Research Article CODEN: IJPRNK Impact Factor: 5.567 ISSN: 2277-8713
Krunal N. Patel, IJPRBS, 2017; Volume 6(2): 59-74 IJPRBS

INTERNATIONAL JOURNAL OF
PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

SYNTHESIS OF SOME 1,2,3-TRIAZOLYL PYRIDYL SUBSTITUTED COUMARINS AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITY

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Accepted Date: 12/03/2017; Published Date: 27/04/2017

Abstract: The synthesis of various 3-[6-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)-4-arylpyridin-2-yl]coumarins **(5a-r)** has been carried out. The target compounds have been synthesized by reacting 3-cinnamoyl coumarins **(3a-f)** with 1,2,3-triazoloyl methyl pyridinium salt **(4a-c)** in the presence of ammonium acetate in glacial acetic acid under Krohnke's reaction condition. All the synthesized compounds were characterized by IR, ^{1}H-NMR, ^{13}C-APT and representative mass spectral analysis. All the compounds were screened for their antimicrobial activity.

Keywords: 1,2,3-triazole, pyridyl coumarin, *Krohnke* reaction, antimicrobial activity.

PAPER-QR CODE

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Access Online On:

www.ijprbs.com

How to Cite This Article:

Krunal N. Patel, IJPRBS, 2017; Volume 6(2): 59-74

INTRODUCTION

Coumarins constitute an important class of benzopyrones, exhibiting a broad range of biological activities such as antimicrobial[1], anticoagulant[2], antiinflammatory[3] and antioxidant[4]. Pyridyl substituted coumarins are reported to have interesting biological activities such as CNS depressant[5], antifungal[6] and antibacterial[7]. The 1,2,3-triazole based derivatives have received much attention due to their wide coverage of biological properties including antiviral[8], anti-HIV[9], anticonvulsants[10], and anti-allergic[11]. In addition, compounds having 1,2,3-triazole group have found industrial applications as dyes, corrosion inhibitors, sensors and photo-stabilizers[12]. Encouraged by the interesting biological properties of pyridyl substituted coumarins and 1,2,3-triazoles, in the present work synthesis of some triazolyl pyridyl substituted coumarins using a *Krohnke's* reaction has been reported.

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 FT-IR 8400-S spectrometer. ^{1}H NMR and ^{13}C APT spectra were recorded on Bruker Advance400 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. Column chromatography was performed with silica gel 60–120 mesh (Merck, Mumbai, India.). The rection was monitored using silica gel 60 F254 TLC plates and their spots were visualized by exposure to a UV lamp, iodine vapour or $KMnO_4$ reagents.

The required 3-acetyl coumarins **(1a-c)**[13], 3-cinnamoyl coumarins **(3a-f)**[14,15 and] 2-bromo-1-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)ethanone[16] were prepared using reported procedures.

2.1. Preparation of 1,2,3-triazoloyl methyl pyridinium bromide salts (4a-c) :

The following general procedure was used.

In a 100 mL round bottom flask fitted with a reflux condenser, a solution of appropriate 2-bromo-1-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)ethanone (0.01 mol) in dry THF (30 mL) was taken and pyridine (0.01 mol) was added. The reaction mixture was refluxed in an oil bath for 2 hours. It was then allowed to come to room temperature and was left for 4 to 5 hours. The pyridinium bromide salt was separated out which was filtered out, washed with THF and dried.

Compound 4a: R_3 =H, Yield = 78 %, Mp:221-222°C

Molecular Formula : $C_{16}H_{15}BrN_4O$

Analysis	% C	% H	% N
Found	54.45	4.16	15.55
Calculated	53.50	4.21	15.60
IR (cm^{-1})	ν_{max} 1694 (C=O stretching of carbonyl group), 1602 and 1534 (aromatic C=C and C=N stretchings), 695 and 745 (C-H out of plane bending vibrations for mono substituted benzene ring), 3063 (aromatic C-H stretching), 2932 (aliphatic C-H stretching).		
^{1}H-NMR (, ppm) **($CDCl_3$)**	2.47 (3H, singlet, CH_3), 6.16 (2H, singlet, CH_2), 7.01-9.06 (10H, multiplet, fourteen aromatic protons).		

Compound 4b[16] : R_3 =CH_3, Yield = 71 %, Mp:268-270°C

Compound 4c : R_3 =OCH_3, Yield = 74 %, Mp:248-250°C

Molecular Formula : $C_{17}H_{17}BrN_4O$

Analysis	% C	% H	% N
Found	54.51	4.35	14.44
Calculated	52.46	4.40	14.39
IR (cm^{-1})	ν_{max} 1702 (C=O stretching of carbonyl group), 1610 and 1545 (aromatic C=C and C=N stretchings), 833 (C-H bending vibrations of p-disubstituted benzene ring), 3061 (aromatic C-H stretching), 2928 (aliphatic C-H stretching).		
^{1}H-NMR (, ppm) **($CDCl_3$)**	2.49 (3H, singlet, CH_3), 3.91 (3H, singlet, OCH_3), 6.21 (2H, singlet, CH_2), 7.60-9.02 (9H, multiplet, fourteen aromatic protons).		

2.1. General procedure for the synthesis of 3-[6-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)-4-phenylpyridin-2-yl]coumarins (5a-r) :

The following general procedure was used.

In a 100 mL round bottom flask equipped with a dropping funnel, condenser, guard tube and magnetic needle, appropriate 1,2,3-triazoloyl methyl pyridinium salt **(4a-c)** (0.003 mol) in glacial acetic acid (15 mL) was taken. To this ammonium acetate (0.03 mol) was added with stirring at room temperature. Then a solution of appropriate 3-cinnamoyl coumarin **(3a-f)** (0.003 mol) in glacial acetic acid (15 mL) was added with stirring at room temperature during 15 minutes. The reaction mixture was further stirred for 1 hour at room temperature and then it was 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 sulfate. The removal of chloroform under reduced pressure gave crude material which was subjected to column chromatography using silica gel and chloroform-pet ether (6:4) as an eluent to give products **(5a-r)**. The compounds were recrystallized from chloroform-hexane.

The physical, analytical and spectral data for the compounds **(5a-r)** are given below.

Compound 5a : white solid; yield 68 %; mp 278-280°C; Anal. Calcd. for $C_{29}H_{20}N_4O_2$: C, 76.30; H, 4.42; N, 12.27%. Found: C, 76.25; H, 4.37; N, 12.32%. IR (KBr, ν_{max}, cm^{-1}); 1714 (C=O stretching of □-lactone of coumarin), 1606 and 1550 (aromatic C=C and C=N stretchings), 3060 (aromatic C-H stretching), 2936 (aliphatic C-H stretching), 701 and 750 (C-H out of plane bending vibrations for mono substituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 2.78 (3H, singlet, CH_3), 7.18-7.79 (14H, multiplet, aromatic protons), 8.46 and 8.57 (2H, poorly resolved doublet, C'$_3$-H and C'$_5$-H), 8.73 (1H, singlet, C_4-H); ^{13}C APT (100MHz, $CDCl_3$, δ): 10.31(CH_3), 108.13(CH), 110.26(CH), 111.31(CH), 111.94(CH), 112.51(CH), 115.46(C), 117.59(CH), 120.68(CH), 122.65(CH), 125.78(CH), 126.35(CH), 127.68(C), 129.31(CH), 130.57(C), 132.87(CH), 139.56(CH), 144.28(C), 147.31(C), 149.46(C), 149.96(C), 152.82(C), 153.56(C), 157.11(C), 160.63(CO of coumarin). The mass spectrum of compound showed M^+ peak at 456(38%) (m/z%) along with some fragments peaks at 428(100%), 324(22%),130(23%) and 77(%). The appearance of molecular ion peak at 456 mass unit supports the structure of compound 5a.

Compound 5b : white solid; yield 72 %; mp 263-265°C; Anal. Calcd. for $C_{30}H_{22}N_4O_2$: C, 76.58; H, 4.71; N, 11.91%. Found: C, 76.63; H, 4.67; N, 11.86%. IR (KBr, ν_{max}, cm^{-1}); 1720 (C=O stretching of □-lactone of coumarin), 1604 and 1544 (aromatic C=C and C=N stretchings), 3063 (aromatic C-H stretching), 2932 (aliphatic C-H stretching), 826 (C-H bending vibrations of p-disubstituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 2.53 (3H, singlet, CH_3), 2.86 (3H, singlet, CH_3), 7.04-

7.85 (13H, multiplet, aromatic protons), 8.57 and 8.61 (2H, poorly resolved doublet, C'$_3$-H and C'$_5$-H), 8.73 (1H, singlet, C$_4$-H); ^{13}C APT (100MHz, CDCl$_3$, δ): 10.25(CH_3), 22.37(CH_3), 108.35(CH), 110.26(CH), 112.48(CH), 115.30(C), 120.97(CH), 123.18(CH), 125.21(CH), 126.20(CH), 127.51(C), 128.50(CH), 129.03(C), 129.07(CH), 129.48(C), 133.37(CH), 138.12(C), 139.12(CH), 144.17(CH), 148.09(C), 152.87(C), 153.25(C), 153.34(C), 157.12(C), 159.46(C), 162.59(CO of coumarin).

Compound 5c : white solid; yield 74 %; mp >300°C; Anal. Calcd. for $C_{30}H_{22}N_4O_3$: C, 74.06; H, 4.56; N, 11.52%. Found: C, 74.11; H, 4.61; N, 11.47%. IR (KBr, ν_{max}, cm^{-1}); 1722 (C=O stretching of □-lactone of coumarin), 1610 and 1549 (aromatic C=C and C=N stretchings), 3059 (aromatic C-H stretching), 2934 (aliphatic C-H stretching), 831 (C-H bending vibrations of p-disubstituted benzene ring); ^{1}H NMR (400MHz, CDCl$_3$, δ): 2.87 (3H, singlet, CH_3), 3.91 (3H, singlet, OCH_3), 7.06-7.87 (13H, multiplet, aromatic protons), 8.52 and 8.63 (2H, poorly resolved doublet, C'$_3$-H and C'$_5$-H), 8.82 (1H, singlet, C$_4$-H); ^{13}C APT (100MHz, CDCl$_3$, δ): 10.29(CH_3), 56.13(OCH_3), 107.53(CH), 110.19(CH), 110.29(CH), 111.42(CH), 112.21(CH), 115.29(C), 116.69(CH), 119.70(CH), 122.46(CH), 123.88(CH), 126.81(CH), 129.91(C), 133.60(CH), 134.20(CH), 148.13(C), 149.78(C), 150.53(C), 150.69(C), 150.96(C), 151.72(C), 153.47(C), 153.61(C), 154.65(C), 160.20(CO of coumarin).

Compound 5d : white solid; yield 70 %; mp >300°C; Anal. Calcd. for $C_{30}H_{22}N_4O_3$: 74.06; H, 4.56; N, 11.52%. Found: C, 74.10; H, 4.62; N, 11.48%. IR (KBr, ν_{max}, cm^{-1}); 1720 (C=O stretching of □-lactone of coumarin), 1612 and 1542 (aromatic C=C and C=N stretchings), 3064 (aromatic C-H stretching), 2928 (aliphatic C-H stretching), 833 (C-H bending vibrations of p-disubstituted benzene ring); ^{1}H NMR (400MHz, CDCl$_3$, δ): 2.82 (3H, singlet, CH_3), 3.92 (3H, singlet, OCH_3), 7.11-7.88 (13H, multiplet, aromatic protons), 8.54 and 8.65 (2H, poorly resolved doublet, C'$_3$-H and C'$_5$-H), 8.83 (1H, singlet, C$_4$-H); ^{13}C APT (100MHz, CDCl$_3$, δ): 11.17(CH_3), 56.57(OCH_3), 111.68(CH), 116.63(CH), 119.51(C), 120.74(CH), 121.25(CH), 123.38(CH), 124.55(CH), 124.77(C), 125.22(CH), 126.41(CH), 127.19(C), 128.79(C), 128.85(CH), 128.90(C), 132.33(CH), 133.87(CH), 136.36(C), 142.67(CH), 146.47(C), 149.22(C), 151.35(C), 154.15(C), 155.77(C), 160.24(CO of coumarin).

Compound 5e : white solid; yield 72 %; mp 280-282°C; Anal. Calcd. for $C_{31}H_{24}N_4O_3$: 74.38; H, 4.83; N, 11.19%. Found: C, 74.43; H, 4.78; N, 11.24%. IR (KBr, ν_{max}, cm^{-1}); 1726 (C=O stretching of □-lactone of coumarin), 1608 and 1546 (aromatic C=C and C=N stretchings), 3060 (aromatic C-H stretching), 2930 (aliphatic C-H stretching), 828 (C-H bending vibrations of p-disubstituted benzene ring); ^{1}H NMR (400MHz, CDCl$_3$, δ): 2.68 (3H, singlet, CH_3), 2.85 (3H, singlet, CH_3), 3.91 (3H, singlet, OCH_3), 6.98-8.38 (12H, multiplet, aromatic protons), 8.51 and 8.63 (2H, poorly resolved doublet, C'$_3$-H and C'$_5$-H), 8.82 (1H, singlet, C$_4$-H); ^{13}C APT (100MHz, CDCl$_3$, δ): 10.34(CH_3), 22.11(CH_3), 56.17(OCH_3), 112.63(CH), 113.49(C), 115.32(C), 117.60(CH), 118.68(CH), 119.08(CH), 120.68(C), 124.56(CH), 125.36(CH), 126.11(CH), 127.82(C), 129.42(C), 130.96(C),

132.90(CH), 133.70(CH), 134.27(CH), 136.90(CH), 143.00(C), 149.34(C), 151.05(C), 151.97(C), 153.91(C), 154.31(C), 160.36(CO of coumarin).

Compound 5f : white solid; yield 67 %; mp >300°C; Anal. Calcd. for $C_{31}H_{24}N_4O_4$: 72.08; H, 4.68; N, 10.85%. Found: C, 72.13; H, 4.72; N, 10.90%. IR (KBr, ν_{max}, cm^{-1}); 1714 (C=O stretching of □-lactone of coumarin), 1610 and 1551 (aromatic C=C and C=N stretchings), 3058 (aromatic C-H stretching), 2934 (aliphatic C-H stretching), 833 (C-H bending vibrations of p-disubstituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 2.82 (3H, singlet, CH_3), 3.91 (3H, singlet, OCH_3), 3.93 (3H, singlet, OCH_3), 7.05-7.85 (12H, multiplet, aromatic protons), 8.50 and 8.62 (2H, poorly resolved doublet, C'$_3$-H and C'$_5$-H), 8.81 (1H, singlet, C_4-H); ^{13}C APT (100MHz, $CDCl_3$, δ): 10.87(CH_3), 55.37(OCH_3), 56.41(OCH_3), 111.74(CH), 116.37(C), 119.37(CH), 119.57(C), 121.02(C), 121.62(CH), 123.29(CH), 123.79(CH), 124.72(C), 125.33(CH), 125.41(CH), 127.57(CH), 128.39(C), 130.15(CH), 130.29(CH), 133.08(CH), 139.03(C), 141.26(C), 143.14(CH), 149.63(C), 151.11(C), 153.89(C), 154.37(C), 159.74(CO of coumarin).

Compound 5g : yellow solid; yield 74 %; mp 276-278°C; Anal. Calcd. for $C_{30}H_{22}N_4O_3$: 74.06; H, 4.56; N, 11.52%. Found: C, 74.11; H, 4.60; N, 11.47%. IR (KBr, ν_{max}, cm^{-1}); 1720 (C=O stretching of □-lactone of coumarin), 1608 and 1548 (aromatic C=C and C=N stretchings), 3062 (aromatic C-H stretching), 2934 (aliphatic C-H stretching), 829 (C-H bending vibrations of p-disubstituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 2.87 (3H, singlet, CH_3), 3.92 (3H, singlet, OCH_3), 7.04-7.85 (13H, multiplet, aromatic protons), 8.54 and 8.62 (2H, poorly resolved doublet, C'$_3$-H and C'$_5$-H), 8.73 (1H, singlet, C_4-H); ^{13}C APT (100MHz, $CDCl_3$, δ): 11.02(CH_3), 55.57(OCH_3), 111.45(CH), 116.38(C), 119.47(C), 120.73(CH), 121.10(CH), 121.62(CH), 123.39(CH), 124.91(CH), 125.10(CH), 125.24(C), 126.63(CH), 127.36(C), 128.60(C), 128.79(C), 129.18(CH), 129.60(CH), 132.52(CH), 142.71(CH), 147.25(C), 149.11(C), 151.66(C), 154.09(C), 155.35(C), 160.13(CO of coumarin).

Compound 5h : white solid; yield 70 %; mp 270-272°C; Anal. Calcd. for $C_{31}H_{24}N_4O_3$: 74.38; H, 4.83; N, 11.19%. Found: C, 74.43; H, 4.79; N, 11.23%. IR (KBr, ν_{max}, cm^{-1}); 1722 (C=O stretching of □-lactone of coumarin), 1605 and 1552 (aromatic C=C and C=N stretchings), 3064 (aromatic C-H stretching), 2930 (aliphatic C-H stretching), 834 (C-H bending vibrations of p-disubstituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 2.50 (3H, singlet, CH_3), 2.84 (3H, singlet, CH_3), 4.03 (3H, singlet, OCH_3), 7.11-7.87 (12H, multiplet, aromatic protons), 8.52 and 8.66 (2H, poorly resolved doublet, C'$_3$-H and C'$_5$-H), 8.79 (1H, singlet, C_4-H); ^{13}C APT (100MHz, $CDCl_3$, δ): 11.07(CH_3), 21.38(CH_3), 56.65(OCH_3), 112.18(CH), 115.33(C), 118.74(CH), 119.42(CH), 120.25(C), 122.64(CH), 126.42(CH), 126.58(CH), 126.75(CH), 127.62(CH), 128.40(C), 129.16(C), 129.58(CH), 129.65(CH), 129.76(CH), 129.88(C), 137.81(C), 138.23(C), 139.45(C), 143.65(C), 151.25(C), 153.44(C), 153.84(C), 160.60(CO of coumarin).

Compound 5i : white solid; yield 68 %; mp >300°C; Anal. Calcd. for $C_{31}H_{24}N_4O_4$: 72.08; H, 4.68; N, 10.85%. Found: C, 72.12; H, 4.73; N, 10.91%. IR (KBr, ν_{max}, cm^{-1}); 1714 (C=O stretching of □-lactone of coumarin), 1609 and 1544 (aromatic C=C and C=N stretchings), 3060 (aromatic C-H stretching), 2928 (aliphatic C-H stretching), 830 (C-H bending vibrations of p-disubstituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 2.83 (3H, singlet, CH_3), 3.93 (3H, singlet, OCH_3), 4.03 (3H, singlet, OCH_3), 7.10-7.88 (12H, multiplet, aromatic protons), 8.53 and 8.67 (2H, poorly resolved doublet, C'$_3$-H and C'$_5$-H), 8.80 (1H, singlet, C_4-H); ^{13}C APT (100MHz, $CDCl_3$, δ): 10.66(CH_3), 55.53(OCH_3), 56.35(OCH_3), 111.47(CH), 114.00(CH), 114.28(CH), 116.54(CH), 120.07(C), 120.16(C), 120.42(CH), 121.71(CH), 123.38(CH), 124.22(CH), 125.53(CH), 125.13(C), 128.33(CH), 128.73(C), 130.13(C), 143.09(CH), 146.87(C), 149.37(C), 149.83(C), 151.63(C), 155.27(C), 155.16(C), 159.56(C), 160.47(CO of coumarin).

Compound 5j : white solid; yield 72 %; mp 284-286°C; Anal. Calcd. for $C_{31}H_{24}N_4O_4$: 72.08; H, 4.68; N, 10.85%. Found: C, 72.13; H, 4.73; N, 10.91%. IR (KBr, ν_{max}, cm^{-1}); 1718 (C=O stretching of □-lactone of coumarin), 1612 and 1548 (aromatic C=C and C=N stretchings), 3062 (aromatic C-H stretching), 2933 (aliphatic C-H stretching), 835 (C-H bending vibrations of p-disubstituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 2.83 (3H, singlet, CH_3), 4.04 (3H, singlet, OCH_3), 4.07 (3H, singlet, OCH_3), 7.07-7.84 (12H, multiplet, aromatic protons), 8.46 and 8.68 (2H, poorly resolved doublet, C'$_3$-H and C'$_5$-H), 8.71 (1H, singlet, C_4-H); ^{13}C APT (100MHz, $CDCl_3$, δ): 10.56(CH_3), 55.74(OCH_3), 56.62(OCH_3), 107.32(CH), 113.89(CH), 114.32(CH), 116.60(C), 116.68(CH), 119.48(C), 120.68(CH), 123.56(CH), 124.36(CH), 125.11(C), 128.52(CH), 128.72(CH), 130.56(C), 130.90(C), 132.24(CH), 142.67(CH), 145.90(C), 149.20(C), 149.34(C), 151.75(C), 153.97(C), 155.91(C), 157.31(C), 160.56(CO of coumarin).

Compound 5k : white solid; yield 68 %; mp >300°C; Anal. Calcd. for $C_{32}H_{26}N_4O_4$: 72.44; H, 4.94; N, 10.56%. Found: C, 72.39; H, 4.89; N, 10.62%. IR (KBr, ν_{max}, cm^{-1}); 1722 (C=O stretching of □-lactone of coumarin), 1615 and 1552 (aromatic C=C and C=N stretchings), 3057 (aromatic C-H stretching), 2925 (aliphatic C-H stretching), 831 (C-H bending vibrations of p-disubstituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 2.56 (3H, singlet, CH_3), 2.84 (3H, singlet, CH_3), 3.94 (3H, singlet, OCH_3), 3.97 (3H, singlet, OCH_3), 7.05-7.86 (11H, multiplet, aromatic protons), 8.58 and 8.62 (2H, poorly resolved doublet, C'$_3$-H and C'$_5$-H), 8.74 (1H, singlet, C_4-H); ^{13}C APT (100MHz, $CDCl_3$, δ): 10.57(CH_3), 21.68(CH_3), 55.70(OCH_3), 56.83(OCH_3), 117.40(CH), 119.44(C), 120.34(C), 121.62(CH), 124.08(CH), 124.87(CH), 125.06(C), 127.65(CH), 129.22(CH), 129.43(CH), 129.65(CH), 130.65(C), 132.27(CH), 138.02(C), 143.11(CH), 145.21(C), 148.19(C), 149.19(C), 150.22(C), 151.68(C), 153.22(C), 154.12(C), 155.65(C), 160.34(CO of coumarin).

Compound 5l : white solid; yield 74 %; mp >300°C; Anal. Calcd. for $C_{32}H_{26}N_4O_5$: 70.32; H, 4.79; N, 10.25%. Found: C, 70.27; H, 4.84; N, 10.19%. IR (KBr, ν_{max}, cm^{-1}); 1714 (C=O stretching of □-lactone of coumarin), 1606 and 1545 (aromatic C=C and C=N stretchings), 3062 (aromatic C-H

stretching), 2932 (aliphatic C-H stretching), 836 (C-H bending vibrations of p-disubstituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 2.82 (3H, singlet, CH_3), 3.93 (3H, singlet, OCH_3), 4.03 (3H, singlet, OCH_3), 4.04 (3H, singlet, OCH_3), 7.04-7.84 (11H, multiplet, aromatic protons), 8.48 and 8.63 (2H, poorly resolved doublet, C'$_3$-H and C'$_5$-H), 8.78 (1H, singlet, C_4-H); ^{13}C APT (100MHz, $CDCl_3$, δ): 10.87(CH_3), 56.27(OCH_3), 57.31(OCH_3), 57.42(OCH_3), 116.39(CH), 117.45(C), 119.43(C), 121.18(CH), 121.65(C), 123.17(CH), 124.56(CH), 125.29(C), 125.43(CH), 127.49(CH), 128.76(C), 129.08(CH), 129.20(CH), 129.42(C), 132.33(CH), 138.23(C), 143.21(CH), 149.19(C), 150.12(C), 151.64(C), 153.91(C), 155.38(C), 155.43(C), 160.27(CO of coumarin).

Compound 5m : white solid; yield 67 %; mp 268-270°C; Anal. Calcd. for $C_{29}H_{19}BrN_4O_2$: 65.06; H, 3.58; N, 10.46%. Found: C, 65.11; H, 3.63; N, 10.51%. IR (KBr, ν_{max}, cm^{-1}); 1708 (C=O stretching of □-lactone of coumarin), 1614 and 1552 (aromatic C=C and C=N stretchings), 3056 (aromatic C-H stretching), 2936 (aliphatic C-H stretching), 832 (C-H bending vibrations of p-disubstituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 2.86 (3H, singlet, CH_3), 7.03-7.87 (13H, multiplet, aromatic protons), 8.53 and 8.64 (2H, poorly resolved doublet, C'$_3$-H and C'$_5$-H), 8.75 (1H, singlet, C_4-H); ^{13}C APT (100MHz, $CDCl_3$, δ): 11.04(CH_3), 112.56(C), 112.82(CH), 115.66(C), 118.35(CH), 118.82(CH), 119.01(C), 121.64(CH), 123.12(CH), 125.14(CH), 127.21(C), 127.51(CH), 127.84(CH), 129.47(CH), 130.35(CH), 132.95(CH), 134.09(C), 140.79(C), 144.34(C), 145.87(C), 147.26(C), 149.01(CH), 156.64(C), 159.13(C), 160.48(CO of coumarin).

Compound 5n : white solid; yield 69 %; mp 272-274°C; Anal. Calcd. for $C_{30}H_{21}BrN_4O_2$: 65.58; H, 3.85; N, 10.20%. Found: C, 65.63; H, 3.90; N, 10.15%. IR (KBr, ν_{max}, cm^{-1}); 1712 (C=O stretching of □-lactone of coumarin), 1605 and 1546 (aromatic C=C and C=N stretchings), 3064 (aromatic C-H stretching), 2930 (aliphatic C-H stretching), 833 (C-H bending vibrations of p-disubstituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 2.51 (3H, singlet, CH_3), 2.84 (3H, singlet, CH_3), 7.05-7.85 (12H, multiplet, aromatic protons), 8.53 and 8.62 (2H, poorly resolved doublet, C'$_3$-H and C'$_5$-H), 8.74 (1H, singlet, C_4-H); ^{13}C APT (100MHz, $CDCl_3$, δ): 10.86(CH_3), 21.83(CH_3), 113.12(CH), 116.40(C), 116.61(CH), 117.32(C), 119.47(C), 121.43(CH), 124.19(CH), 124.66(CH), 124.89(C), 127.26(C), 128.12(CH), 129.01(CH), 129.22(CH), 129.40(CH), 130.59(C), 132.23(CH), 137.64(C), 143.07(CH), 148.64(C), 150.02(C), 151.66(C), 154.11(C), 156.57(C), 160.19(CO of coumarin).

Compound 5o : white solid; yield 65 %; mp 262-264°C; Anal. Calcd. for $C_{30}H_{21}BrN_4O_3$: 63.73; H, 3.74; N, 9.91%. Found: C, 63.67; H, 3.68; N, 9.86%. IR (KBr, ν_{max}, cm^{-1}); 1721 (C=O stretching of □-lactone of coumarin), 1612 and 1551 (aromatic C=C and C=N stretchings), 3061 (aromatic C-H stretching), 2932 (aliphatic C-H stretching), 830 (C-H bending vibrations of p-disubstituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 2.86 (3H, singlet, CH_3), 3.93 (3H, singlet, OCH_3), 7.04-7.83 (12H, multiplet, aromatic protons), 8.56 and 8.64 (2H, poorly resolved doublet, C'$_3$-H and C'$_5$-H), 8.74 (1H, singlet, C_4-H); ^{13}C APT (100MHz, $CDCl_3$, δ): 10.57(CH_3), 56.90(CH_3), 113.15(CH), 113.90(CH), 116.33(C), 117.65(CH), 119.72(C), 120.16(C), 120.42(CH), 121.57(CH),

123.83(C), 124.22(CH), 124.65(CH), 125.13(C), 127.03(C), 127.29(C), 128.13(CH), 129.09(CH), 129.37(CH), 130.63(C), 137.87(C), 143.26(CH), 150.34(C), 152.13(C), 154.29(C), 160.32(CO of coumarin).

Compound 5o : white solid; yield 65 %; mp 262-264°C; Anal. Calcd. for $C_{30}H_{21}BrN_4O_3$: 63.73; H, 3.74; N, 9.91%. Found: C, 63.67; H, 3.68; N, 9.86%. IR (KBr, ν_{max}, cm^{-1}); 1721 (C=O stretching of -lactone of coumarin), 1612 and 1551 (aromatic C=C and C=N stretchings), 3061 (aromatic C-H stretching), 2932 (aliphatic C-H stretching), 830 (C-H bending vibrations of p-disubstituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 2.86 (3H, singlet, CH_3), 3.93 (3H, singlet, OCH_3), 7.04-7.83 (12H, multiplet, aromatic protons), 8.56 and 8.64 (2H, poorly resolved doublet, C'$_3$-H and C'$_5$-H), 8.74 (1H, singlet, C_4-H); ^{13}C APT (100MHz, $CDCl_3$, δ): 10.57(CH_3), 56.90(CH_3), 113.15(CH), 113.90(CH), 116.33(C), 117.65(CH), 119.72(C), 120.16(C), 120.42(CH), 121.57(CH), 123.83(C), 124.22(CH), 124.65(CH), 125.13(C), 127.03(C), 127.29(C), 128.13(CH), 129.09(CH), 129.37(CH), 130.63(C), 137.87(C), 143.26(CH), 150.34(C), 152.13(C), 154.29(C), 160.32(CO of coumarin).

Compound 5p : white solid; yield 70 %; mp >300°C; Anal. Calcd. for $C_{30}H_{21}BrN_4O_3$: 63.73; H, 3.74; N, 9.91%. Found: C, 63.67; H, 3.69; N, 9.86%. IR (KBr, ν_{max}, cm^{-1}); 1708 (C=O stretching of -lactone of coumarin), 1604 and 1542 (aromatic C=C and C=N stretchings), 3059 (aromatic C-H stretching), 2925 (aliphatic C-H stretching), 829 (C-H bending vibrations of p-disubstituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 2.84 (3H, singlet, CH_3), 3.92 (3H, singlet, OCH_3), 7.11-7.88 (12H, multiplet, aromatic protons), 8.57 and 8.69 (2H, poorly resolved doublet, C'$_3$-H and C'$_5$-H), 8.83 (1H, singlet, C_4-H); ^{13}C APT (100MHz, $CDCl_3$, δ): 10.30(CH_3), 53.46(CH_3), 114.47(CH), 118.65(CH), 119.29(C), 120.12(CH), 120.26(C), 123.75(C), 124.64(CH), 124.79(CH), 124.94(CH), 126.28(CH), 129.30(CH), 129.93(CH), 131.64(CH), 134.06(C), 139.56(C), 139.93(C), 141.40(CH), 145.06(C), 147.07(C), 151.65(C), 154.45(C), 155.51(C), 158.39(C), 160.15(CO of coumarin).

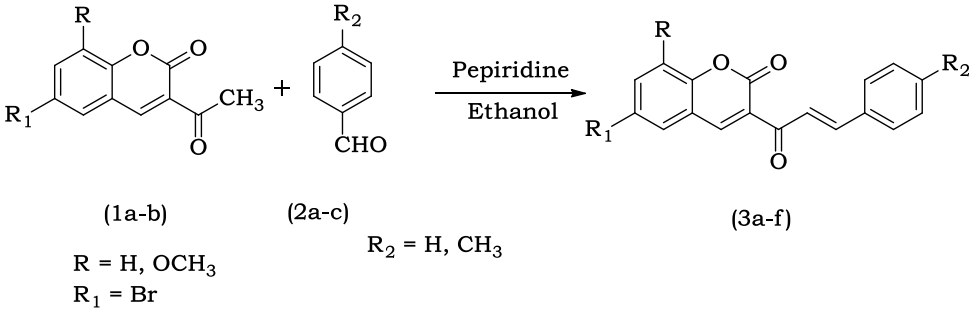
Compound 5q : white solid; yield 66 %; mp 276-278°C; Anal. Calcd. for $C_{31}H_{23}BrN_4O_3$: 64.26; H, 4.00; N, 9.67%. Found: C, 64.31; H, 3.95; N, 9.72%. IR (KBr, ν_{max}, cm^{-1}); 1712 (C=O stretching of -lactone of coumarin), 1609 and 1546 (aromatic C=C and C=N stretchings), 3065 (aromatic C-H stretching), 2932 (aliphatic C-H stretching), 836 (C-H bending vibrations of p-disubstituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 2.51 (3H, singlet, CH_3), 2.84 (3H, singlet, CH_3), 3.91 (3H, singlet, OCH_3), 7.05-7.85 (11H, multiplet, aromatic protons), 8.53 and 8.62 (2H, poorly resolved doublet, C'$_3$-H and C'$_5$-H), 8.74 (1H, singlet, C_4-H); ^{13}C APT (100MHz, $CDCl_3$, δ): 10.53(CH_3), 21.45(CH_3), 56.58(OCH_3), 112.25(CH), 115.41(C), 118.66(CH), 119.38(CH), 120.25(C), 122.64(CH), 126.55(CH), 126.58(CH), 126.65(CH), 127.62(C), 128.40(C), 129.16(C), 129.48(CH), 129.65(CH), 129.76(CH), 129.88(C), 137.81(C), 138.23(C), 139.45(C), 143.65(C), 151.25(C), 153.35(C), 153.77(C), 160.73(CO of coumarin).

Compound 5r : white solid; yield 65 %; mp >300°C; Anal. Calcd. for $C_{31}H_{23}BrN_4O_4$: 62.53; H, 3.89; N, 9.41%. Found: C, 62.48; H, 3.94; N, 9.36%. IR (KBr, ν_{max}, cm^{-1}); 1719 (C=O stretching of -lactone of coumarin), 1611 and 1552 (aromatic C=C and C=N stretchings), 3061 (aromatic C-H stretching), 2924 (aliphatic C-H stretching), 829 (C-H bending vibrations of p-disubstituted benzene ring); ^{1}H NMR (400MHz, $CDCl_3$, δ): 2.84 (3H, singlet, CH_3), 3.92 (3H, singlet, OCH_3), 3.92 (3H, singlet, OCH_3), 7.02-7.89 (11H, multiplet, aromatic protons), 8.56 and 8.67 (2H, poorly resolved doublet, C'$_3$-H and C'$_5$-H), 8.82 (1H, singlet, C_4-H); ^{13}C APT (100MHz, $CDCl_3$, δ): 10.51(CH_3), 56.37(OCH_3), 56.90(OCH_3), 111.41(CH), 114.00(CH), 114.51(CH), 116.54(CH), 120.25(C), 120.38(C), 120.76(CH), 121.66(CH), 123.22(C), 124.41(CH), 125.22(CH), 125.39(C), 128.37(CH), 128.90(C), 130.19(C), 143.07(CH), 146.95(C), 149.16(C), 149.53(C), 151.63(C), 155.21(C), 155.36(C), 159.68(C), 160.65(CO of coumarin).

3. RESULTS AND DISCUSSION:

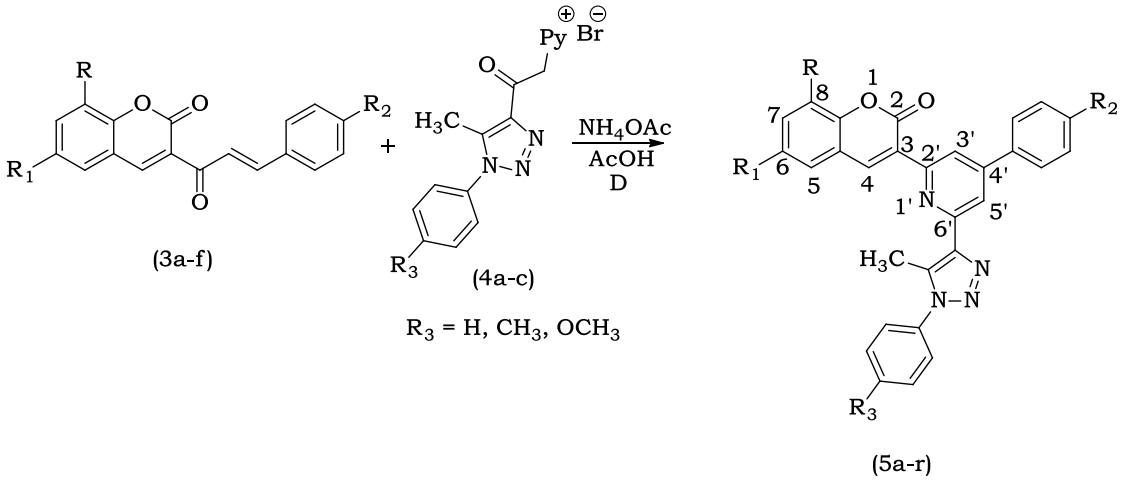
3.1. CHEMISTRY:

In the present work, various 3-[6-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)-4-arylpyridin-2-yl]coumarins **(5a-r)** have been synthesized by the reaction of 3-cinnamoyl coumarins **(3a-f)** with1,2,3-triazoloyl methyl pyridinium salt **(4a-c)** in the presence of ammonium acetate in glacial acetic acid under Krohnke's reaction condition[17] **(Scheme 1)**. The starting material 3-{3-[1-aryl-3-(benzofuran-2-yl)-1*H*-pyrazol-4-yl]acryloyl}coumarins **(3a-f)** were prepared by the reaction of 3-acetyl coumarins **(1a-c)** with appropriate aryl aldehyde **(2a-b)** in the presence of piperidine in ethanol. The plausible mechanism for the formation of target compounds **(5a-r)** is shown in **Scheme 2**



(1a-b) (2a-c) (3a-f)

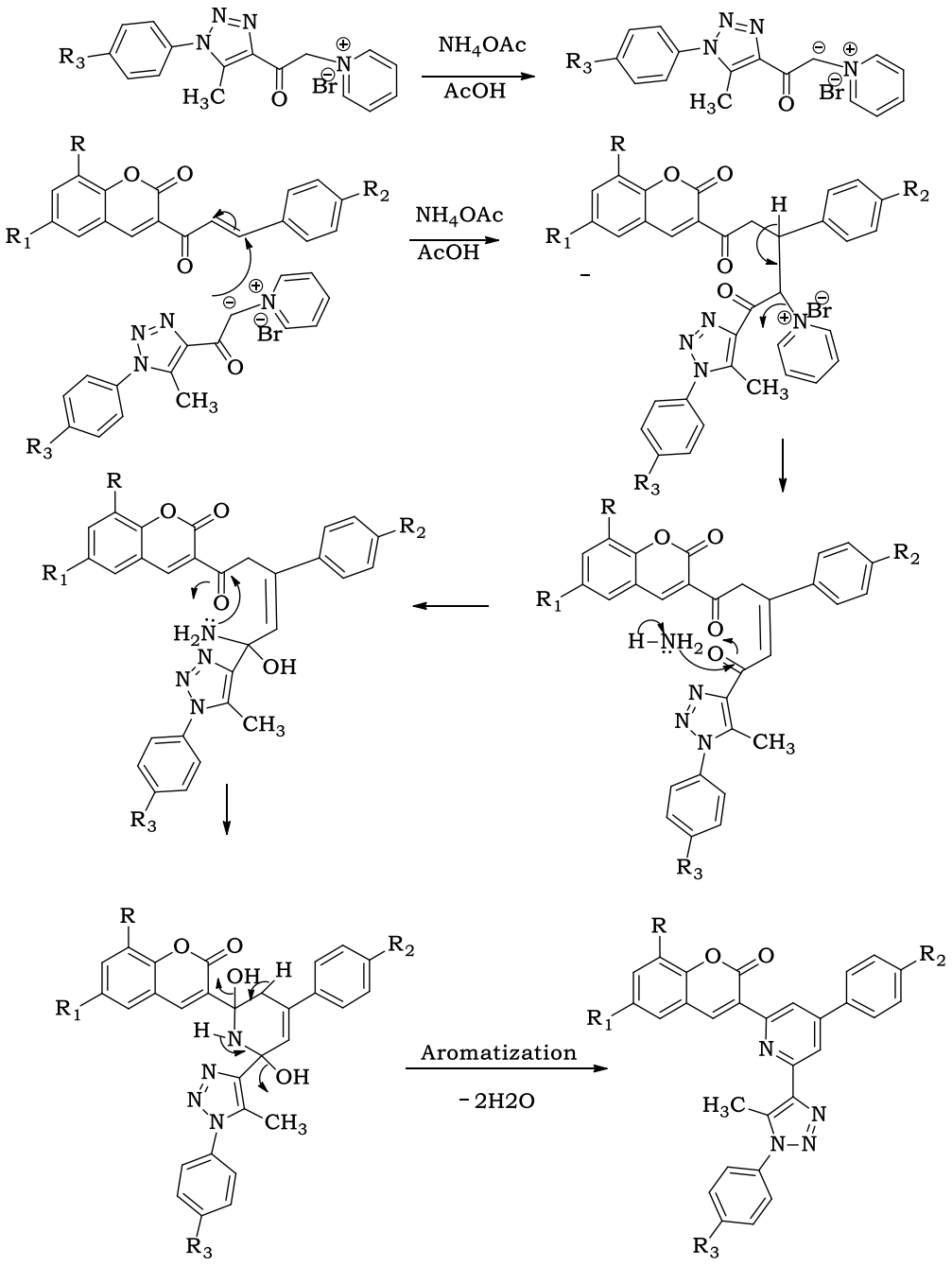
R = H, OCH_3
R_1 = Br
R_2 = H, CH_3



R_3 = H, CH_3, OCH_3

Compounds	R	R_1	R_2	R_3	Compounds	R	R_1	R_2	R_3
5a:	H	H	H	H	**5j:**	OCH_3	H	OCH_3	**H**
5b:	H	H	H	CH_3	**5k:**	OCH_3	H	OCH_3	**CH_3**
5c:	H	H	H	OCH_3	**5l:**	OCH_3	H	OCH_3	**OCH_3**
5d:	H	H	OCH_3	H	**5m:**	H	Br	H	**H**
5e:	H	H	OCH_3	CH_3	**5n:**	H	Br	H	**CH_3**
5f:	H	H	OCH_3	OCH_3	**5o:**	H	Br	H	**OCH_3**
5g:	OCH_3	H	H	H	**5p:**	H	Br	OCH_3	**H**
5h:	OCH_3	H	H	CH_3	**5q:**	H	Br	OCH_3	**CH_3**
5i:	**OCH_3**	**H**	**H**	**OCH_3**	**5r:**	**H**	**Br**	**OCH_3**	**OCH_3**

Scheme-1 : Synthetic scheme for compounds (5a-r)



Scheme-2. Plausible mechanism for the formation of target compounds (5a-r)

3.2. BIOLOGICAL RESULTS:

3.2.1. ANTIMICROBIAL ACTIVITY

The newly synthesized target compounds **(5a-r)** 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[18]. 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 *µg/*mL concentration as a stock solution. The results were recorded in the form of primary and secondary screening. The synthesized compounds **(5a-r)** 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 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.

3.2.2. ANTIMICROBIAL EVALUTION

The compounds **(5a-r)** 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**.

Upon evaluating the antimicrobial activity data, it was observed that compound **5g** and **5p** (MIC = 62.5*µg/*mL) exhibited excellent activity compared to Ampicillin (MIC = 250*µg/*mL) and Norfloxacin (MIC = 100*µg/*mL) against gram positive bacteria *B. subtilis.* Compounds **5d, 5f, 5j and 5m** (MIC = 100*µg/*mL) showed excellent activity towards the gram positive bacteria *B. subtilis* as compared to Ampicillin (MIC = 250*µg/*mL) and showed equipotent activity to Norfloxacin (MIC = 100*µg/*mL). Against gram positive bacteria *B. subtilis* compound **5i** and **5n**

(MIC = 125 *µg/*mL) showed better activity as compared to Ampicillin (MIC = 250*µg/*mL). Compounds **5a, 5c,** and **5k** (MIC = 200*µg/*mL) showed better activity towards the gram positive bacteria *B. subtilis* as compared to Ampicillin (MIC = 250*µg/*mL). Compounds **5b, 5e, 5h, 5l, 5o, 5q** and **5r** (MIC = 250*µg/*mL) exerted equipotent activity against gram positive bacteria *B. subtilis*. Compounds **5c, 5g, 5k** and **5n** (MIC = 100*µg/*mL) exhibited excellent activity compared to Ampicillin (MIC = 250*µg/*mL) against gram positive bacteria *S. aureus.* Compounds **5d, 5f, 5j, 5m** and **5q** (MIC = 125*µg/*mL) and Compounds **5i, 5l, 5o** and **5p** (MIC = 200*µg/*mL) exhibited better activity against gram positive bacteria *S. aureus* as compared to Ampicillin (MIC = 250*µg/*mL). Compounds 5**a, 5b, 5e, 5h** and **5r** (MIC = 250*µg/*mL) were found equipotent to Ampicillin (MIC = 250*µg/*mL) against gram positive bacteria *S. aureus*. Compound **5c** and **5h** (MIC = 62.5*µg/*mL) exhibited better activity compared to Ampicillin (MIC = 100*µg/*mL) against gram negative bacteria *E. coli*. Compounds **5d, 5j** and **5l** (MIC = 100*µg/*mL) were found equipotent compared to Ampicillin (MIC = 100*µg/*mL) against *E. coli*. Compounds **5l** (MIC = 62.5*µg/*mL) exhibited better activity compared to Ampicillin (MIC = 100*µg/*mL) against gram negative bacteria *S. typhi*. Compounds **5e, 5h, 5n** and **5q** (MIC = 100*µg/*mL) were found equipotent compared to Ampicillin (MIC = 100*µg/*mL) against *S. typhi*. Compounds **5g, 5i, 5l, 5o** and **5p** (MIC = 500*µg/*mL) were found equipotent to Griseofulvin (MIC = 500*µg/*mL) against *C. albicans.*

It is perceived from the antimicrobial data that almost all the tested derivatives **3a-l** were found to be potent against the gram positive bacterial strains. Among all the tested compounds, the compounds **5c, 5g, 5h, 5l** and **5p** were found to be more efficient members of the series.

Table-1 : In *vitro* Antimicrobial activity of compounds (5a-r)

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.***
5a	**200**	**250**	200	200	1000	**1000**
5b	**250**	**250**	200	250	1000	**1000**
5c	**200**	**100**	**62.5**	200	500	**>1000**
5d	**100**	**125**	**100**	125	500	**1000**
5e	**250**	**250**	200	**100**	500	**1000**
5f	**100**	**125**	250	250	250	**>1000**
5g	**62.5**	**100**	200	250	1000	**500**
5h	**250**	**250**	**62.5**	**100**	500	**1000**
5i	**125**	**200**	250	200	1000	**500**
5j	**100**	**125**	**100**	200	500	**>1000**
5k	**200**	**100**	250	200	>1000	**1000**
5l	**250**	**200**	**100**	**62.5**	1000	**500**
5m	**100**	**125**	200	200	1000	**1000**

5n	**125**	**100**		125	**100**		500	**1000**
5o	**250**	**200**		200	250		>1000	**500**
5p	**62.5**	**200**		250	200		500	**500**
5q	**250**	**125**		200	**100**		1000	**1000**
5r	**250**	**250**		200	125		1000	**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:

From present study, we summarized that employed synthetic strategy provide efficient route for the synthesis of 3-[6-(5-methyl-1-aryl-1*H*-1,2,3-triazol-4-yl)-4-arylpyridin-2-yl] coumarins by Krohnke's protocol. Moreover the starting precursors were also easy to prepare from synthesis point of view. Antimicrobial study on target compounds concluded that the all the compounds exerted promising activity against gram positive bacteria and gram negative. Compounds **5c, 5g, 5h, 5l** and **5p** were found to be the most efficient members of the series.

5. ACKNOWLEDGEMENT:

The authors are thankful to the Head, Department of Chemistry, Sardar Patel University for providing research facilities. Financial assistance as the BSR fellowship to KNP from the UGC , New Delhi, India, is highly acknowledged.

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