Synthesis of Some 3-(4-Aryl-benzofuro[3,2-*b*]pyridin-2-yl)coumarins and Their Antimicrobial Screening

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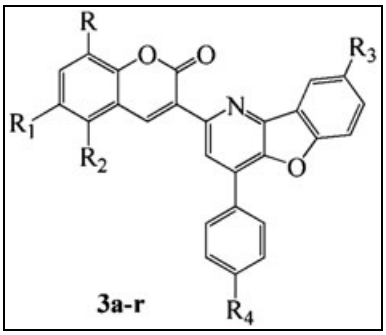

The synthesis of some 3-(4-aryl-benzofuro[3,2-*b*]pyridin-2-yl)coumarins **3a–r** has been carried out by the reaction of 3-coumarinoyl methyl pyridinium salts **1a–c** with 2-arylidene aurones **2a–f** in the presence of ammonium acetate and acetic acid under Kröhnke's reaction conditions. All the synthesized compounds were characterized by analytical and spectral data. They have been screened for their antibacterial activity against *Escherichia coli (ATCC 25922)* as Gram-negative bacteria, *Bacillus subtillis (ATCC 1633)* as Gram-positive bacteria and antifungal activity against *Aspergillus niger (ATCC 9029)*.

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INTRODUCTION

Coumarin and its derivatives are important heterocyclic compounds that are found in many natural products [1]; many of them exhibit remarkable physiological and biological activities [2–4]. Many compounds containing the coumarin skeleton have shown antihelmintic, hypnotic, insecticidal, anticoagulant, and antifungal properties [5]. The incorporation of other bioactive heterocyclic component in the parent coumarin moiety either as a substituent group or as a fused component converts it into useful derivative.

Benzofuropyridines are important heterocyclic derivatives, and many of them are reported to have interesting biological activities. Benzofuro[3,2-*b*]pyridines have been reported as endothelin receptor antagonists [6]. Certain benzofuro[3,2-*b*]pyridine derivatives possess an antibacterial and antimalerial activity [7,8]. A benzofuro[3,2-*b*]pyridine, Elbfluorene and its derivatives are interesting leads as cyclin-dependent kinase inhibitors [9].

As described above, both the coumarins and benzofuropyridines possess important bioactivities that render them useful substances in drug research. On this basis, it appeared expedient to synthesize new heterocyclic compounds bearing both coumarin moiety and benzofuropyridine unit. Keeping this objective in mind, in this work, we have synthesized some 3-(4-aryl-benzofuro [3,2-*b*]pyridin-2-yl)coumarins **3a–r** by reacting 3-coumarinoyl methyl pyridinium salts **1a–c** with 2-arylidene aurones **2a–f** under Kröhnke's reaction conditions. A synthetic path for the preparation of the compounds **3a–r** is depicted in Scheme 1. The compounds have been tested for their *in vitro* antimicrobial screening.

RESULTS AND DISCUSSION

The condensation of 3-coumarinoyl methyl pyridinium salts **1a–c** with 2-arylidene aurones **2a–f** under Kröhnke's reaction conditions afforded expected products 3-(4-aryl-benzofuro[3,2-*b*]pyridin-2-yl)coumarins **3a–r** in moderate yield (52–61%). A plausible reaction mechanism for the formation of the compounds **3a–r** is depicted in Scheme 2**.** The structures of all the compounds **3a–r** were established on the basis of analytical and spectral data.

In IR spectra, compounds **3a–r** showed a very strong band between 1715 and 1730 cm^{-1} for δ-lactone carbonyl (C=O) stretching vibrations. The strong bands for aromatic C=C, C=N stretching vibrations, and asymmetric C—O—C stretching of furan moiety were observed between 1590–1610, 1465–1485, and 1095–1105 cm^{-1}, respectively. The aromatic C—H stretching vibrations were observed between 3050 and 3060 cm^{-1} in the form of a medium band.

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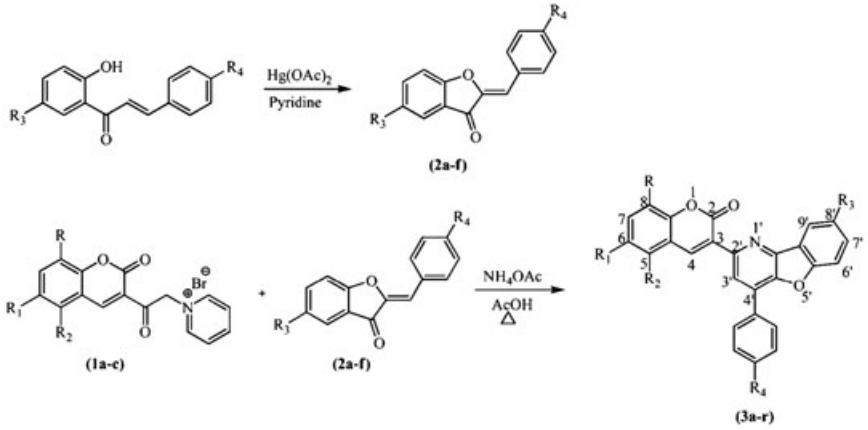
Scheme 1. The synthetic scheme for the preparation of compounds **3a–r**.



Entry	R	R_1	R_2	R_3	R_4	Entry	R	R_1	R_2	R_3	R_4
3a	H	H	H	H	H	**3j**	OCH_3	H	H	CH_3	H
3b	H	H	H	H	CH_3	**3k**	OCH_3	H	H	CH_3	CH_3
3c	H	H	H	H	OCH_3	**3l**	OCH_3	H	H	CH_3	OCH_3
3d	H	H	H	CH_3	H	**3m**	H	Benzo		H	H
3e	H	H	H	CH_3	CH_3	**3n**	H	Benzo		H	CH_3
3f	H	H	H	CH_3	OCH_3	**3o**	H	Benzo		H	OCH_3
3g	OCH_3	H	H	H	H	**3p**	H	Benzo		CH_3	H
3h	OCH_3	H	H	H	CH_3	**3q**	H	Benzo		CH_3	CH_3
3i	OCH_3	H	H	H	OCH3	**3r**	H	Benzo		CH_3	OCH_3

In the ^{1}H-NMR spectra of compounds **3a–r**, the aromatic protons appeared in the form of multiplets between 7.11 and 8.64 δ. The pyridine proton C_3'-H and coumarin proton C_4-H showed signals in downfield region compared to aromatic protons. The C_3' proton of pyridine ring appeared as a singlet in the region 8.72–8.89 δ. The C_3' proton of pyridine ring appears in the downfield region due to the peri effect of carbonyl group of δ-lactone. In all the compounds, the C_4-H signal was observed in the most downfield region. In case of compounds **3a–l**, the C_4-H signal appeared in the region 8.93–8.99 δ, whereas in compounds **3m–r**, it appeared in the region 9.78–9.81 δ. This downfield shifting of this signal in compounds **3m–r** compared to compounds **3a–l** is due to the presence of an additional fused benzene ring in the coumarin moiety, which causes diamagnetic anisotropy.

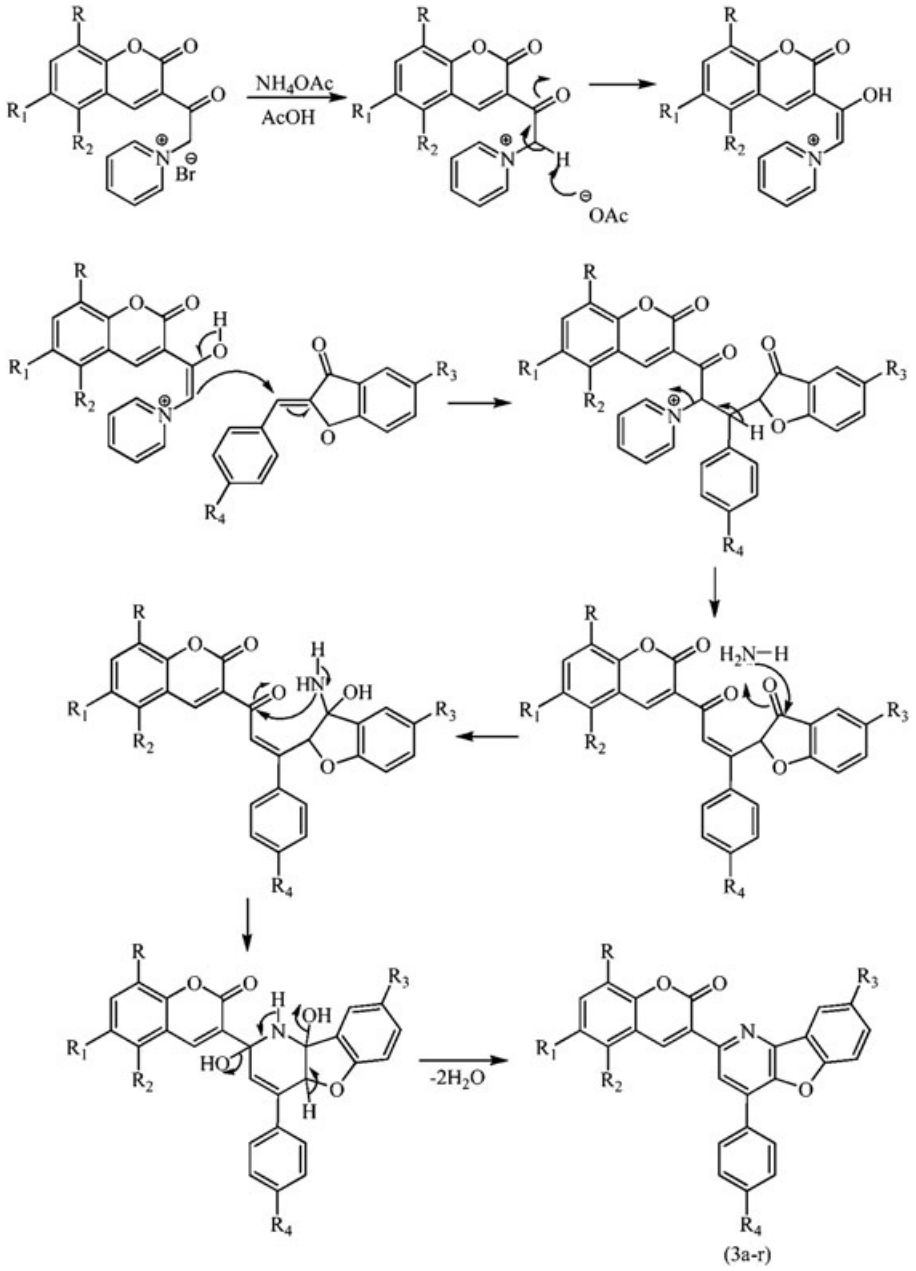
The ^{13}C-NMR spectra of compounds **3a–r** showed expected signals for non equivalent carbons present in the respective compound. Similarly, the DEPT 90 spectra gave the expected non equivalent tertiary carbon signals.

The mass spectra of compounds **3a–r** showed expected molecular ion peak which supports the structure of respective compound. All compounds gave satisfactory analytical and spectral data.

Biological evaluation. The antimicrobial activity of compounds **3a–r** was evaluated *in vitro* against bacteria strains *Escherichia coli ATCC 25922, Bacillus subtilis ATCC 1633, and* fungi *Aspergillus niger ATCC 9029*. Antimicrobial activity of all synthesized compounds were carried out by the agar cup diffusion method [10]. The result of antimicrobial screening is summarized in Table 1. All MIC determinations were carried out using NCCLS guidelines [11]. MIC values are given in μg/mL and were compared with MIC values for the standard drugs. Ciprofloxacin and Ampicillin were used as antibacterial standard, and Griseofulvin was used as antifungal standard. (Table 2)

The screening data reveal that the compounds having parent coumarin and 8-methoxy coumarin moieties **3a–l** showed activity against bacteria *E coli*, but compounds having 5,6-benzo coumarin moiety **3m–r** did not show activity against

Scheme 2. The plausible mechanism for the formation of the compounds **3a–r**.



bacteria *E coli*. However, though all the compounds **3a–r** showed activity against bacteria *B subtillis*, the compounds having 5,6-benzo coumarin moiety **3m–r** showed relatively poor activity compared to other compounds **3a–l**. All the compounds were found inactive toward fungi *A niger*. None of the compound showed better activity compared to standard drugs.

EXPERIMENTAL

All the melting points reported are uncorrected. The IR spectra (KBr disc) were recorded on Shimadzu FT-IR 8400-S spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR. The chemical shift (δ) is reported in ppm using chloroform-d as a solvent and calibrated standard solvent signal. Elemental analyses were carried out with a Hareaus CHNO analyzer. Mass spectrum of compound **3a** was recorded on Shimadzu QP 2010 spectrometer. The required starting materials, 3-coumarinoyl methyl pyridinium salts **1a–c** were prepared according to literature procedures [12].

General procedure for the synthesis of 2-arylidene aurones (2a–f). In a 50-mL round-bottom flask equipped with a magnetic stirrer, $Hg(OAc)_2$ (0.001 mol) was dissolved in

Table 1

Antimicrobial activity of synthesized compounds **3a-r** at 1000 μg/mL concentration against tested bacterial and fungal strains.

	Diameter of inhibition zone in mm		
	Bacteria		Fungi
Compd. no.	*E coli ATCC 25922*	*B subtillis ATCC 1633*	*A niger ATCC 9029*
3a	12	11	-
3b	11	09	-
3c	14	13	-
3d	13	12	-
3e	12	11	-
3f	15	14	-
3g	14	13	-
3h	12	11	-
3i	11	12	-
3j	12	13	-
3k	14	12	-
3l	13	11	-
3m	-	09	-
3n	-	10	-
3o	-	10	-
3p	-	08	-
3q	-	10	-
3r	-	11	-
Ciprofloxacin	18	25	NT
Ampicillin	20	27	NT
Griseofulvin	NT	NT	25

-, no inhibition zone observed; NT, not tested.

pyridine (10 mL) and to this a solution of appropriate 2'-hydroxy chalcone (0.001 mol) in pyridine (10 mL) was added with stirring and the reaction mixture was then refluxed for 10–15 min. The reaction mixture was cooled and treated with dil. HCl (1:1). The separated solid was filtered out and washed with water. It was recrystallized from ethanol to get pure aurone **(2a–f)**.

Compound 2a. $R_3 = R_4 = H$; Yield: 75%, m.p. 100°C (ref. [13]; m.p. 100–101°C)

Compound 2b. $R_3 = H, R_4 = CH_3$; Yield: 70%, m.p. 99–100°C (ref. [14]; m.p. 98.5–100°C)

Compound 2c. $R_3 = H$, $R_4 = OCH_3$; Yield: 69%, m.p. 136–37°C (ref. [13]; m.p. 137–138°C)

Compound 2d. $R_3 = CH_3, R_4 = H$; Yield: 72%, m.p. 117–118°C (ref. [13]; m.p. 118–119°C)

Compound 2e. $R_3 = R_4 = CH_3$; Yield = 71%, m.p. 118–119°C; ^{1}H-NMR: δ 2.42 (6H, s, 2 × CH_3), 6.87 (1H, s, benzylidene proton), 7.22–7.84 (7H, m, Ar-H). Anal. Calcd. for $C_{17}H_{14}O_2$: C, 81.58; H, 5.64. Found: C, 81.70; H, 5.60; MS: *m/z* 250 (M^+)

Compound 2f. $R_3 = CH_3$, $R_4 = OCH_3$; Yield: 65%, m.p. 155–56°C (ref. [13]; m.p. 154–155°C)

General procedure for the synthesis of 3-(4-aryl-benzofuro [3,2-*b*]pyridin-2-yl) coumarins (3a–r). In a 100-mL three-necked round-bottom flask equipped with a magnetic stirrer, appropriate 3-coumarinoyl methyl pyridinium salt **(1a–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 2-arylidene aurone **(2a–f)** (0.003 mol) in glacial acetic acid (15 mL) was added with stirring at room temperature during 15 min. The reaction mixture was further stirred for 1 h and then refluxed for 12 h 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 × 30 mL). The chloroform extract was washed with 5% sodium bicarbonate solution (3 × 20 mL), water (2 × 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 ethyl acetate-petroleum ether (60–80) (3:7) as an eluent to give product **(3a–r)**. The compounds were recrystallized from chloroform-hexane.

3-(4-Phenylbenzofuro[3,2-b]pyridin-2-yl)coumarin (3a). Yield 59%; m.p. 209–210°C; IR: cm^{-1} 1723, 1593, 1468, 1100; ^{1}H-NMR: δ 7.30–8.37 (13H, m, Ar-H), 8.80 (1H, s, C_3'-H), 8.99 (1H, s, C_4-H); ^{13}C-NMR: δ 112.42(CH), 116.40(CH), 119.78(C), 120.81(CH), 121.45(CH), 123.34(C), 123.71(CH), 124.61(CH), 125.67(C), 128.85(CH), 129.01(CH), 129.48(CH), 131.95(CH), 132.10(C), 133.90(C), 142.35(CH), 147.78(C), 153.90(C), 157.90(C), 160.62(C). Anal. Calcd. for $C_{26}H_{15}NO_3$: C, 80.19; H, 3.88; N, 3.60. Found: C, 80.03; H, 3.72; N, 3.50; MS: *m/z* 389 (M^+)

3-(4-(4-Methylphenyl)benzofuro[3,2-b]pyridin-2-yl)coumarin (3b). Yield 61%; m.p. 226–228°C; IR: cm^{-1} 1720, 1595, 1475, 1100; ^{1}H-NMR: δ 2.49 (3H, s, CH_3), 7.34–8.34 (12H, m, Ar-H), 8.80 (1H, s, C_3'-H), 8.99 (1H, s, C_4-H); ^{13}C-NMR: δ 21.69

Table 2

Minimum inhibitory concentration (MIC) μg/mL of synthesized compounds **3a–r** against tested bacterial and fungal strains.

	Minimum inhibitory concentration (MIC) (μg/mL)		
	Bacteria		Fungi
Compd. no.	*E coli ATCC 25922*	*B subtillis ATCC 1633*	*A niger ATCC 9029*
3a	500	500	>1000
3b	500	1000	>1000
3c	250	250	>1000
3d	500	500	>1000
3e	500	500	>1000
3f	250	250	>1000
3g	250	250	>1000
3h	500	500	>1000
3i	500	500	>1000
3j	500	500	>1000
3k	250	500	>1000
3l	250	500	>1000
3m	>1000	1000	>1000
3n	>1000	1000	>1000
3o	>1000	1000	>1000
3p	>1000	1000	>1000
3q	>1000	1000	>1000
3r	>1000	500	>1000
Ciprofloxacin	125	62.5	NT
Ampicillin	125	62.5	NT
Griseofulvin	NT	NT	62.5

NT, not tested.

(CH_3), 112.38(CH), 116.38(CH), 119.75(C), 120.55(CH), 121.40 (CH), 123.33(C), 123.63(CH), 124.57(CH), 125.72(C), 128.82 (CH), 128.85(CH), 129.38(CH), 129.71(CH), 130.94(C), 131.91 (CH), 132.07(C), 139.63(C), 142.30(CH), 144.75(C), 147.00(C), 147.72(C), 153.86(C), 157.84(C), 160.63(C). Anal. Calcd. for $C_{27}H_{17}NO_3$: C, 80.38; H, 4.25; N, 3.47. Found: C, 80.28; H, 4.13; N, 3.53; MS: *m/z* 403 (M^+)

3-(4-(4-Methoxyphenyl)benzofuro[3,2-b]pyridin-2-yl)coumarin (3c). Yield 58%; m.p. 228–230°C; IR: cm^{-1} 1730, 1600, 1465, 1100; ^{1}H-NMR: δ 3.94 (3H, s, OCH_3), 7.13–8.35 (12H, m, Ar-H), 8.75 (1H, s, C_3'-H), 8.97 (1H, s, C_4-H); ^{13}C-NMR: δ 55.44(OCH_3), 112.36 (CH), 114.44(CH), 116.37(CH), 119.79(C), 120.19(CH), 121.40 (CH), 123.39(C), 123.63(CH), 124.57(CH), 125.79(C), 126.16(C), 128.81(CH), 129.34(CH), 130.35(CH), 131.75(C), 131.88(CH), 142.29(CH), 144.71(C), 146.90(C), 147.72(C), 153.88(C), 157.80 (C), 160.67(C). Anal. Calcd. for $C_{27}H_{17}NO_4$: C, 77.32; H, 4.09; N, 3.34. Found: C, 77.45; H, 4.19; N, 3.23; MS: *m/z* 419 (M^+)

3-(8-Methyl-4-phenylbenzofuro[3,2-b]pyridin-2-yl)coumarin (3d). Yield 57%; m.p. 252–253°C; IR: cm^{-1} 1725, 1600, 1475, 1100; ^{1}H-NMR: δ 2.58 (3H, s, CH_3), 7.36–8.12 (12H, m, Ar-H), 8.76 (1H, s, C_3'-H), 8.96 (1H, s, C_4-H); ^{13}C-NMR: δ 21.39(CH_3), 111.89(CH), 116.38(CH), 119.77(C), 120.59(CH), 121.23(CH), 123.22(C), 124.59(CH), 125.67(C), 128.79(CH), 128.98(CH), 129.40(CH), 130.64(CH), 131.90(CH), 133.37(C), 133.93(C), 135.19(C), 142.21(CH), 144.85(C), 147.51(C), 153.85(C), 156.28(C), 156.32(C), 160.63(C). Anal. Calcd. for $C_{27}H_{17}NO_3$: C, 80.38; H, 4.25; N, 3.47. Found: C, 80.28; H, 4.31; N, 3.45; MS: *m/z* 403 (M^+)

3-(8-Methyl-4-(4-methylphenyl)benzofuro[3,2-b]pyridin-2-yl) coumarin (3e). Yield 59%; m.p. 218–219°C; IR: cm^{-1} 1730, 1605, 1475, 1100; ^{1}H-NMR: δ 2.48 and 2.58 (6H, two s, 2 × CH_3), 7.34–8.11 (11H, m, Ar-H), 8.73 (1H, s, C_3'-H), 8.94 (1H, s, C_4-H); ^{13}C-NMR: δ 21.35(CH_3), 21.44(CH_3), 111.86(CH), 116.35(CH), 119.79 (C), 120.35(CH), 121.18(CH), 123.28(C), 124.54(CH), 125.78(C), 128.77(CH), 128.83(CH), 129.67(CH), 130.53(CH), 131.00(C), 131.81(CH), 131.90(C), 133.27(C), 139.53(C), 142.13(CH), 144.74 (C), 147.47(C), 153.85(C), 156.27(C), 160.60(C). Anal. Calcd. for $C_{28}H_{19}NO_3$: C, 80.56; H, 4.59; N, 3.36. Found: C, 80.64; H, 4.68; N, 3.47; MS: *m/z* 417 (M^+)

3-(8-Methyl-4-(4-methoxyphenyl)benzofuro[3,2-b]pyridin-2-yl)coumarin (3f). Yield 54%; m.p. 223–224°C; IR: cm^{-1} 1725, 1605, 1480, 1105; ^{1}H-NMR: δ 2.57 (3H, s, CH_3), 3.93 (3H, s, OCH_3), 7.11–8.11 (11H, m, Ar-H), 8.72 (1H, s, C_3'-H), 8.94 (1H, s, C_4-H); ^{13}C-NMR: δ 21.37(CH_3), 55.43(OCH_3), 111.84(CH), 114.39(CH), 116.35(CH), 119.79(C), 119.96(CH), 121.19(CH), 123.27(C), 124.56(CH), 125.76(C), 126.21(C), 128.78(CH), 130.33(CH), 130.51(CH), 131.55(C), 131.83(CH), 133.27(C), 142.16(CH), 144.69(C), 147.14(C), 147.43(C), 153.83(C), 156.22(C), 160.59(C), 160.64(C). Anal. Calcd. for $C_{28}H_{19}NO_4$: C, 77.59; H, 4.42; N, 3.23. Found: C, 77.48; H, 4.30; N, 3.17; MS: *m/z* 433 (M^+)

8-Methoxy-3-(4-phenylbenzofuro[3,2-b]pyridin-2-yl) coumarin (3g). Yield 61%; m.p. 248–249°C; IR: cm^{-1} 1715, 1605, 1485, 1100; ^{1}H-NMR: δ 4.04 (3H, s, OCH_3), 7.14–8.37 (12H, m, Ar-H), 8.81 (1H, s, C_3'-H), 8.97 (1H, s, C_4-H); ^{13}C-NMR: δ 56.31(OCH_3), 112.44(CH), 113.74(CH), 120.28(CH), 120.44(C), 120.91(CH), 121.45(CH), 123.35(C), 123.71(CH), 124.43(CH), 125.92(C), 128.94(CH), 129.02(CH), 129.45(CH), 132.18(C), 133.91(C), 142.53(CH), 143.58(C), 144.84(C), 146.96(C), 147.06(C), 147.83(C), 157.92(C), 160.06(C). Anal. Calcd. for $C_{27}H_{17}NO_4$: C, 77.32; H, 4.09; N, 3.34. Found: C, 77.48; H, 4.13; N, 3.40; MS: *m/z* 419 (M^+)

8-Methoxy-3-(4-(4-methylphenyl)benzofuro[3,2-b]pyridin-2-yl) coumarin (3h). Yield 52%; m.p. 263–264°C; IR: cm^{-1} 1720, 1600, 1480, 1105; ^{1}H-NMR: δ 2.49 (3H, s, CH_3), 4.04 (3H, s, OCH_3), 7.14–8.36 (11H, m, Ar-H), 8.78 (1H, s, C_3'-H), 8.96 (1H, s, C_4-H); ^{13}C-NMR: δ 21.44(CH_3), 56.33(OCH_3), 112.41(CH), 113.68(CH), 120.28(CH), 120.46(CH), 120.59(C), 120.71(CH), 121.40(CH), 122.63(C), 123.39(C), 123.63(CH), 124.41(CH), 126.04(C), 128.90(CH), 129.37(C), 129.73(CH), 130.95(C), 132.19(C), 138.73(C), 139.61(C), 142.46(CH), 144.73(C), 146.95 (C), 157.87(C), 160.06(C). Anal. Calcd. for $C_{28}H_{19}NO_4$: C, 77.59; H 4.42; N, 3.23. Found: C, 77.42; H, 4.29; N, 3.11; MS: *m/z* 433 (M^+)

8-Methoxy-3-(4-(4-methoxyphenyl)benzofuro[3,2-b]pyridin-2-yl)coumarin (3i). Yield 54%; m.p. 268–270°C; IR: cm^{-1} 1715, 1600, 1480, 1105; ^{1}H-NMR: δ 3.94 and 4.04 (6H, two s, 2 × OCH_3), 7.13–8.63 (11H, m, Ar-H), 8.78 (1H, s, C_3'-H), 8.96 (1H, s, C_4-H); ^{13}C-NMR: δ 55.44(OCH_3), 56.32(OCH_3), 110.19(C), 112.39(CH), 113.72(CH), 114.45(CH), 120.27(CH), 120.30(CH), 120.46(C), 121.44(CH), 123.66(CH), 124.43(CH), 126.16(C), 129.34(CH), 130.41(CH), 131.86(C), 136.15(C), 136.69(C), 142.26(C), 142.49(CH), 144.18(C), 146.96(C), 147.74(C), 156.28(C), 157.83(C), 160.67(C). Anal. Calcd. for $C_{28}H_{19}NO_5$: C, 74.82; H, 4.26; N, 3.12. Found: C, 74.98; H, 4.38; N, 3.28; MS: *m/z* 449 (M^+)

8-Methoxy-3-(8-methyl-4-phenylbenzofuro[3,2-b]pyridin-2-yl) coumarin (3j). Yield 52%; m.p. 218–220°C; IR: cm^{-1} 1715, 1605, 1480, 1100; ^{1}H-NMR: δ 2.57 (3H, s, CH_3), 4.02 (3H, s, OCH_3), 7.11–8.11 (11H, m, Ar-H), 8.76 (1H, s, C_3'-H), 8.93 (1H, s, C_4-H); ^{13}C-NMR: δ 21.35(CH_3), 56.28(OCH_3), 111.87 (CH), 113.61(CH), 120.23(CH), 120.41(C), 120.63(CH), 121.21 (CH), 123.24(C), 124.37(CH), 125.86(C), 128.93(CH), 129.00 (CH), 129.35(CH), 130.60(CH), 131.91(C), 133.33(C), 133.95 (C), 142.33(CH), 143.52(C), 144.79(C), 146.91(C), 147.27(C), 147.51(C), 156.31(C), 160.01(C). Anal. Calcd. for $C_{28}H_{19}NO_4$: C, 77.59; H, 4.42; N, 3.23. Found: C, 77.67; H, 4.51; N, 3.37; MS: *m/z* 433 (M^+)

8-Methoxy-3-(8-methyl-4-(4-methylphenyl)benzofuro[3,2-b] pyridin-2-yl)coumarin (3k). Yield 55%; m.p. 255–257°C; IR: cm^{-1} 1715, 1595, 1480, 1105; ^{1}H-NMR: δ 2.48 and 2.58 (6H, two s, 2 × CH_3), 4.03 (3H, s, OCH_3), 7.13–8.13 (10H, m, Ar-H), 8.75 (1H, s, C_3'-H), 8.93 (1H, s, C_4-H); ^{13}C-NMR: δ 21.35(CH_3), 21.43(CH_3), 56.30(OCH_3), 109.69(C), 111.88 (CH), 113.62(CH), 120.23(CH), 120.47(CH), 121.19(CH), 123.31(C), 124.37(CH), 126.04(C), 128.88(CH), 129.67(CH), 130.53(CH), 131.02(C), 132.00(C), 133.28(C), 139.52(C), 142.33(CH), 143.54(C), 144.74(C), 146.93(C), 147.33(C), 147.53(C), 156.29(C), 160.05(C). Anal. Calcd. for $C_{29}H_{21}NO_4$: C, 77.84; H, 4.73; N, 3.13. Found: C, 77.98; H, 4.86; N, 3.27; MS: *m/z* 447 (M^+)

8-Methoxy-3-(8-methyl-4-(4-methoxyphenyl)benzofuro[3,2-b]pyridin-2-yl)coumarin (3l). Yield 52%; m.p. 262–264°C; IR: cm^{-1} 1715, 1600, 1475, 1100; ^{1}H-NMR: δ 2.58 (3H, s, CH_3), 3.93 and 4.04 (6H, two s, 2 × OCH_3), 7.12–8.13 (10H, m, Ar-H), 8.75 (1H, s, C_3'-H), 8.94 (1H, s, C_4-H); ^{13}C-NMR: δ 21.34(CH_3), 55.43(OCH_3), 56.31(OCH_3), 111.85(CH), 113.63 (CH), 114.40(CH), 120.09(CH), 120.23(CH), 120.47(C), 121.20(CH), 123.34(C), 124.37(CH), 126.07(C), 126.25(C), 130.36(CH), 130.49(CH), 133.63(C), 133.27(C), 142.31(CH), 143.55(C), 144.71(C), 146.94(C), 147.21(C), 147.51(C), 156.25(C), 160.06(C), 160.61(C). Anal. Calcd. for $C_{29}H_{21}NO_5$: C, 75.15; H, 4.57; N, 3.02. Found: C, 75.28; H, 4.68; N, 3.12; MS: *m/z* 463 (M^+).

3-(4-Phenylbenzofuro[3,2-b]pyridin-2-yl)benzo[f]coumarin (3m). Yield 61%; m.p. 278°C; IR: cm^{-1} 1717, 1603, 1470, 1095; ^{1}H-NMR: δ 7.51–8.61 (15H, m, Ar-H), 8.89 (1H, s, C_3'-H), 9.81 (1H, s, C_4-H); ^{13}C-NMR: δ 112.42(CH), 114.07(C), 116.61(CH), 120.79(CH), 121.56(CH), 122.15(CH), 123.36(C), 123.68(CH), 124.32(C), 126.15(CH), 128.33(CH), 129.02(CH), 129.47(CH), 129.60(CH), 130.41(C), 132.13(C), 133.41(CH), 133.91(C), 138.12(CH), 144.87(C), 147.00(C), 148.02(C), 153.72(C), 157.92(C), 160.70(C). Anal. Calcd. for $C_{30}H_{17}NO_3$: C, 81.99; H, 3.90; N, 3.19. Found: C, 81.82; H, 3.80; N, 3.07; MS: *m/z* 439 (M^+).

3-(4-(4-Methylphenyl)benzofuro[3,2-b]pyridin-2-yl)benzo[f] coumarin (3n). Yield 52%; m.p. 281°C; IR: cm^{-1} 1720, 1590, 1470, 1100; ^{1}H-NMR: δ 2.50 (3H, s, CH_3), 7.43–8.62 (14H, m, Ar-H), 8.87 (1H, s, C_3'-H), 9.81 (1H, s, C_4-H); ^{13}C-NMR: δ 21.47(CH_3), 112.44(CH), 114.11(C), 116.66(CH), 117.37(C), 120.63(CH), 121.55(CH), 122.19(CH), 123.41(C), 123.64(CH), 124.48(C), 126.16(CH), 128.34(CH), 128.90(CH), 129.06(CH), 129.41(CH), 129.74(CH), 130.43(C), 130.98(C), 132.22(C), 133.40(CH), 138.16(CH), 139.66(C), 148.03(C), 152.38(C), 152.79(C), 153.73(C), 157.90(C), 160.76(C). Anal. Calcd. for $C_{31}H_{19}NO_3$: C, 82.10; H, 4.22; N, 3.09. Found: C, 82.22; H, 4.30; N, 3.01; MS: *m/z* 453 (M^+).

3-(4-(4-Methoxyphenyl)benzofuro[3,2-b]pyridin-2-yl)benzo[f]coumarin (3o). Yield 54%; m.p. 279–280°C; IR: cm^{-1} 1725, 1600, 1475, 1100; ^{1}H-NMR: δ 3.94 (3H, s, OCH_3), 7.13–8.61 (14H, m, Ar-H), 8.85 (1H, s, C_3'-H), 9.79 (1H, s, C_4-H); ^{13}C-NMR: δ 55.43(OCH_3), 112.38(CH), 114.10(C), 114.45 (CH), 116.62(CH), 116.64(C), 120.21(CH), 121.56(CH), 122.21 (CH), 123.39(C), 123.64(CH), 126.15(CH), 128.34(CH), 129.03 (CH), 129.38(CH), 129.62(C), 130.38(CH), 133.40(CH), 138.14 (CH), 146.89(C), 147.93(C), 153.70(C), 157.84(C), 160.67(C), 160.75(C). Anal. Calcd. for $C_{31}H_{19}NO_4$: C, 79.31; H, 4.08; N, 2.98. Found: C, 79.38; H, 4.20; N, 2.88; MS: *m/z* 469 (M^+).

3-(8-Methyl-4-phenylbenzofuro[3,2-b]pyridin-2-yl)benzo[f] coumarin (3p). Yield 52%; m.p. 280°C; IR: cm^{-1} 1725, 1600, 1480, 1105; ^{1}H-NMR: δ 2.62 (3H, s, CH_3), 7.43–8.63 (14H, m, Ar-H), 8.86 (1H, s, C_3'-H), 9.80 (1H, s, C_4-H); ^{13}C-NMR: δ 21.40(CH_3), 111.91(CH), 114.10(C), 116.62(CH), 120.63(CH), 121.35(CH), 122.20(CH), 123.28(C), 124.02(C), 124.42(C), 126.15(CH), 128.32(CH), 128.97(CH), 129.01(CH), 129.04 (CH), 129.41(CH), 129.63(C), 130.42(C), 130.69(CH), 132.05 (C), 133.36(CH), 134.01(C), 138.07(CH), 147.32(C), 147.81 (C), 153.72(C), 156.40(C), 160.72(C). Anal. Calcd. for $C_{31}H_{19}NO_3$: C, 82.10; H, 4.22; N, 3.09. Found: C, 82.22; H, 4.34; N, 3.01; MS: *m/z* 453 (M^+).

3-(8-Methyl-4-(4-methylphenyl)benzofuro[3,2-b]pyridin-2-yl) benzo[f]coumarin (3q). Yield 55%; m.p. 281–282°C; IR: cm^{-1} 1730, 1610, 1475, 1100; ^{1}H-NMR: δ 2.49 and 2.62 (6H, two s, 2 × CH_3), 7.41–8.62 (13H, m, Ar-H), 8.84 (1H, s, C_3'-H), 9.78 (1H, s, C_4-H); ^{13}C-NMR: δ 21.41(CH_3), 21.47(CH_3), 111.90(CH), 114.11(C), 116.64(CH), 120.40(CH), 121.31(CH), 122.21(CH), 123.31(C), 124.51(C), 126.13(CH), 128.29(CH), 128.87(CH), 129.04(CH), 129.61(C), 129.70(CH), 130.41(C), 130.57(CH), 131.04(C), 132.01(C), 133.28(C), 133.32(CH), 138.00(CH), 139.56(C), 144.81(C), 147.30(C), 147.76(C), 153.67(C), 156.32(C), 160.74(C). Anal. Calcd. for $C_{32}H_{21}NO_3$: C, 82.21; H, 4.53; N, 3.00. Found: C, 82.14; H, 4.36; N, 2.89; MS: *m/z* 467 (M^+).

3-(8-Methyl-4-(4-methoxyphenyl)benzofuro[3,2-b]pyridin-2-yl)benzo[f]coumarin (3r). Yield 52%; m.p. 274–276°C; IR: cm^{-1} 1720, 1605, 1475, 1095; ^{1}H-NMR: δ 2.63 (3H, s, CH_3), 3.94 (3H, s, OCH_3), 7.14–8.64 (13H, m, Ar-H), 8.84 (1H, s, C_3'-H), 9.81 (1H, s, C_4-H); ^{13}C-NMR: δ 21.40(CH_3), 55.43 (OCH_3), 111.89(CH), 114.13(C), 114.43(C), 116.66(CH), 120.04(CH), 121.33(CH), 122.22(CH), 123.37(CH), 126.13 (CH), 126.30(C), 126.80(C), 128.29(CH), 129.05(CH), 129.63 (C), 130.37(CH), 130.43(CH), 130.54(C), 133.29(C), 133.32 (CH), 135.43(C), 138.06(CH), 147.25(C), 147.83(C), 153.41 (C), 153.71(C), 156.32(C), 160.63(C), 160.79(C). Anal. Calcd. for $C_{32}H_{21}NO_4$: C, 79.49; H, 4.38; N, 2.90. Found: C, 79.38; H, 4.31; N, 2.78; MS: *m/z* 483 (M^+).

BIOLOGY

Antimicrobial activity. All the synthesized compounds **3a–r** were screened for their antibacterial activities against bacteria strains *Escherichia coli ATCC 25922, Bacillus subtilis ATCC 1633,* and fungi *Aspergillus niger ATCC 9029* by the agar cup diffusion method [10]. The zone of inhibition was measured in mm and was compared with standard drug. Dimethyl formamide was used as blank, Ciprofloxacin and Ampicillin were used as antibacterial standard and Griseofulvin was used as antifungal standard. All the compounds were tested at 1000 μg/mL concentration.

Minimum inhibitory concentration (MIC). The antimicrobial activity was assayed *in vitro* by two-fold broth dilution [15] against bacteria strains *Escherichia coli ATCC 25922, Bacillus subtilis ATCC 1633,* and fungi *Aspergillus niger ATCC 9029*. The minimum inhibitory concentrations (MIC, in μg/mL) were defined as the lowest concentrations of compounds that completely inhibited the growth of each strain. All compounds dissolved in dimethylsulfoxide were added to the culture media. Mueller-Hinton Broth for bacteria and Sabouraud Liquid media for fungi were used to obtain the final concentrations ranging from 1000 to 62.5 μg/mL. The amount of dimethylsulfoxide never exceeded 1% v/v. Inocula consisted of 5.0×10^4 bacteria/mL and 1.0×10^4 fungi/mL. The MICs were read after incubation at 37°C for 24 h (bacteria) and at 30°C for 48 h (fungi). Media and media with 1% v/v dimethylsulfoxide were used as growth controls. Ciprofloxacin and Ampicillin were used as antibacterial drugs, whereas Griseofulvin was used as antifungal drug. To detect the type of antimicrobial activity, subcultures were performed by transferring 100 μL of each mixture remaining clear in 1 mL of fresh medium. The minimum bactericidal concentrations (MBC, μg/mL) and minimum fungicidal concentrations (MFC, μg/mL) were read after incubation at 37°C for 24 h and at 30°C for 48 h, respectively.

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