic C-H stretching)., ^{1}H-NMR (400MHz, $CDCl_3$,δ): 3.72(3H, singlet, OCH_3), 6.53-8.06 (13H, multiplet, aromatic protons). ^{13}C-NMR (100MHz,$CDCl_3$,δ): 55.24(OCH_3), 114.56(CH), 114.61(CH), 116.12(CH), 117.12(C), 118.06(CH), 118.62(C), 119.59(C), 120.44(CH), 121.73(CH), 123.23(C), 123.92(CH), 124.63(C), 125.38(CH), 128.17(CH), 129.46(C), 130.74(C), 134.04(CH), 136.70(CH), 140.67(C), 142.86(C), 144.63(C) ,148.05(C), 152.38(C), 160.10(CO of coumarin).

2-(7-Methoxybenzofuran-2-yl)-7-methoxy-4-phenyl pyrido[3,4-*c*]coumarin (4i):White solid; yield = 79%; mp 200˚C, Anal. Calcd. for $C_{28}H_{19}NO_5$: C, 74.82; H, 4.26; N, 3.12%, Found: C, 74.75; H, 4.21; N, 3.18%. IR (KBr, ν_{max}, cm^{-1}): 1735 (C=O stretching of δ-lactone of coumarin), 1600 and 1543 (aromatic C=C and C=N stretchings), 700 and 725 (C-H bending vibrations of mono substituted benzene ring), 2930 (aliphatic C-H stretching), 3022 (aromatic C-H stretching).,^{1}H-NMR (400MHz, $CDCl_3$,δ): 4.02 and 4.13 (6H, two singlets, 2 X OCH_3), 6.93-8.53 (13 H, multiplet, aromatic protons)., ^{13}C-NMR (100MHz, $CDCl_3$, δ): 55.77(OCH_3), 55.91(OCH_3), 108.69(CH), 111.77(CH), 114.69(C), 116.59(CH), 118.98(C), 122.10(CH), 122.25(CH), 123.64(CH), 125.20(CH),

126.41(CH), 128.06(CH), 128.55(C), 128.86(CH), 133.30(CH), 134.09(C), 136.36(C), 138.50(C), 151.01(C), 152.50(C), 153.48(C), 155.77(C), 155.91(C), 157.71(C), 160.66(CO of coumarin).

2-(Furan-2-yl)-4-phenyl pyrido[3,4-*c*]coumarins (5a): White solid; yield = 78%; mp 215-217˚C, Anal. Calcd. for $C_{22}H_{13}NO_3$: C, 77.87; H, 3.36; N, 4.13%, Found: C, 77.81; H, 3.31; N, 4.19%. IR (KBr, ν_{max}, cm^{-1}): ν_{max} 1739 (C=O stretching of δ-lactone of coumarin), 1595 and 1536 (aromatic C=C and C=N stretchings), 699 and 750 (C-H bending vibrations of mono substituted benzene ring), 3048(aromatic C-H stretching)., ^{1}H-NMR (400MHz, $CDCl_3$,δ): 6.63-8.30 (13H, multiplet, aromatic protons), ^{13}C-NMR (100MHz,$CDCl_3$,δ): 106.39(CH), 108.35(CH), 112.12(C), 112.72(C), 117.23(CH), 121.16(CH), 121.68(CH), 123.02(CH), 124.68(CH), 124.99(CH), 128.82(C), 129.05(C), 131.31(CH), 141.16(C), 141.78(CH), 142.72(C), 146.39(C), 152.79(C), 159.36(CO of coumarin). The mass spectrum of compound showed M+1peak at 340(m/z%) mass unit supports the structure of compound **5a**.

9-Bromo-2-(furan-2-yl)-4-phenyl pyrido[3,4-*c*]coumarins (5b): White solid; yield = 84%; mp 235-238˚C, Anal. Calcd. for $C_{22}H_{12}BrNO_3$: C, 63.18; H, 2.89; N, 3.35%, Found: C, 63.11; H, 2.85; N, 3.29%. IR (KBr, ν_{max}, cm^{-1}): ν_{max} 1731 (C=O stretching of δ-lactone of coumarin), 1594 and 1534 (aromatic C=C and C=N stretchings), 695 and 747 (C-H bending vibrations of mono substituted benzene ring), 3048 (aromatic C-H stretching).,^{1}H-NMR (400MHz, CDCl3,δ): 7.32-8.16(12H, multiplet, aromatic protons), ^{13}C-NMR(100MHz,$CDCl_3$,δ): 109.12(CH), 115.60(CH), 115.65(C), 117.12(CH), 124.05(CH), 125.36(CH), 125.99(CH), 128.95(CH), 129.55(C), 130.01(CH), 133.70(CH), 141.94(CH), 143.56(C), 147.12(C), 150.11(C), 152.56(C), 155.65(C), 157.01(C), 157.99(C), 161.70(CO of coumarin).

2-(Furan-2-yl)-7-methoxy-4-phenyl pyrido[3,4-*c*]coumarins (5c): White solid; yield = 82%; mp 215-217˚C Anal. Calcd. for $C_{23}H_{15}NO_4$: C, 74.79; H, 4.09; N, 3.79%, Found: C, 74.81; H, 4.02; N, 3.75%. IR (KBr, ν_{max}, cm^{-1}): 1728 (C=O stretching of δ-lactone of coumarin), 1590 and 1545 (aromatic C=C and C=N stretchings), 698 and 751 (C-H bending vibrations of mono substituted benzene ring), 2928 (aliphatic C-H stretching), 3045 (aromatic C-H stretching). ^{1}H-NMR (400MHz, $CDCl_3$,δ): 4.04(3H, singlet, OCH_3), 6.66-8.30 (12H, multiplet, aromatic protons), 56.34(OCH_3), 108.66(CH), 109.40(CH), 111.60(CH), 114.29(C), 115.16(CH), 117.20(CH), 122.35(C), 123.59(C), 124.33(CH), 126.28(CH), 127.87(CH), 128.64(CH), 129.06(C), 129.13(CH), 140.38(C), 144.81(C), 147.91(C), 151.23(C), 155.85(C), 157.94(CO of coumarin).

4-Phenyl-2-(thiophen-2-yl) pyrido[3,4-*c*]coumarins (5d): White solid; yield = 85%; mp 267˚C Anal. Calcd. for $C_{22}H_{13}NO_2S$:C, 74.35; H, 3.69; N, 3.94%, Found: C, 74.28; H, 3.75; N, 3.99%. IR (KBr, ν_{max}, cm^{-1}): ν_{max} 1734(C=O stretching of δ-lactone of coumarin), 1587 and 1532 (aromatic C=C and C=N stretchings), 697 and 753 (C-H bending vibrations of mono substituted benzene ring), 3061 (aromatic C-H stretching), ^{1}H-NMR (400MHz, $CDCl_3$,δ): 6.96-8.05 (13H, multiplet,

aromatic protons). ^{13}C-NMR (100MHz, $CDCl_3$, δ): 109.99(CH), 112.06(CH), 113.35(C), 113.85(C), 115.54(CH), 116.41(C), 119.20(CH), 121.03(CH), 123.28(CH), 127.74(CH), 129.46(CH), 131.05(C), 132.04(C), 132.96(CH), 135.02(CH), 137.52(CH), 141.79(C), 149.78(C), 150.07(C), 160.08(CO of coumarin).

9-Bromo-4-phenyl-2-(thiophen-2-yl) pyrido[3,4-*c*]coumarins (5e):: White solid; yield = 84%; mp 215-217˚C, Anal. Calcd. for $C_{22}H_{12}BrNO_2S$:C, 60.84; H, 2.78 N, 3.23%, Found: C, 60.89; H, 2.71; N, 3.29%, IR (KBr, ν_{max},cm^{-1}): ν_{max} 1725 (C=O stretching of δ-lactone of coumarin), 1595 and 1547 (aromatic C=C and C=N stretchings), 701 and 753 (C-H bending vibrations of mono substituted benzene ring), 3061 (aromatic C-H stretching).,^{1}H-NMR (400MHz, $CDCl_3$,δ). 7.43-8.16 (12 H, multiplet, aromatic protons), ^{13}C-NMR (100MHz, $CDCl_3$, δ): 109.95(CH), 111.86(CH), 113.25(C), 113.75(C), 115.44(CH), 116.43(C), 119.21(CH), 121.00(CH), 123.29(CH), 124.78(CH), 129.46(C), 130.95(C), 132.14(C), 132.94(CH), 135.02(CH), 137.51(CH), 141.78(C), 149.74(C), 150.05(C), 159.48(CO of coumarin).

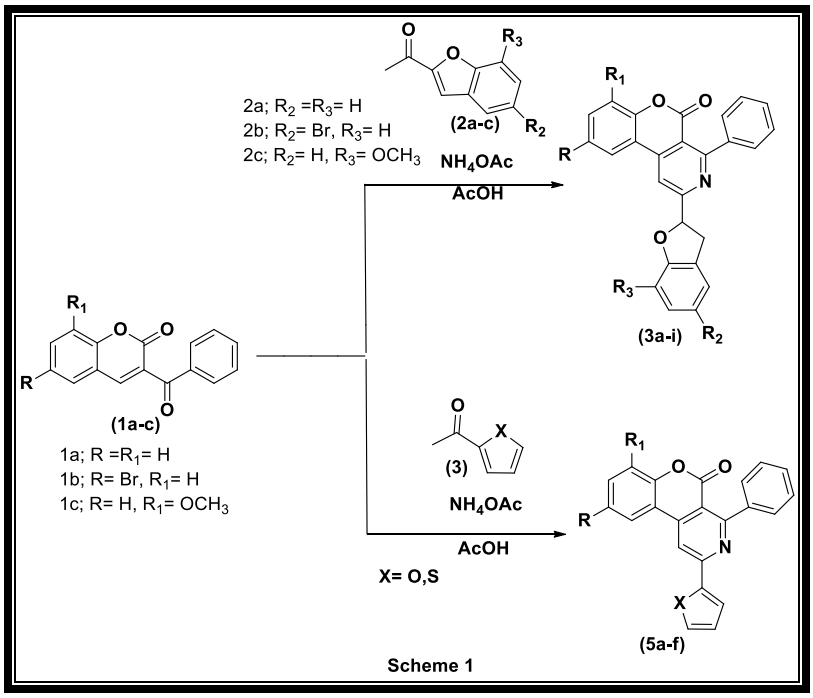
7-Methoxy-4-phenyl-2-(thiophen-2-yl) pyrido[3,4-c]coumarins (5f): White solid; yield = 84%; mp 219-220 ˚C, Anal. Calcd. for $C_{23}H_{15}NO_3S$:C, 71.67; H, 3.92 N, 3.63%, Found: C, 71.69; H, 3.95; N, 3.65%, IR (KBr, ν_{max},cm^{-1}): 1724 (C=O stretching of δ-lactone of coumarin), 1589 and 1534 (aromatic C=C and C=N stretchings), 702 and 753 (C-H bending vibrations of mono substituted benzene ring), 2945 (aliphatic C-H stretching), 3061 (aromatic C-H stretching)., ^{1}H-NMR (400MHz, $CDCl_3$,δ), 3.94 (3H, singlet, OCH_3), 6.99-8.17 (12H, multiplet, aromatic protons)., ^{13}C-NMR (100MHz, $CDCl_3$, δ): 56.30(OCH_3), 107.23(CH), 108.09(C), 110.79(CH), 117.01(C), 117.27(CH), 120.83(CH), 121.49(C), 121.73(CH), 123.02(CH), 124.97(CH), 128.85(CH), 129.10(C), 132.41(CH), 134.61(C), 141.66(CH), 143.17(C), 145.19(C), 147.09(C), 149.86(C), 159.11(CO of coumarin)

In case of compound **5a** the number of non-equivalent carbon signals observed is one less and in compound **4d**, the number of non-equivalent carbon signals observed is two less than expected. This may be due to identical chemical shift of certain carbons which may appear at the same position.

3. RESULTS AND DISCUSSION:

3.1 CHEMISTRY

Reaction of various 3-benzoyl coumarins **(1a-c)** with 2-acetylbenzofuran **(2a-c)** and 2-acetyl furan/thiophene **(3)** in the presence of ammonium acetate in refluxing acetic acid gave target compounds (**4a-i)** and (**5a-f)** respectively in 65-80% yield (**Scheme 1**). The formation of pyridine nucleus follows Krohnke's reaction mechanism [17]. The structures of all the synthesized compounds (**4a-i)** and (**5a-f)** were established by IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data.



Scheme 1

Compounds	R	R_1	R_2	R_3
4a	H	H	H	H
4b	Br	H	H	H
4c	H	OCH_3	H	H
4d	H	H	Br	H
4e	Br	H	Br	H
4f	H	OCH_3	Br	H
4g	H	H	H	OCH_3
4h	Br	H	H	OCH_3
4i	H	OCH_3	H	OCH_3

Compounds	R	R_1	X
5a	H	H	O
5b	Br	H	O
5c	H	OCH_3	O
5d	H	H	S
5e	Br	H	S
5f	H	OCH_3	S

Scheme 1. Synthetic scheme for the preparation of compounds 4a-i and 5a-f.

3.2 EVALUATION OF ANTIMICROBIAL ACTIVITY

All the compounds **4a-i** and **5a-f** were assayed for their *in vitro* antimicrobial activity against Gram-positive bacteria viz. *Bacillus subtilis*, *Staphylococcus aureus*, Gram-negative bacteria viz. *Escherichia coli*, *Salmonella typhi* and antifungal activity against *Aspergillus niger* and *Candida albicans* by Broth dilution method [18]

Table 1. Antimicrobial activity of compounds 4a-i and 5a-f

Compound	Minimum Inhibitory Concentration (MIC,µgmL^{-1})					
	Gram +ve Bacteria		Gram -ve Bacteria		Fungi	
	B.s.	*S.a.*	*E.c.*	*S.t.*	*A.n.*	*C.a.*
4a	200	100	125	250	>1000	>1000
4b	250	200	200	250	1000	500
4c	125	100	250	200	500	250
4d	200	100	100	100	250	1000
4e	125	250	125	200	1000	500
4f	100	125	200	100	250	>1000
4g	100	200	100	250	500	1000

4h	125	100	250	250	1000	250
4i	62.5	100	125	200	>1000	500
5a	100	125	200	200	250	1000
5b	125	200	200	250	500	250
5c	200	250	250	125	250	>1000
5d	100	200	200	250	>1000	>1000
5e	250	250	100	125	1000	250
5f	250	250	100	100	1000	500
Ampicillin	250	250	100	100	-	-
Chloramphenicol	50	50	50	50	-	-
Ciprofloxacin	50	50	25	25	-	-
Norfloxacin	100	10	10	10	-	-
Griseofulvin	-	-	-	-	100	500
Nystatin	-	-	-	-	100	100

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

Upon evaluating the antimicrobial activity data, it was observed that compound **4i** (MIC = 62.5*µg/*mL) exhibited excellent activity against gram positive bacteria *B. subtilis* compared to Ampicillin (MIC = 250*µg/*mL) and Norfloxacin (MIC = 100*µg/*mL). Compounds **4f, 4g, 5a** and **5d** (MIC = 100*µg/*mL) were found to be more potent compared to Ampicillin (MIC = 250*µg/*mL) and equipotent to Norfloxacin (MIC = 100*µg/*mL) against gram positive bacteria *B. subtilis.* The Compounds **4c, 4h, 4e and 5b** (MIC = 125*µg/*mL) exerted good activity against gram positive bacteria *B. subtilis* compared to Ampicillin (MIC = 250*µg/*mL). Compounds **4a, 4d and 5c** (MIC = 200*µg/*mL) exhibited moderate activity compared to Ampicillin (MIC = 250*µg/*mL) against gram positive bacteria bacteria *B. subtilis.*

Compounds **4a, 4c, 4d, 4h** and **4i** (MIC = 100*µg/*mL) and compounds **4f** and **5a** (MIC = 125*µg/*mL) exhibited better activity compared to Ampicillin (MIC = 250*µg/*mL) against gram

positive bacteria *S. aureus.* Compounds **4b, 4g, 5b** and **5d** (MIC = 200*µg/*mL) showed moderate activity compared to Ampicillin (MIC = 250*µg/*mL) against gram positive bacteria *S. aureus* .

Compounds **4d, 4g, 5e** and **5f** (MIC = 100*µg/*mL) and compounds **4d, 4f** and **5f** (MIC = 100*µg/*mL) were found equipotent compared to Ampicillin (MIC = 100*µg/*mL) against *E. coli and S. typhi* respectively.

Antifungal activity data of target compounds revealed that compounds **4c, 4h, 5b** and **5e** (MIC = 250*µg/*mL) were found to be more active against *C. albicans* compared to Griseofulvin (MIC = 500*µg/*mL). Compounds **4b, 4e, 4i** and **5f** (MIC = 500*µg/*mL) were found equipotent to Griseofulvin (MIC = 500*µg/*mL) against *C. albicans.* No compound showed good activity against fungis *A. Niger*.

It is perceived from the antimicrobial data that almost all the tested derivatives **4a-i** and **5a-f** were found to be potent against the gram positive bacterial strains. Among all the tested compounds, the compounds **4c, 4d, 4f, 4g, 4i, 4h, 5a** and **5d** were found to be more efficient members of the series.

4. CONCLUSION:

The present study reports expedient synthesis of varios pyrido[3,4-c]coumarins. The synthesized compounds were subjected to antibacterial and antifungal studies. The screening results revealed that all the compounds exhibited moderate to excellent activities against the pathogenic strains. The antimicrobial data revealed that compounds **4a-i** and 5**a-f** were found to be potent against the gram positive bacterial strains. Among all the tested compounds, the compounds **4c, 4d, 4f, 4g, 4i, 4h, 5a** and **5d** were found to be more efficient members of the series, hence the compounds are ideal for further modifications to obtain more efficacious antimicrobial agents.

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