A Novel and Efficient Synthesis of [1,2,3]-Triazolyl Substituted Benzo[c]Coumarins and Evaluation of their Antimicrobial Activity

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Abstract. A series of novel [1,2,3]-triazolyl substituted benzo[c]coumarins have been synthesized by reacting various 3-coumarinoyl methyl pyridinium bromide salts with 1-(5-methyl-1-phenyl-1*H*-1,2,3-triazol-4-yl)ethanones in the presence of sodium acetate in refluxing acetic acid. The structures of the synthesized compounds have been elucidated by IR, ¹H-NMR, ¹³C-NMR and Mass spectral data. All the synthesized compounds have been screened for their *in vitro* anti-bacterial and anti-fungal activities. Some of the compounds have been found to be active against some bacterial and fungal pathogens compared to standard drugs.

1. Introduction

Benzo[c]coumarins form an important class of coumarin derivatives and are isolated from plant and animal sources and are mainly metabolic products of microorganisms [1]-[3]. In literature many reports are there for isolation of natural products having benzo[c]coumarin skeleton such as urolitin B [4,5], alternariol [6], autumnariol, autumnariniol [7], altenuisol [8], arnottin [9], defucogilvocarcin [10] etc. Certain benzo[c]coumarins are reported to be used as an intermediate for the synthesis of variety of pharmaceutically interesting compounds including progesterone, androgen, glucocorticoid modulators [11]-[13], endothelial cell proliferation inhibitors [14] and anti-dyslipidemic agents [15]. Many benzo[c]coumarin derivatives are reported to possess various antibiotic [16], anticancer [17], antiviral [18], anti-mutagenic [19], anti-proliferative [20], anti-allergic [21], cytotoxic [22], AChE inhibitory activity [23], antioxidant and free radical scavenging activity [24] etc.

A literature survey for the synthesis of benzo[c]coumarins revealed that in majority of the reports on the synthesis of benzo[c]coumarins, the compounds have been synthesized by the lactonization of appropriately substituted biphenyl derivatives [25]-[27]. In the present work we have synthesized some new benzo[c]coumarin derivatives utilizing a novel and distinct approach.

Triazoles form an important class in heterocyclic chemistry. Many [1,2,3]-triazole derivatives are known for their varied biological activity such as anti-HIV [28], antimicrobial [29], antihistaminic [30], anti-proliferative [31], antiviral and cytotoxic properties [32], anti-inflammatory agents [33], β -lactamase inhibitors [34], agonists of muscarine receptors [35], antagonists of GABA receptors [36] etc. In addition to above biological activities certain [1,2,3]-triazoles are also known for their fungicidal and plant growth regulator properties and are used as agrochemicals. Certain triazole derivatives are also reported to be used in industry as dyes, corrosion inhibitors and photo stabilizers [37].

Thus considering the importance of benzo[c]coumarins and triazoles, it was thought worthwhile to synthesize a new class compounds which are hybrid of benzo[c]coumarin and [1,2,3]-triazole. Keeping this objective in mind in the present work we have synthesized some [1,2,3]-triazolyl substituted benzo[c]coumarins using a new synthetic approach.

2. Experimental

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. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker Advance 400 spectrometer operating at 400 MHz for ^1H 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. 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. Compounds 3-coumarinoyl methyl pyridinium bromide salts (**1a-c**) [38]-[40] and 1-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)ethanones (**2a-d**) [41] were prepared according to literature procedure.

2.1. General procedure for the synthesis of 7-hydroxy-9-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl) benzo[*c*]coumarins (**3a-l**)

A solution of an appropriate 3-coumarinoyl methyl pyridinium bromide salt (**1a-c**) (0.004 mol) in glacial acetic acid (40 mL) was added in a round bottom flask. To this, sodium acetate (0.012 mol) and appropriate 1-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)ethanone (0.004 mol) (**2a-d**) was added with stirring at room temperature during 10 minutes. The reaction mixture was further stirred for 20 minutes at room temperature and then refluxed for 8 hours. It was then allowed to cool to room temperature and poured into ice-cold water and extracted with chloroform (3 x 30 mL). The combined chloroform extract was washed with water (2 x 20 mL) and then dried over anhydrous sodium sulphate. The removal of chloroform under reduced pressure gave a solid product, which were subjected to column chromatography using chloroform-petroleum ether (60-80) (8:2) as an eluent to obtain the pure product (**3a-l**) (scheme-1).

2.2. Analytical and Spectral Characterization

The structure of all the twelve synthesized (**3a-l**) compounds were confirmed by their ^1H -NMR, ^{13}C - NMR, IR, elemental analysis and representative mass spectral data given below.

7-Hydroxy-9-[5'-methyl-1'-phenyl-1*H*-1,2,3-triazole-4'-yl]benzo[*c*]coumarin (3a**):** White solid; yield = 80% ; mp 227-229°C; Anal. Calcd. For $\text{C}_{22}\text{H}_{15}\text{N}_3\text{O}_3$: C, 71.54; H, 4.09; N, 11.38%. Found: C, 71.58; H, 4.14; N, 11.42%. IR (KBr, ν_{max} , cm^{-1}): 1680 (C=O stretching of δ -lactone of coumarin), 1628 (aromatic C=C stretching), 3068 (aromatic C-H stretching), 2923 (aliphatic C-H stretching), 3443 (O-H stretching). ^1H NMR (400MHz, CDCl_3 , δ) : 2.62 (3H, singlet, CH_3 of triazole ring), 7.38-8.28 (11H, multiplet, aromatic protons), 11.48 (1H, singlet, -OH proton). ^{13}C NMR (100MHz, CDCl_3 , δ) : 10.75(CH_3), 105.05(C), 111.10(CH), 113.63(CH), 117.75(CH), 118.30(C), 123.76(CH), 125.29(CH), 125.47(CH), 129.73(CH), 129.96(CH), 130.87(CH), 131.62(C), 135.71(C), 135.90(C), 140.25(C), 143.01(C), 150.75(C), 162.62(C), 165.31(CO of coumarin).

7-Hydroxy-9-[5'-methyl-1'-(*p*-tolyl)-1*H*-1,2,3-triazole-4'-yl]benzo[*c*]coumarin (3b**):** White solid; yield = 82% ; mp 206-208°C; Anal. Calcd. For $\text{C}_{23}\text{H}_{17}\text{N}_3\text{O}_3$: C, 72.05; H, 4.47; N, 10.96%. Found: C, 72.01; H, 4.42; N, 10.92%. IR (KBr, ν_{max} , cm^{-1}): 1682 (C=O stretching of δ -lactone of coumarin), 1620 (aromatic C=C stretching), 3070 (aromatic C-H stretching), 2932 (aliphatic C-H stretching), 3418 (O-H stretching). ^1H NMR (400MHz, CDCl_3 , δ): 2.50 (3H, singlet, CH_3 of phenyl ring), 2.60 (3H, singlet, CH_3 of triazole ring), 7.32-8.29 (10H, multiplet, aromatic protons), 11.49 (1H, singlet, -OH proton). ^{13}C NMR (100MHz, CDCl_3 , δ) : 10.49(CH_3), 21.59(CH_3), 111.18(CH), 113.63(CH), 117.72(CH), 123.80(CH), 125.09(CH), 125.32(CH), 130.23(CH), 130.88(CH), 131.17(C), 131.64(C), 132.05(C), 133.39(C), 135.73(C), 140.22(C), 140.35(C), 142.93(C), 145.17(C), 150.78(C), 162.70(CO of coumarin).

7-Hydroxy-9-[1'-(*p*-methoxyphenyl)-5'-methyl-1*H*-1,2,3-triazole-4'-yl]benzo[*c*]coumarin (3c**):** White solid; yield = 74% ; mp 245-247°C; Anal. Calcd. For $\text{C}_{23}\text{H}_{17}\text{N}_3\text{O}_4$: C, 69.17; H, 4.29; N, 10.52%. Found: C, 69.22; H, 4.35; N, 10.56%. IR (KBr, ν_{max} , cm^{-1}): 1674 (C=O stretching of δ -lactone of coumarin), 1620 (aromatic C=C stretching), 3063 (aromatic C-H stretching), 2932

(aliphatic C-H stretching), 3418 (O-H stretching). ^1H NMR (400MHz, CDCl_3 , δ) : 2.59 (3H, singlet, CH_3), 3.93 (3H, singlet, OCH_3), 7.09-8.31 (10H, multiplet, aromatic protons), 11.50 (1H, singlet, -OH proton). ^{13}C NMR (100MHz, CDCl_3 , δ) : 10.65(CH_3), 55.71(OCH_3), 111.09(CH), 112.32(C), 113.65(CH), 114.76(CH), 117.69(CH), 118.48(C), 123.77(CH), 125.35(CH), 126.85(CH), 128.67(C), 130.81(CH), 131.75(C), 134.60(C), 135.86(C), 140.53(C), 142.19(C), 150.88(C), 160.68(C), 162.74(CO of coumarin).

7-Hydroxy-9-(1'-(p-chlorophenyl)-5'-methyl-1*H*-1,2,3-triazole-4'-yl]benzo[c]coumarin (3d):

White solid; yield = 81% ; mp 218-220°C; Anal. Calcd. For $\text{C}_{22}\text{H}_{14}\text{ClN}_3\text{O}_3$: C, 65.43; H, 3.49; N, 10.41%. Found: C, 65.47; H, 3.45; N, 10.46%. IR (KBr, ν_{max} , cm^{-1}); 1713 (C=O stretching of δ -lactone of coumarin), 1636 (aromatic C=C stretching), 3094 (aromatic C-H stretching), 2962 (aliphatic C-H stretching), 3425 (O-H stretching). ^1H NMR (400MHz, CDCl_3 , δ) : 2.62 (3H, singlet, CH_3), 7.28-8.27 (10H, multiplet, aromatic protons), 11.49 (1H, singlet, -OH proton). ^{13}C NMR (100MHz, CDCl_3 , δ) : 10.70(CH_3), 105.19(C), 111.13(CH), 113.69(CH), 117.79(CH), 118.28(C), 123.73(CH), 125.30(CH), 126.65(CH), 130.00(CH), 130.92(CH), 131.51(C), 134.37(C), 135.79(C), 136.10(C), 139.99(C), 143.27(C), 150.78(C), 162.66(C), 165.29(CO of coumarin).

7-Hydroxy-4-methoxy-9-[5'-methyl-1'-phenyl-1*H*-1,2,3-triazole-4'-yl]benzo[c]coumarin (3e):

White solid; yield = 78% ; mp 257-259°C; Anal. Calcd. For $\text{C}_{23}\text{H}_{17}\text{N}_3\text{O}_4$: C, 69.17; H, 4.29; N, 10.52%. Found: C, 69.23 ; H, 4.33; N, 10.58%. IR (KBr, ν_{max} , cm^{-1}); 1682 (C=O stretching of δ -lactone of coumarin), 1628 (aromatic C=C stretching), 3070 (aromatic C-H stretching), 2978 (aliphatic C-H stretching), 3425 (O-H stretching). ^1H NMR (400MHz, CDCl_3 , δ) : 2.61 (3H, singlet, CH_3), 4.00 (3H, singlet, OCH_3), 6.90-8.40 (10H, multiplet, aromatic protons), 11.42 (1H, singlet, -OH proton). ^{13}C NMR (100MHz, CDCl_3 , δ) : 10.78(CH_3), 56.28(OCH_3), 105.05(C), 111.55(CH), 112.54(CH), 113.68(CH), 114.99(CH), 119.10(C), 124.70(CH), 124.93(CH), 125.46(C), 129.71(CH), 129.94(CH), 131.63(C), 135.86(C), 135.90(C), 140.11(C), 143.00(C), 148.03(C), 162.56(C), 164.74(CO of coumarin).

7-Hydroxy-4-methoxy-9-[5'-methyl-1'-(p-tolyl)-1*H*-1,2,3-triazole-4'-yl]benzo[c]coumarin (3f):

White solid; yield = 76% ; mp 197-199°C; Anal. Calcd. For $\text{C}_{24}\text{H}_{19}\text{N}_3\text{O}_4$: C, 69.72; H, 4.63; N, 10.16%. Found: C, 69.76; H, 4.68; N, 10.21%. IR (KBr, ν_{max} , cm^{-1}); 1713 (C=O stretching of δ -lactone of coumarin), 1636 (aromatic C=C stretching), 3032 (aromatic C-H stretching), 2939 (aliphatic C-H stretching), 3418 (O-H stretching). ^1H NMR (400MHz, CDCl_3 , δ) : 2.60 (3H, singlet, CH_3 of phenyl ring), 2.61 (3H, singlet, CH_3 of triazole ring), 4.01 (3H, singlet, OCH_3), 7.19-8.26 (9H, multiplet, aromatic protons), 11.51 (1H, singlet, -OH proton). ^{13}C NMR (100MHz, CDCl_3 , δ) : 10.33(CH_3), 21.51(CH_3), 56.22(OCH_3), 102.40(C), 116.08(CH), 119.74(CH), 120.16(CH), 120.31(C), 122.64(CH), 125.16(CH), 127.17(C), 130.09(CH), 130.25(CH), 132.21(C), 133.75(C), 139.48(C), 139.78(C), 142.90(C), 144.57(C), 146.93(C), 152.71(C), 162.61(CO of coumarin).

7-Hydroxy-4-methoxy-9-[1'-(p-methoxyphenyl)-5'-methyl-1*H*-1,2,3-triazole-4'-yl]benzo[c]coumarin (3g):

White solid; yield = 85% ; mp 248-250°C; Anal. Calcd. For $\text{C}_{24}\text{H}_{19}\text{N}_3\text{O}_5$: C, 67.13; H, 4.46; N, 9.79%. Found: C, 67.08 ; H, 4.42; N, 9.75%. IR (KBr, ν_{max} , cm^{-1}); 1682 (C=O stretching of δ -lactone of coumarin), 1597 (aromatic C=C stretching), 3032 (aromatic C-H stretching), 2939 (aliphatic C-H stretching), 3448 (O-H stretching). ^1H NMR (400MHz, CDCl_3 , δ) : 2.69 (3H, singlet, CH_3), 3.40 and 4.05 (6H, two singlets, OCH_3 of phenyl ring and OCH_3 of phenyl ring of coumarin), 7.20-8.07 (9H, multiplet, aromatic protons), 10.84 (1H, singlet, -OH proton). ^{13}C NMR (100MHz, CDCl_3 , δ) : 10.17(CH_3), 55.95(OCH_3), 56.52(OCH_3), 110.00(C), 112.61(CH), 112.83(C), 114.05(CH), 114.82(CH), 114.98(CH), 115.60(CH), 115.66(C), 117.54(C), 118.09(C), 118.50(C), 126.11(CH), 126.70(CH), 137.55(C), 140.91(C), 147.75(C), 151.60(C), 161.01(C), 162.47(CO of coumarin).

7-Hydroxy-4-methoxy-9-(1'-(p-chlorophenyl)-5'-methyl-1*H*-1,2,3-triazole-4'-yl)benzo[*c*]coumarin (3h): White solid; yield = 79% ; mp 267-269°C; Anal. Calcd. For C₂₃H₁₆ClN₃O₄: C, 63.67; H, 3.72; N, 9.69%. Found: C, 63.71 ; H, 3.76; N, 9.75%. IR (KBr, $\nu_{\max}$, cm⁻¹): 1713 (C=O stretching of δ -lactone of coumarin), 1597 (aromatic C=C stretching), 3032 (aromatic C-H stretching), 2939 (aliphatic C-H stretching), 3448 (O-H stretching). ¹H NMR (400MHz, CDCl₃, δ) : 2.61 (3H, singlet, CH₃), 4.02 (3H, singlet, OCH₃), 7.09-8.25 (9H, multiplet, aromatic protons), 11.52 (1H, singlet, -OH proton). ¹³C NMR (100MHz, CDCl₃, δ) : 10.90(CH₃), 56.50(OCH₃), 105.20(C), 111.66(CH), 112.50(CH), 113.70(CH), 114.95(CH), 119.12(C), 125.01(CH), 126.68(CH), 128.29(C), 130.00(CH), 131.49(C), 134.37(C), 135.82(C), 135.96(C), 136.10(C), 140.00(C), 148.12(C), 148.98(C), 162.63(CO of coumarin).

2-Bromo-7-hydroxy-9-[5'-methyl-1'-phenyl-1*H*-1,2,3-triazole-4'-yl]benzo[*c*]coumarin (3i): White solid; yield = 75% ; mp 244-246°C; Anal. Calcd. For C₂₂H₁₄BrN₃O₃: C, 58.95; H, 3.15; N, 9.37%. Found: C, 58.91 ; H, 3.11; N, 9.32%. IR (KBr, $\nu_{\max}$, cm⁻¹): 1713 (C=O stretching of δ -lactone of coumarin), 1628 (aromatic C=C stretching), 3078 (aromatic C-H stretching), 3009 (aliphatic C-H stretching), 3448 (O-H stretching). ¹H NMR (400MHz, CDCl₃, δ) : 2.62 (3H, singlet, CH₃), 7.25-8.28 (10H, multiplet, aromatic protons), 11.35 (1H, singlet, -OH proton). ¹³C NMR (100MHz, CDCl₃, δ) : 10.76(CH₃), 104.80(C), 111.19(CH), 114.24(CH), 118.35(C), 119.42(CH), 120.05(C), 125.47(CH), 126.53(CH), 129.73(CH), 130.01(CH), 131.79(C), 133.65(CH), 134.32(C), 135.82(C), 140.43(C), 142.66(C), 149.57(C), 162.65(C), 164.70(CO of coumarin).

2-Bromo-7-hydroxy-9-[5'-methyl-1'-(p-tolyl)-1*H*-1,2,3-triazole-4'-yl]benzo[*c*]coumarin (3j): White solid; yield = 71% ; mp 252-254°C; Anal. Calcd. For C₂₃H₁₆BrN₃O₃: C, 59.76; H, 3.49; N, 9.09%. Found: C, 59.80 ; H, 3.53; N, 9.13%. IR (KBr, $\nu_{\max}$, cm⁻¹): 1682 (C=O stretching of δ -lactone of coumarin), 1628 (aromatic C=C stretching), 3078 (aromatic C-H stretching), 2901 (aliphatic C-H stretching), 3441 (O-H stretching). ¹H NMR (400MHz, CDCl₃, δ) : 2.50 (3H, singlet, CH₃ of phenyl ring), 2.60 (3H, singlet, CH₃ of triazole ring), 7.27-8.31 (9H, multiplet, aromatic protons), 11.38 (1H, singlet, -OH proton). ¹³C NMR (100MHz, CDCl₃, δ) : 10.75(CH₃), 21.33(CH₃), 104.81(C), 111.21(CH), 114.24(CH), 118.36(C), 119.44(CH), 120.09(C), 125.28(CH), 126.57(CH), 130.28(CH), 131.83(C), 133.30(C), 133.66(CH), 134.35(C), 140.32(C), 140.54(C), 142.56(C), 149.59(C), 162.68(C), 164.77(CO of coumarin).

2-Bromo-7-hydroxy-9-[1'-(p-methoxyphenyl)-5'-methyl-1*H*-1,2,3-triazole-4'-yl]benzo[*c*]coumarin (3k): White solid; yield = 71% ; mp 234-236°C; Anal. Calcd. For C₂₃H₁₆BrN₃O₄: C, 57.76; H, 3.37; N, 8.79%. Found: C, 57.81 ; H, 3.42; N, 8.74%. IR (KBr, $\nu_{\max}$, cm⁻¹): 1713 (C=O stretching of δ -lactone of coumarin), 1628 (aromatic C=C stretching), 3070 (aromatic C-H stretching), 2970 (aliphatic C-H stretching), 3448 (O-H stretching). ¹H NMR (400MHz, CDCl₃, δ) : 2.59 (3H, singlet, CH₃), 3.91 (3H, singlet, OCH₃), 7.10-8.33 (9H, multiplet, aromatic protons), 11.39 (1H, singlet, -OH proton). ¹³C NMR (100MHz, CDCl₃, δ) : 10.72(CH₃), 55.73(OCH₃), 104.75(C), 111.29(CH), 114.24(CH), 114.98(CH), 118.41(C), 119.48(CH), 120.05(C), 126.52(CH), 127.01(CH), 128.81(C), 132.08(C), 133.80(CH), 134.45(C), 140.76(C), 142.47(C), 149.59(C), 153.11(C), 160.64(C), 162.69(CO of coumarin).

2-Bromo-7-hydroxy-9-[1'-(p-chlorophenyl)-5'-methyl-1*H*-1,2,3-triazole-4'-yl]benzo[*c*]coumarin (3l): White solid; yield = 76% ; mp 291-293°C; Anal. Calcd. For C₂₃H₁₄BrClN₃O₃: C, 54.74; H, 2.71; N, 8.70%. Found: C, 54.70 ; H, 2.67; N, 8.65%. IR (KBr, $\nu_{\max}$, cm⁻¹): 1705 (C=O stretching of δ -lactone of coumarin), 1628 (aromatic C=C stretching), 3078 (aromatic C-H stretching), 3009 (aliphatic C-H stretching), 3448 (O-H stretching). ¹H NMR (400MHz, CDCl₃, δ) : 2.68 (3H, singlet, CH₃), 7.34-8.26 (9H, multiplet, aromatic protons), 10.87 (1H, singlet, -OH proton). ¹³C NMR (100MHz, CDCl₃, δ) : 10.14(CH₃), 109.97(C), 112.19(CH), 112.80(C), 115.56(CH), 115.63(C), 118.46(C), 119.07(C), 119.37(C), 119.59(CH), 126.43(CH), 126.58(CH), 130.72(CH), 132.25(C), 134.67(C), 134.89(CH), 135.89(C), 138.61(C), 149.27(C), 165.58(CO of coumarin).

3. Results and discussion

3.1. Chemistry

With a view to synthesizing some new [1,2,3] triazolyl benzo[*c*]coumarins adopting new synthetic route the present work was carried out. In the present work, various 7-hydroxy-9-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl) benzo[*c*]coumarins (**3a-l**) have been synthesized by reacting appropriate 3-coumarinoyl methyl pyridinium bromide salt (**1a-c**) with various 1-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)ethanones (**2a-d**) in the presence of sodium acetate in refluxing acetic acid. The required triazole derivatives (**2a-d**) were prepared by reacting appropriate aryl azide with acetyl acetone in the presence of potassium carbonate (**Scheme-1**). The structures of all the synthesized compounds (**3a-l**) were established by IR, ¹H-NMR, ¹³C-NMR and selected mass spectral data are shown in experimental section 2.2.

Compound	R	R ₁	R ₂	Compound	R	R ₁	R ₂
3a	H	H	H	3g	OCH ₃	H	OCH ₃
3b	H	H	CH ₃	3h	OCH ₃	H	Cl
3c	H	H	OCH ₃	3i	H	Br	H
3d	H	H	Cl	3j	H	Br	CH ₃
3e	OCH ₃	H	H	3k	H	Br	OCH ₃
3f	OCH ₃	H	CH ₃	3l	H	Br	Cl

Scheme 1: Synthetic scheme for the compounds (**3a-l**)

3.2. Mechanism

It is reported that reaction of α,β -unsaturated ketone with methyl ketone or ketone with active methylene group in the presence of sodium acetate results in a formation of 1,3,5-trisubstituted benzene derivatives [42]. This novel reaction is utilized in the preparation of present compounds. Here the benzene ring has been built up between 3 and 4 position of coumarin nucleus by utilizing this reaction. The plausible mechanism for the synthesis of target compounds (**3a-l**) is shown in **Scheme-2**. The reaction proceeds by the anion generation from the acetyl group of compound (**2a-d**). This anion further adds to the 3,4-double bond of coumarin (**1a-c**) and results in the formation of intermediate (**A**) having 1,5-dione functionality. The active methylene group in (**A**) then gets cyclized with carbonyl group of [1,2,3]-triazole moiety resulting in the formation of intermediate (**B**) which finally gets converted into the product by loss of water molecule and subsequent aromatization. The reaction is smooth and results the triazolyl substituted benzo[*c*]coumarins (**3a-l**) in good yield.

Scheme 2: Plausible mechanism for the synthesis of target compounds (3a-l)

3.3. Biological results

3.3.1 Antimicrobial activity

The newly synthesized target compounds (**3a-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 [43]. 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 $\mu\text{g/mL}$ concentration as a stock solution. The results were recorded in the

form of primary and secondary screening. The synthesized compounds (**3a-l**) were screened for their antibacterial and antifungal activity at the concentration of 1000, 500 and 250 $\mu\text{g/mL}$ 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 $\mu\text{g/mL}$. The suspension 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.

3.3.2. Antimicrobial evaluation

The compounds (**3a-l**) 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**.

Review of the antimicrobial activities of synthesized compounds (**3a-l**) in (**Table-1**) indicated that compounds **3e** (MIC=100, $\mu\text{g/mL}$) exhibited excellent activity toward Gram-positive bacteria *Bacillus subtilis* as compared to Ampicillin (MIC=250, $\mu\text{g/mL}$) and showed equipotent activity to Norfloxacin (MIC=100, $\mu\text{g/mL}$). Against Gram-positive bacteria *Bacillus subtilis*, compound **3k** (MIC=150, $\mu\text{g/mL}$) showed activity higher than that of Ampicillin (MIC=250, $\mu\text{g/mL}$). Compounds **3b**, **3f** and **3j** (MIC=200, $\mu\text{g/mL}$) displayed better activity than Ampicillin (MIC=250, $\mu\text{g/mL}$) toward Gram-positive bacteria *Bacillus subtilis*. Compounds **3a**, **3h** and **3i** (MIC=250, $\mu\text{g/mL}$) showed results equivalent to that of Ampicillin (MIC=250, $\mu\text{g/mL}$) toward Gram-positive bacteria *Bacillus subtilis*. Against Gram-positive bacteria *Staphylococcus aureus*, compound **3e** (MIC=62.5, $\mu\text{g/mL}$) showed excellent activity as compared to Ampicillin (MIC=250, $\mu\text{g/mL}$). Compounds **3f** and **3k** (MIC=100, $\mu\text{g/mL}$) were found to be more effective against Gram-positive bacteria *Staphylococcus aureus* than Ampicillin (MIC=250, $\mu\text{g/mL}$). Compounds **3b**, **3g** and **3j** (MIC=200, $\mu\text{g/mL}$) showed good activity against Gram-positive bacteria *Staphylococcus aureus* as compared to Ampicillin (MIC=250, $\mu\text{g/mL}$). Against Gram-positive bacteria *Staphylococcus aureus*, compound **3a** and **3i** (MIC=250, $\mu\text{g/mL}$) showed equipotent activity to that of Ampicillin (MIC=250, $\mu\text{g/mL}$).

Moreover, Against Gram-negative bacteria *Escherichia coli*, compounds **3i** and **3j** (MIC=62.5, $\mu\text{g/mL}$) showed excellent activity as compared to Ampicillin (MIC=100, $\mu\text{g/mL}$). Where as compounds **3a**, **3h** and **3k** (MIC=100, $\mu\text{g/mL}$) showed activity comparable to Ampicillin (MIC=100, $\mu\text{g/mL}$) toward Gram-negative bacteria *Escherichia coli*. Against Gram-negative bacteria *Salmonella typhi*, compounds **3c**, **3h**, **3i**, **3j** and **3k** (MIC=100, $\mu\text{g/mL}$) were found to be equipotent with Ampicillin (MIC=100, $\mu\text{g/mL}$).

Furthermore, against *Candida albicans* fungal pathogen, however compound **3f** (MIC=250, $\mu\text{g/mL}$) showed better inhibition action as compare to the standard drug Griseofulvin (MIC=500, $\mu\text{g/mL}$). Where as compounds **3b**, **3h** and **3i** (MIC=500, $\mu\text{g/mL}$) showed activity comparable to Griseofulvin (MIC=500, $\mu\text{g/mL}$) against fungal pathogen *Candida albicans*.

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).

Table 1: *In vitro* Antimicrobial activity of compounds (3a-l)

Compound	Minimum Inhibitory Concentration (MIC, $\mu\text{g mL}^{-1}$)					
	Gram +ve bacteria			Gram –ve bacteria		Fungi
	<i>B.s.</i>	<i>S.a.</i>		<i>E.c.</i>	<i>S.t.</i>	
	MTCC441	MTCC96		MTCC443	MTCC98	MTCC282 MTCC227
3a	250	250		100	125	500 1000
3b	200	200		200	200	1000 500
3c	500	500		500	100	200 >1000
3d	500	500		250	200	250 >1000
3e	100	62.5		250	250	500 1000
3f	200	100		200	125	>1000 250
3g	500	200		200	200	1000 >1000
3h	250	500		100	100	1000 500
3i	250	250		62.5	100	1000 500
3j	200	200		62.5	100	250 >1000
3k	150	100		100	100	1000 1000
3l	500	500		200	250	>1000 1000
Ampicillin	250	250		100	100	- -
Chloramphenicol	50	50		50	50	- -
Norfloxacine	100	10		10	10	- -
Griseofulvin	-	-		-	-	100 500
Nystatin	-	-		-	-	100 100

4. Conclusions

A novel and efficient protocol for the synthesis of a series of [1,2,3]-triazolyl substituted benzo[*c*]coumarin derivatives was described and the synthesized compounds were screened for their *in vitro* antimicrobial evaluation. The results indicated that all the synthesized compounds shown good antibacterial activity. In particular, compounds **3e**, **3f**, **3i**, **3j**, and **3k** exhibited the more potent inhibitory activity against bacterial and fungal pathogens as compared to other compounds and emerged as potential lead compounds for further investigations.

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