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Synthesis, Characterization and In Vitro Antimicrobial Evaluation of Some New Benzofuranyl Substituted Pyrido [3, 2-C] Coumarins

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Abstract: ***In the present study, a series of benzofuranyl substituted pyrido [3, 2-c] coumarins (4a-l) were synthesized by the reaction of various 4-hydroxycoumarins (1a-c) and appropriate 1- (benzofuran-2-yl) -3-aryl prop-2-en-1-ones (chalcones) (3a-d) under Krohnke's reaction condition. The structure of all the synthesized compounds elucidated by elemental analysis, IR, ^{1}H-NMR, ^{13}C-APT and some representative mass spectral data. The compounds were subjected to in vitro antimicrobial screening against selected bacterial (Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Salmonella typhi) and fungal (Candida albicans and Aspergillus niger) pathogens. Compounds 4b, 4e, 4f, 4g, 4i, 4j and 4k displayed excellent antimicrobial activity and served as the most promising leads of the series.***

Keywords: Coumarin, benzofuran, antimicrobial screening, broth dilution method, spectral analysis

1. Introduction

A great number of heterocyclic compounds and heterocyclic fragments are present in many drugs due to their versatility and unique physicochemical properties and have become an important basis for medicinal chemistry [1]. Coumarin is an aromatic compound that has a bicyclic structure with lactone carbonyl groups widely distributed in the plant kingdom. The isolation of coumarin was first reported by Vogel in 1820 [2]. The name coumarin originates from a Caribbean word coumarou for the Tonka tree (Dipteryx odorata Willd, Leguminosae or Fabaceae), which shares the characteristic smell of these compounds and was known botanically at one time as Coumarouna odorata Aubl. Naturally occurring coumarins, which are classified by their benzopyran-2-one nucleus, have been isolated from numerous plants, particularly members of the Apiaceae, Rutaceae, and Fucaceae, as well as from some genera of Leguminosae. Many natural and synthetic coumarin derivatives display a remarkable array of biochemical and pharmacological activity [3-9].

The incorporation of another heterocyclic moiety like pyridine, indole, imidazole, diazole, thiazole, triazole etc in coumarin nucleus as a substituent or fused component can bring about an extensive modification in the properties of the parent coumarins and converts them into more useful derivatives. Among these, pyridine fused coumarin derivatives have drawn considerable attention due to their varied biological activities like antitumor [10], antidiabetic [11], analgesic [12], antiallergic [13], antimicrobial [14] properties, being characterized by a phenanthrene like structure as found in tetrahydrocannabinol. Thus, due to these biological activities considerable efforts have been made toward the preparation of pyrido fused coumarin derivatives. In literature four isomeric pyrido fused coumarins are reported i.e. pyrido [2, 3-c] coumarins, pyrido [3, 2-c] coumarins, pyrido [3, 4-c] coumarins and pyrido [4, 3-c] coumarins, in which pyrido [3, 2-c] coumarins have been widely studied. Variety of methods have been reported for the synthesis of pyrido [3, 2-c] coumarins [15].

Moreover, Benzofuran is also an important heterocyclic moiety found in variety of biologically active natural products as well as synthetic materials [16]. Benzofuran derivatives possess several biological properties such as anti-inflammatory [17], antiviral [18], antioxidant [19], antimicrobial [20], antidiabetic [21], anti-HIV [22], anti-arrhythmic [23], β-adrenocepter blocking activity [24], antihyperglycemic [25], antiparasitic [26], antitumor activities [27]. Such a wide range of biological properties inherent in benzofuran scaffold justifies the extensive interest in using benzofuran as building blocks of pharmacological agents. Many of the clinically approved drugs are synthetic or naturally occurring substituted and fused benzofuran derivatives with other heterocyclic moieties [28].

Thus considering the biological importance of pyrido fused coumarins and benzofurans, it was thought worthwhile to incorporate both of these biologically active moieties into a single scaffold and therefore in the present work, synthesis of various 4-aryl-2- (benzofuranyl) pyrido [3, 2-c] coumarins (**4a-l)** has been carried out.

2. 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 FTIR 8400-S spectrometer. ^{1}H-NMR and ^{13}C-APT spectra were recorded on Bruker Advance 400 spectrometer operating at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-APT. The chemical shift (δ) is reported in ppm using chloroform-d as a solvent and calibrated standard solvent signal. Mass spectra were recorded on Shimadzu QP 2010 spectrometer. Elemental

analysis was carried out on Perkin-Elmer 2400 C-H-N-S-O Analyser Series-II. All the compounds were routinely checked for completion of the reaction on silica gel 60 F254 TLC plates and their spots were visualized by exposure to a UV lamp, iodine vapour or $KMnO_4$ reagents. In the present work, various 4-aryl-2- (benzofuranyl) pyrido [3, 2-c] coumarins (**4a-l)** have been synthesized by reacting various 4-hydroxy coumarins (**1a-c)** with appropriate 1- (benzofuran-2-yl) -3-aryl-prop-2-en-1-ones (chalcones) (**3a-d)** in the presence of ammonium acetate in refluxing acetic acid. Compounds (**1a-c)** were prepared according to literature procedure [29-30].

2.1. General procedure for the preparation of 1- (benzofuran-2-yl) -3-aryl prop-2-en-1-ones (3a-d).

In a 100 ml three necked flask equipped with a thermometer and magnetic needle, an aqueous 10% sodium hydroxide solution (25 ml) and ethanol (25 ml) were taken and cooled to 0-10°C in an ice bath. An appropriate 2-acetyl benzofuran (0.02 mol) was added in small portions over a period of 10 minutes. Then appropriate aromatic aldehyde (0.02 mol) was introduced in one portion. The reaction mixture was stirred fir three hours at 10°C, whereby a solid product was separated out. It was filtered out and washed with cold ethanol. The product was then dried and recrystallized from ethanol to give yellow crystals. Chalcones **3a** and **3b** were prepared according to literature procedure [31].

4, 5-Benzo-1- (benzofuran-2-yl) -3- (p-tolyl) prop-2-en-1-one (3c): Amorphous powder: IR (KBr, ν_{max}, cm^{-1}): 1680 (C=O stretching), 1589 (C=C stretching), 748 (C-H bending vibrations of p-disubstituted benzene ring), 2893 (aliphatic C-H stretching), 3055 (aromatic C-H stretching). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 2.41 (3H, singlet, CH_3), 7.24-8.20 (13H, multiplet, ten aromatic protons + two olefinic protons + C_3 proton of benzofuran ring).

4, 5-Benzo-1- (benzofuran-2-yl) -3- (4methoxyphenyl) prop-2-en-1-one (3d): Amorphous powder: IR (KBr, ν_{max}, cm^{-1}): 1674 (C=O stretching), 1582 (C=C stretching), 810 (C-H bending vibrations of p-disubstituted benzene ring), 2970 (aliphatic C-H stretching), 3078 (aromatic C-H stretching). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.86 (3H, singlet, OCH_3), 6.94-8.20 (13H, multiplet, ten aromatic protons + two olefinic protons + C_3 proton of benzofuran ring).

2.2 General procedure for the synthesis of 4-aryl-2- (benzofuranyl) pyrido [3, 2-c] coumarins (4a-l).

A solution of an appropriate 4-hydroxy coumarin (**1a-c) (**0.005 mol) in glacial acetic acid (15 mL) was added in a round bottom flask. To this, ammonium acetate (0.05 mol) and appropriate 1- (benzofuran-2-yl) -3-aryl prop-2-en-1-one (**3a-c) (**0.005 mol) in acetic acid (15 mL) was added with stirring at room temperature. The reaction mixture was added with stirring for 45 minutes at room temperature and then refluxed in an oil bath at 140°C for 12 hours. It was then allowed to come to room temperature and poured into ice cold water and extracted with chloroform (3 x 20 mL). The combined chloroform extract was washed with 10% sodium bicarbonate solution (3 x 30 mL) and then with water (3 x 25 mL). It was dried over anhydrous sodium sulphate. The removal of chloroform under vacuum gave a solid product which were subjected to column chromatography using silica gel and chloroform-hexane (3:7) as an eluent to obtain white colored solid product 4-aryl-2- (benzofuranyl) pyrido [3, 2-c] coumarins (**4a-l).**

2- (benzofuranyl-2-yl) -4- (p-tolyl) pyrido [3, 2-c] coumarin (4a): Yield: 55%., Amorphous powder: m.p.276-278°C., 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), 813 (C-H bending vibrations of p-disubstituted benzene ring), 2924 (aliphatic C-H stretching), 3061 (aromatic C-H stretching). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 2.49 (3H, singlet, CH_3), 7.32-7.87 (12H, multiplet, aromatic protons), 7.95 (1H, singlet, C_3'-H), 8.81 (1H, poorly resolved doublet of doublet, C_{10}-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ): 21.44 (CH_3), 108.76 (CH), 111.71 (CH), 116.85 (CH), 119.44 (C), 122.18 (CH), 122.27 (CH), 123.67 (CH), 123.43 (CH), 125.60 (CH), 126.45 (CH), 127.21 (C), 127.72 (CH), 128.08 (C), 128.55 (CH), 128.89 (C), 130.18 (C), 132.31 (CH), 132.40 (C), 136.27 (C), 138.56 (C), 152.62 (C), 152.91 (C), 153.42 (C), 159.39 (CO of coumarin). Anal.Calcd. for $C_{27}H_{17}NO_3$: C, 80.38; H, 4.25; N, 3.47 %. Found: C, 80.42; H, 4.29; N, 4.51 %.

2- (benzofuranyl-2-yl) -4- (p-methoxyphenyl) pyrido [3, 2-c] coumarin (4b): Yield: 58%., Amorphous powder: m.p.260-262°C., IR (KBr, ν_{max}, cm^{-1}): 1713 (C=O stretching of δ-lactone of coumarin), 1605 and 1504 (aromatic C=C and C=N stretchings), 817 (C-H bending vibrations of p-disubstituted benzene ring), 2901 (aliphatic C-H stretching), 3063 (aromatic C-H stretching). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 4.03 (3H, singlet, OCH_3), 7.14-8.29 (12H, multiplet, aromatic protons), 8.70-8.74 (2H, multiplet, signals for C_{10}-H and C_3'-H merged). ^{13}C-APT (100 MHz, $CDCl_3$, δ): 55.71 (OCH_3), 112.76 (C), 114.66 (CH), 115.59 (C), 117.78 (CH), 118.31 (CH), 118.42 (C), 123.31 (CH), 123.43 (CH), 124.51 (C), 126.95 (CH), 127.44 (CH), 127.85 (C), 128.88 (CH), 129.13 (CH), 129.30 (CH), 129.54 (CH), 129.99 (CH), 130.51 (C), 130.94 (C), 132.03 (C), 134.06 (C), 137.48 (C), 153.14 (C), 161.41 (CO of coumarin). Anal.Calcd. for $C_{27}H_{17}NO_4$: C, 77.32; H, 4.09; N, 3.34 %. Found: C, 77.38; H, 4.13; N, 3.40 %.

4, 5-Benzo-2- (benzofuranyl-2-yl) -4- (p-tolyl) pyrido [3, 2-c] coumarin (4c): Yield: 62%., Amorphous powder: m.p.296-298°C., IR (KBr, ν_{max}, cm^{-1}): 1712 (C=O stretching of δ-lactone of coumarin), 1604 and 1512 (aromatic C=C and C=N stretchings), 825 (C-H bending vibrations of p-disubstituted benzene ring), 2901 (aliphatic C-H stretching), 3055 (aromatic C-H stretching). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 2.54 (3H, singlet, CH_3), 7.14-8.25 (14H, multiplet, aromatic protons), 8.73 (1H, singlet, C_3'-H), 8.78 (1H, poorly resolved doublet of doublet, C_{10}-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ): 21.40 (CH_3), 109.99 (C), 111.89 (CH), 112.83 (C), 115.66 (C), 116.18 (CH), 116.80 (CH), 118.07 (CH), 118.49 (C), 123.49 (CH), 123.53 (CH), 124.47 (C), 124.60 (CH), 126.20 (CH),

126.74 (CH), 127.51 (C), 127.64 (CH), 127.98 (CH), 128.62 (CH), 129.45 (C), 129.56 (C), 130.84 (C), 133.08 (CH), 133.59 (C), 136.66 (CH), 141.83 (C), 153.07 (C), 156.78 (C), 160.94 (CO of coumarin). Anal.Calcd. for $C_{31}H_{19}NO_3$: C, 82.10; H, 4.22; N, 3.09 %. Found: C, 82.16; H, 4.28; N, 3.13 %.

4, 5-Benzo-2- (benzofuranyl-2-yl) -4- (p-methoxyphenyl) pyrido [3, 2-c] coumarin (4d): Yield: 67%., Amorphous powder: m.p.280-282˚C., IR (KBr, ν_{max}, cm^{-1}): 1720 (C=O stretching of δ-lactone of coumarin), 1604 and 1519 (aromatic C=C and C=N stretchings), 810 (C-H bending vibrations of p-disubstituted benzene ring), 2901 (aliphatic C-H stretching), 3062 (aromatic C-H stretching). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 4.01 (3H, singlet, OCH_3), 7.12-8.24 (14H, multiplet, aromatic protons), 8.66 (1H, singlet, C_3'-H), 8.76 (1H, poorly resolved doublet of doublet, C_{10}-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ): 55.66 (OCH_3), 111.94 (CH), 112.94 (C), 114.38 (CH), 114.70 (CH), 114.75 (CH), 115.77 (C), 117.78 (CH), 122.99 (CH), 123.50 (CH), 124.15 (C), 124.39 (C), 124.80 (CH), 125.95 (CH), 126.47 (CH), 127.55 (C), 128.34 (CH), 129.11 (CH), 129.32 (C), 130.34 (CH), 130.73 (C), 131.33 (C), 132.13 (CH), 135.94 (C), 146.36 (C), 149.80 (C), 152.88 (C), 156.19 (C), 160.42 (CO of coumarin). Anal.Calcd. for $C_{31}H_{19}NO_4$: C, 79.31; H, 4.08; N, 2.98 %. Found: C, 79.35; H, 4.14; N, 3.02 %.

2- (Benzofuranyl-2-yl) -9-methyl-4- (p-tolyl) pyrido [3, 2-c] coumarin (4e): Yield: 56%., Amorphous powder: m.p.294-296˚C., IR (KBr, ν_{max}, cm^{-1}): 1728 (C=O stretching of δ-lactone of coumarin), 1620 and 1519 (aromatic C=C and C=N stretchings), 833 (C-H bending vibrations of p-disubstituted benzene ring), 2916 (aliphatic C-H stretchings), 3032 (aromatic C-H stretchings).^{1}H-NMR (400 MHz, $CDCl_3$, δ): 2.48 (3H, singlet, CH_3), 2.56 (3H, singlet, CH_3), 7.27-7.90 (11H, multiplet, aromatic protons), 7.94 (1H, singlet, C_3'-H), 8.59 (1H, poorly resolved doublet of doublet, C_{10}-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ): 21.10 (CH_3), 21.43 (CH_3), 108.69 (CH), 111.77 (CH), 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), 160.66 (CO of coumarin). Anal.Calcd. for $C_{28}H_{19}NO_3$: C, 80.56; H, 4.59; N, 3.36 %. Found: C, 80.62; H, 4.66; N, 3.40 %.

2- (Benzofuranyl-2-yl) -9-methyl-4- (p-methoxyphenyl) pyrido [3, 2-c] coumarin (4f): Yield: 59%., Amorphous powder: m.p.273-275˚C., IR (KBr, ν_{max}, cm^{-1}): 1720 (C=O stretching of δ-lactone of coumarin), 1627 and 1496 (aromatic C=C and C=N stretchings), 817 (C-H bending vibrations of p-disubstituted benzene ring), 2901 (aliphatic C-H stretching), 3062 (aromatic C-H stretching). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 2.56 (3H, singlet, CH_3), 3.92 (3H, singlet, OCH_3), 7.04-7.89 (11H, multiplet, aromatic protons), 7.94 (1H, singlet, C_3'-H), 8.58 (1H, poorly resolved doublet of doublet, C_{10}-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ): 21.13 (CH_3), 55.36 (OCH_3), 108.69 (CH), 111.82 (CH), 113.63 (CH), 116.59 (CH), 119.01 (C), 120.37 (C), 122.11 (CH), 122.25 (CH), 123.67 (CH), 125.24 (CH), 126.41 (CH), 127.10 (C), 128.59 (C), 129.78 (CH), 131.42 (C), 133.29 (CH), 134.11 (C), 142.26 (C), 144.82 (C), 152.46 (C), 153.50 (C), 154.14 (C), 155.48 (C), 160.18 (CO of coumarin). Anal.Calcd. for $C_{28}H_{19}NO_4$: C, 77.59; H, 4.42; N, 3.23 %. Found: C, 77.65; H, 4.48; N, 3.29 %.

4, 5-Benzo-2- (benzofuranyl-2-yl) -9-methyl-4- (p-tolyl) pyrido [3, 2-c] coumarin (4g): Yield: 66%., Amorphous powder: m.p.257-259˚C., IR (KBr, ν_{max}, cm^{-1}): 1712 (C=O stretching of δ-lactone of coumarin), 1612 and 1504 (aromatic C=C and C=N stretchings), 825 (C-H bending vibrations of p-disubstituted benzene ring), 2916 (aliphatic C-H stretching), 3070 (aromatic C-H stretching). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 2.50 (3H, singlet, CH_3), 2.29 (3H, singlet, CH_3), 7.24-8.33 (14H, multiplet, aromatic protons and C_3'-H), 8.61 (1H, poorly resolved doublet of doublet, C_{10}-H). ^{13}C-APT (100 MHz, $CDCl_3$, δ): 21.12 (CH_3), 21.44 (CH_3), 112.41 (CH), 115.99 (C), 116.58 (CH), 119.01 (C), 121.81 (CH), 123.68 (CH), 124.84 (C), 125.19 (CH), 125.22 (CH), 126.35 (C), 126.60 (CH), 126.98 (C), 127.81 (CH), 128.07 (CH), 128.31 (CH), 128.85 (CH), 128.90 (CH), 128.97 (C), 129.89 (C), 130.58 (C), 133.26 (CH), 134.06 (C), 136.44 (C), 138.46 (C), 152.43 (C), 153.54 (C), 153.96 (C), 160.66 (CO of coumarin). Anal.Calcd. for $C_{32}H_{21}NO_3$: C, 82.21; H, 4.53; N, 3.00 %. Found: C, 82.29; H, 4.59; N, 3.08 %.

4, 5-Benzo-2- (benzofuranyl-2-yl) -9-methyl-4- (p-methoxyphenyl) pyrido [3, 2-c] coumarin (4h): Yield: 63%., Amorphous powder: m.p.288-290˚C., IR (KBr, ν_{max}, cm^{-1}): 1728 (C=O stretching of δ-lactone of coumarin), 1604 and 1512 (aromatic C=C and C=N stretchings), 818 (C-H bending vibrations of p-disubstituted benzene ring), 2908 (aliphatic C-H stretchings), 3032 (aromatic C-H stretchings).^{1}H-NMR (400 MHz, $CDCl_3$, δ): 2.59 (3H, singlet, CH_3), 4.01 (3H, singlet, OCH_3), 7.12-8.24 (13H, multiplet, aromatic protons), 8.52 (1H, singlet, proton at C_3'), 8.73 (1H, poorly resolved doublet, C_{10}-H).^{13}C-APT (100 MHz, $CDCl_3$, δ): 20.60 (CH_3), 55.66 (OCH_3), 111.54 (C), 111.85 (CH), 112.67 (C), 114.46 (CH), 115.77 (C), 117.71 (CH), 119.42 (C), 123.11 (CH), 123.40 (CH), 124.04 (CH), 124.43 (C), 126.04 (C), 126.67 (CH), 127.51 (C), 127.82 (CH), 128.55 (C), 129.07 (CH), 130.01 (CH), 130.40 (CH), 130.69 (C), 132.82 (CH), 136.67 (C), 137.74 (CH), 144.64 (C), 149.04 (C), 151.19 (C), 156.56 (C), 162.06 (CO of coumarin). Anal.Calcd. for $C_{32}H_{21}NO_4$: C, 79.49; H, 4.38; N, 2.90 %. Found: C, 79.53; H, 4.44; N, 2.96 %.

2- (Benzofuranyl-2-yl) -9-chloro-4- (p-tolyl) pyrido [3, 2-c] coumarin (4i): Yield: 58%., Amorphous powder: m.p.255-257˚C., IR (KBr, ν_{max}, cm^{-1}): 1720 (C=O stretching of δ-lactone of coumarin), 1627 and 1519 (aromatic C=C and C=N stretchings), 833 (C-H bending vibrations of p-disubstituted benzene ring), 2893 (aliphatic C-H stretchings), 3024 (aromatic C-H stretchings). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 2.49 (3H, singlet, CH_3), 7.31-7.90 (11H, multiplet, aromatic protons), 7.97 (1H, singlet, proton at C_3'), 8.75 (1H, doublet, J = 2.4 Hz, C_{10}-H).^{13}C-APT (100 MHz, $CDCl_3$, δ): 21.54 (CH_3), 109.19 (CH), 111.91 (CH), 118.33 (CH), 118.74 (C), 120.80 (C), 122.36 (CH), 122.77 (CH),

123.76 (CH), 125.16 (CH), 126.81 (CH), 128.13 (CH), 128.62 (C), 129.03 (CH), 130.02 (C), 132.24 (CH), 134.96 (C), 135.95 (C), 138.83 (C), 140.07 (C), 142.62 (C), 152.67 (C), 153.74 (C), 156.12 (C), 158.68 (CO of coumarin). Anal.Calcd. for $C_{27}H_{16}ClNO_3$: C, 74.06; H, 3.68; N, 3.20 %. Found: C, 74.12; H, 3.72; N, 3.26 %.

2- (Benzofuranyl-2-yl) -9-chloro-4- (p-methoxyphenyl) pyrido [3, 2-c] coumarin (4j): Yield: 59%., Amorphous powder: m.p.230-232˚C., IR (KBr, ν_{max}, cm^{-1}): 1713 (C=O stretching of δ-lactone of coumarin), 1620 and 1488 (aromatic C=C and C=N stretchings), 817 (C-H bending vibrations of p-disubstituted benzene ring), 2901 (aliphatic C-H stretchings), 3063 (aromatic C-H stretchings).^{1}H-NMR (400 MHz, $CDCl_3$, δ): 3.98 (3H, singlet, OCH_3), 7.09-7.89 (11H, multiplet, aromatic protons), 7.93 (1H, singlet, proton at C_3'), 8.57 (1H, poorly resolved doublet, C_{10}-H).^{13}C-APT (100 MHz, $CDCl_3$, δ): 55.24 (OCH_3), 114.55 (CH), 114.66 (CH), 116.17 (CH), 117.12 (C), 117.96 (CH), 118.62 (CH), 119.59 (C), 120.44 (C), 121.73 (CH), 123.23 (CH), 123.92 (C), 124.63 (CH), 125.38 (C), 128.17 (CH), 129.46 (CH), 130.74 (C), 134.00 (C), 136.79 (CH), 140.65 (C), 142.86 (C), 144.69 (C), 148.03 (C), 152.30 (C), 161.10 (CO of coumarin). Anal.Calcd. for $C_{27}H_{16}ClNO_4$: C, 71.45; H, 3.55; N, 3.09 %. Found: C, 71.51; H, 3.61; N, 3.13 %.

4, 5-Benzo-2- (benzofuranyl-2-yl) -9-chloro-4- (p-tolyl) pyrido [3, 2-c] coumarin (4k): Yield: 61%., Amorphous powder: m.p.274-276˚C., IR (KBr, ν_{max}, cm^{-1}): 1728 (C=O stretching of δ-lactone of coumarin), 1605 and 1535 (aromatic C=C and C=N stretchings), 833 (C-H bending vibrations of p-disubstituted benzene ring), 2916 (aliphatic C-H stretchings), 3055 (aromatic C-H stretchings).^{1}H-NMR (400 MHz, $CDCl_3$, δ): 2.50 (3H, singlet, CH_3), 7.31-8.35 (14H, multiplet, aromatic protons and C_3'-H), 8.79 (1H, poorly resolved doublet, C_{10}-H).^{13}C-APT (100 MHz, $CDCl_3$, δ): 21.25 (CH_3), 104.68 (CH), 112.49 (CH), 116.03 (CH), 118.34 (CH), 122.35 (C), 123.72 (C), 124.84 (CH), 125.11 (C), 125.30 (CH), 126.35 (CH), 126.61 (C), 126.97 (CH), 127.09 (CH), 128.01 (C), 128.10 (CH), 128.83 (CH), 128.88 (C), 128.97 (CH), 129.98 (C), 130.69 (CH), 132.26 (C), 136.26 (C), 148.91 (C), 150.01 (C), 152.13 (C), 160.70 (CO of coumarin). Anal.Calcd. for $C_{31}H_{18}ClNO_3$: C, 76.31; H, 3.72; N, 2.87 %. Found: C, 76.36; H, 3.76; N, 2.91 %.

4, 5-Benzo-2- (benzofuranyl-2-yl) -9-chloro-4- (p-methoxyphenyl) pyrido [3, 2-c] coumarin (4l): Yield: 62%., Amorphous powder: m.p.296-298˚C., IR (KBr, ν_{max}, cm^{-1}): 1736 (C=O stretching of δ-lactone of coumarin), 1604 and 1520 (aromatic C=C and C=N stretchings), 825 (C-H bending vibrations of p-disubstituted benzene ring), 2901 (aliphatic C-H stretchings), 3070 (aromatic C-H stretchings). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 3.93 (3H, singlet, OCH_3), 7.05-8.38 (14H, multiplet, aromatic protons and C_3'-H), 8.81 (1H, doublet, J = 2.8 Hz, C_{10}-H).^{13}C-APT (100 MHz, $CDCl_3$, δ): 55.27 (OCH_3), 107.02 (C), 108.30 (CH), 112.36 (CH), 113.86 (CH), 116.10 (C), 118.45 (CH), 120.81 (C), 122.41 (CH), 123.91 (CH), 125.51 (CH), 127.01 (CH), 128.18 (CH), 129.15 (CH), 129.79 (C), 132.03 (CH), 133.96 (C), 135.13 (CH), 136.20 (CH), 137.27 (C), 138.45 (C), 139.09 (C), 140.80 (C), 142.08 (C), 155.66 (C), 157.80 (C), 160.15 (CO of coumarin). Anal.Calcd. for $C_{31}H_{18}ClNO_4$: C, 73.89; H, 3.60; N, 2.78 %. Found: C, 73.93; H, 3.66; N, 2.82 %.

3. Results and Discussion

3.1. Chemistry

With a view to synthesize some new benzofuranyl substituted pyrido [3, 2-c] coumarins by adopting a new synthetic route the present work was carried out. In the present work, various 4-aryl-2- (benzofuranyl) pyrido [3, 2-c] coumarins (**4a-l)** have been synthesized by reacting 4-hydroxy coumarins (**1a-c)** with appropriate 1-(benzofuran-2-yl) -3-aryl-prop-2-en-1-ones (chalcones) (**3a-d)** in the presence of ammonium acetate in refluxing acetic acid (**Scheme-1).** The reactions proceeded smoothly and gave expected products (**4a-l)** in moderate yields (55-67%). The structures of all synthesized compounds (**4a-l)** were established by IR, ^{1}H-NMR, ^{13}C-APT and selected mass spectral data are shown in experimental section.

Table 1: Total number of compound synthesized

	R	R_1	R_2	R_3		R	R_1	R_2	R_3
4a:	H	H	H	CH_3	**4g:**	CH_3	-Benzo-		CH_3
4b:	H	H	H	OCH_3	**4h:**	CH_3	-Benzo-		OCH_3
4c:	H	-Benzo-		CH_3	**4i:**	Cl	H	H	CH_3
4d:	H	-Benzo-		OCH_3	**4j:**	Cl	H	H	OCH_3
4e:	CH_3	H	H	CH_3	**4k:**	Cl	-Benzo-		CH_3
4f:	CH_3	H	H	OCH_3	**4l:**	Cl	-Benzo-		OCH_3

3.2. Biological results

3.2.1. Antimicrobial activity

The newly synthesized target compounds (**4a-l)** 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 [32]. 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 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 millilitre by comparing the turbidity. Each synthesized compound was diluted with DMSO so as to have the stock solution of 2000 µg/mL concentration as a stock solution. The results were recorded in the form of primary and secondary screening. The synthesized compounds (**4a-l)** 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 primary screening were further screened in a second set of dilution at concentrations of 200, 100, 62.5, 50 and 25 µg/mL. The suspention of 10 µL from each well was 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-2)** 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.

3.2.2 Antimicrobial evaluation

The compounds (**4a-l)** were screened for their in vitro antibacterial and antifungal evaluation against various bacterial and fungal pathogens by broth dilution method. Ampicillin, Chloramphenicol, Norfloxacin, Ciprofloxacin, Gentamycin, Griseofulvin and Nystatin were used as standard drugs. The values of MIC are summarized in **Table-2**. The antimicrobial activity results reveals that almost all the compounds **4a-l** exerted significant inhibitory activity against gram positive bacteria Bacillus subtilis and Staphylococcus aureus. Compounds **4g** and **4j** (MIC = 50µg/mL) show excellent activity against Bacillus subtilis and Staphylococcus aureus respectively compared to standard drugs Ampicillin (MIC = 250µg/mL) and have equal activity to Chloramphenicol (MIC = 50µg/mL) and Ciprofloxacin (MIC = 50µg/mL). Compounds **4a**, **4b**, **4e**, **4h** and **4i** (MIC = 100µg/mL) showed better activity against Bacillus subtilis as compared to Ampicillin (MIC = 250µg/mL) and have equal activity to Norfloxacin (MIC = 100µg/mL). Compounds **4c, 4j** (MIC = 125µg/mL) and compound **4k (**MIC = 200µg/mL) show good activity against Bacillus subtilis compared to Ampicillin (MIC = 250µg/mL) while compounds **4d**, **4f** and **4l** (MIC = 250µg/mL) were found to be equipotent to Ampicillin (MIC = 250µg/mL) against Bacillus subtilis. Compounds **4b**, **4e**, **4g** and **4i** (MIC = 62.5µg/mL) showed excellent activity against gram positive bacteria Staphylococcus aureus compared to Ampicillin (MIC = 250µg/mL). Compounds **4a**, **4c**, **4f**, **4h** and **4k** (MIC = 100µg/mL) showed better activity against Staphylococcus aureus compared to Ampicillin (MIC = 250µg/mL). Whereas, compounds **4l** (MIC = 125µg/mL) and **4d** (MIC = 200µg/mL) were found to be potent against Staphylococcus aureus as compared to Ampicillin (MIC = 250µg/mL). Compounds **4f** (MIC = 50µg/mL) showed excellent activity against Escherichia coli compared to Ampicillin (MIC = 100µg/mL) and equipotent to Chloramphenicol (MIC = 50µg/mL). Compound **4e** (MIC = 62.5µg/mL) showed excellent activity against gram negative bacteria Escherichia coli and compound **4k** (MIC = 62.5µg/mL) showed excellent activity against gram negative bacteria Salmonella typhi as compared to Ampicillin (MIC = 100µg/mL). Compounds **4a**, **4c**, **4j**, **4l** (MIC = 100µg/mL) and **4c**, **4e** and **4j** (MIC = 100µg/mL) were found to be equipotent to Ampicillin (MIC = 100µg/mL) against Escherichia coli and Salmonella typhi respectively. Compounds **4h** and **4j** (MIC = 250µg/mL) showed better activity than Griseofulvin (MIC = 500µg/mL) whereas, compounds **4c**, **4d**, **4i** and **4k** (MIC = 500µg/mL) were found equipotent to Griseofulvin (MIC = 500µg/mL) against Candida albicans. None of the tested compounds showed better activity against Aspergillus niger than standard drugs. All the compounds **4a-l** possess excellent antibacterial activity against Gram-positive bacteria Bacillus subtilis and Staphylococcus aureus. Examining the antimicrobial data, it has been observed that the derivatization of the parent molecule increased the antimicrobial potency of the synthesized analogs. The observation indicates that varying the substitution on coumarin ring i.e. R= CH_3 and Cl, increased the activity against gram-positive bacteria but decrease in activity against gram-negative bacteria was observed. Some of the compounds showed promising activity against gram-negative bacteria Escherichia coli and Salmonella typhi by altering the substituents on coumarin ring. When varying the substitution on pendant phenyl ring, i.e. R= CH_3 and OCH_3 increased the antibacterial activity. Among all the tested compounds, the compounds **4b**, **4e**, **4f**, **4g**, **4i**, **4j** and **4k** were found to be the most proficient members of the series.

Table 2: Antimicrobial activity data of all synthesized compounds

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	**100**	**100**		**100**	250		500	>1000
4b	**100**	**62.5**		250	125		1000	>1000
4c	**125**	**100**		**100**	**100**		500	**500**
4d	**250**	**200**		250	250		1000	**500**
4e	**100**	**62.5**		**62.5**	**100**		500	1000
4f	**250**	**100**		**50**	250		250	>1000
4g	**50**	**62.5**		250	200		500	>1000
4h	**100**	**100**		200	250		1000	**250**
4i	**100**	**62.5**		250	200		500	**500**
4j	**125**	**50**		**100**	**100**		500	**250**
4k	**200**	**100**		125	**62.5**		1000	**500**
4l	**250**	**125**		**100**	250		500	1000
Ampicillin	250	250		100	100		-	-

Chloramphenicol	50	50		50	50		-	-
Ciprofloxacin	50	50		25	25		-	-
Norfloxacin	100	10		10	10		-	-
Gentamycin	1	0.25		0.05	5		-	-
Griseofulvin	-	-		-	-		100	500
Nystatin	-	-		-	-		100	100
B.s.: Bacillus subtilis, **S.a.:** Staphylococcus aureus, **E.c.:** Escherichia coli, **S.t.:** Salmonella typhi, **A.n.:** Aspergillus niger, **C.a.:** Candida albicans								

4. Conclusion

The widespread distribution and potent bioactivity of various coumarins have led scientists to carry out research involving this ring system for decades. Coumarin derived compounds are a potential source of anti-microbial drugs that need further researches and it is obvious that they will be an important group in the development of new antimicrobial agents. In the structure activity studies of pyrido [3, 2-c] coumarins, significant positive results were obtained in antibacterial activity screening with the addition of substituents at different position of the coumarin core. Therefore, the development of new antimicrobial molecules by attaching appropriate functional groups to different positions around the coumarin core is an important research area.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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