A Convenient Synthesis of Bipyrido-Fused Coumarins and Their Biological Evaluation

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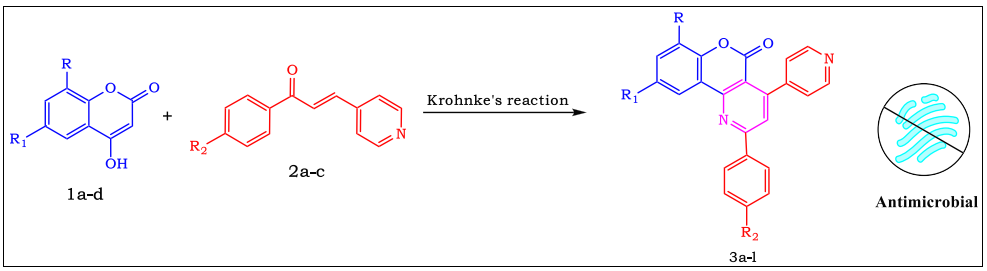
Received April 13, 2019
DOI 10.1002/jhet.3672
Published online 00 Month 2019 in Wiley Online Library (wileyonlinelibrary.com).



A convenient and efficient strategy has been devised for the synthesis of bipyrido-fused coumarins employing Kröhnke's pyridine synthesis approach. In the present work, 4-hydroxycoumarins **1a–d** were reacted with appropriate chalcones **2a–c** to afford desired bipyridyl-fused coumarins **3a–l**. The structures of all the newly synthesized compounds **3a–l** were ascertained by IR, ^{1}H NMR, ^{13}C NMR, mass spectral data, and elemental analyses. The compounds were further evaluated for their antimicrobial response against representative panel of pathogens, and the results thus obtained were compared with those of standard drugs. Few of the derivatives **3c**, **3f**, and **3i** exhibited promising potency.

J. Heterocyclic Chem., **00,** 00 (2019).

INTRODUCTION

Coumarin derivatives, natural as well as synthetic, belong to a cardinal class of heterocycles that possess a diverse range of applications. Coumarins, along with possessing varied biological activities such as antidepressant [1], anticancer [2], antibacterial [3], anti-HIV [4], and anti-inflammatory [5] activities, also have been extensively studied for their other applications such as photochemical [6], chemiluminescence [7], growth regulators in plant [8], cosmetics [9], laser dyes, nonlinear optical chromophores, fluorescent whiteners, fluorescent probes, polymers, optical recording, and solar energy collectors [10,11]. Among coumarin derivatives, pyrido-fused coumarins constitute an important category that is known to have properties such as wound healing [12], anti-allergic [13], anticoagulant [14], antidiabetic [15], and analgesic [16] properties. During our literature survey, we came to know that bipyridine moieties are components of molecules that are of varied applications such as in supramolecular chemistry [17], photocatalysis [18], nonlinear optical chromophore [19], fungicidal activity [20], and cardiotonic drugs [21]. Despite possessing such astonishing properties, no attempt has been made to synthesize compounds having both these scaffolds, namely, pyrido-fused coumarins and bipyridines. An earlier report on the synthesis of pyrido-fused coumarin [22–26] suffers from several shortcomings such as operational complexity, vigorous reaction condition, and lower yield, and also the reported methods have very limited scope of introducing substitution in coumarin ring or pyridine ring.

Earlier, we have reported the synthesis of bipyridyl-substituted coumarins [27–29] and pyrido-fused coumarins [30,31]. Therefore, in continuation of our effort to synthesize new heterocycles and study their biological responses, herein, we report the synthesis of 2-aryl-4-(pyridin-4-yl)-5*H*-chromeno[4,3-*b*]pyridin-5-one (**3a–l**) (bipyrido-fused coumarins) and their antimicrobial activity.

RESULTS AND DISCUSSION

The required α,β-unsaturated carbonyls (chalcones) **2a–c** were prepared by aldol condensation of appropriate acetophenone and 4-formyl pyridine. These chalcones were then reacted with appropriate 4-hydroxycoumarin **1a–d** in the presence of ammonium acetate and acetic acid to afford the target compounds **3a–l** (Scheme 1) in 55–67% yield (Table 1). A plausible mechanism for the synthesis of title compounds **3a–l** is depicted in Scheme 2. The structures of all compounds **3a–l** were established on the basis of IR, ^{1}H-NMR, ^{13}C NMR, and selected mass spectral data.

The IR spectra of compounds **3a–l** showed the characteristic band between 1728 and 1738 cm^{-1} for

Scheme 1. Synthesis strategy for the target compounds **3a–l**.

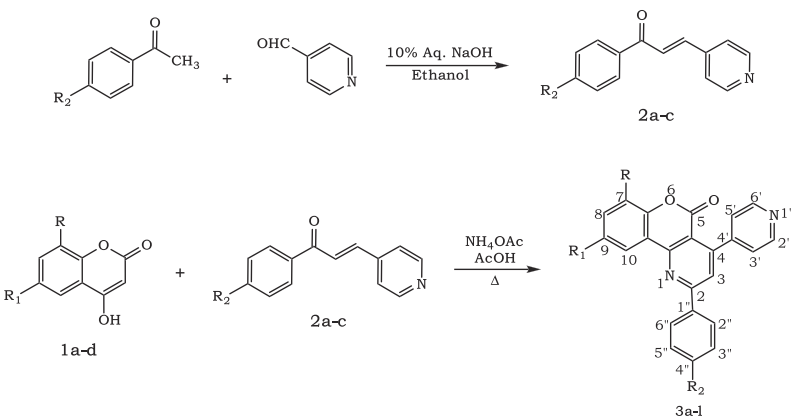


Table 1

Physicochemical parameters of synthesized derivatives **3a–l**.

Compound	R	R_1	R_2	Yield (%)	Melting point (°C)
3a	H	H	CH_3	64	263–264
3b	H	H	OCH_3	67	246
3c	H	H	Cl	63	223–225
3d	H	CH_3	CH_3	59	199–201
3e	H	CH_3	OCH_3	57	179
3f	H	CH_3	Cl	61	185–187
3g	CH_3	H	CH_3	55	277
3h	CH_3	H	OCH_3	62	219–221
3i	CH_3	H	Cl	64	271–273
3j	H	Cl	CH_3	58	245
3k	H	Cl	OCH_3	62	250–252
3l	H	Cl	Cl	63	229

coumarin carbonyl stretching. The band observed between 1581 and 1601 cm^{-1} was assigned to aromatic C=C stretching. The stretching band for C=N was observed between 1528 and 1547 cm^{-1}. The bands for aliphatic and aromatic C–H stretchings were observed between 2921–2939 and 3031–3043 cm^{-1}, respectively. The band observed between 814 and 832 cm^{-1} was assigned to C–H bending of *p*-disubstituted benzene ring.

The ^1H NMR spectra of compounds **3a–l** exhibited a doublet of doublet integrating for one proton between 7.58 and 8.01 δ was attributed to C_{10}–H. The signal for C_2''–H and C_6''–H appeared as doublet integrating for two proton between 7.69 and 8.24 δ. The most deshielded doublet observed between 8.63 and 8.80 δ, integrating for two protons, was assigned to C_2'–H and C_6'–H. The other aromatic protons resonated between 6.59 and 7.74 δ. The compounds bearing CH_3 group gave singlet for methyl between 2.41 and 2.75 δ. Similarly, compounds bearing methoxyl (OCH_3) functionality exhibited a singlet around 3.9 δ. The signals for C_{10}–H, C_2''–H, and C_6''–H of compounds **3d** and **3e** became merged with other aromatic protons and hence could not be assigned separately. The signals for C_2'–H and C_6'–H appear in most downfield regions owing to their attachment to the carbons that are directly attached to N_1'. The signals for C_{10}–H, C_2''–H, and C_6''–H appear in the downfield region owing to the peri effect of N_1.

In the ^{13}C NMR spectra of compounds **3a–l**, the coumarin carbonyl carbons, appeared as the most deshielded signal between 160.61 and 162.75 δ. The signals for other aromatic carbons were observed between 105.24 and 162.11 δ. The compounds having methyl (CH_3) group exhibited the corresponding signal between 15.60 and 21.61 δ, and those bearing methoxyl (OCH_3) functionality depicted a signal between 55.36 and 55.52 δ.

The mass spectrum of compound **3a** showed M^+ peak at 364 (100%) *m/z* (%) along with other fragment peaks at 336 (17%), 321 (2%), 182 (11%), 91 (4%), 44 (4%), and so on. The appearance of molecular ion peak at 364 mass unit supports the structure of compound **3a**.

Evaluation of antimicrobial activity. All compounds **3a–l** were assayed for their *in vitro* antimicrobial activity against Gram-positive bacteria [viz. *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 96)] and Gram-negative bacteria [viz. *Escherichia coli* (MTCC 443) and *Salmonella typhimurium* (MTCC 98)] and antifungal activity against *Aspergillus niger* (MTCC 282) and *Candida albicans* (MTCC 227) by broth dilution method [32].

The antimicrobial activity results presented in Table 2 reveal that compound **3f** [minimum inhibitory concentration (MIC) = 100 μg/mL] and compounds **3c** and **3i** (MIC = 125 μg/mL) showed admirable activity against *B. subtilis* than did ampicillin (MIC = 250 μg/mL). Compounds **3a**, **3c**, and **3d**

Scheme 2. Plausible mechanism for the synthesis of compounds **3a–l**.

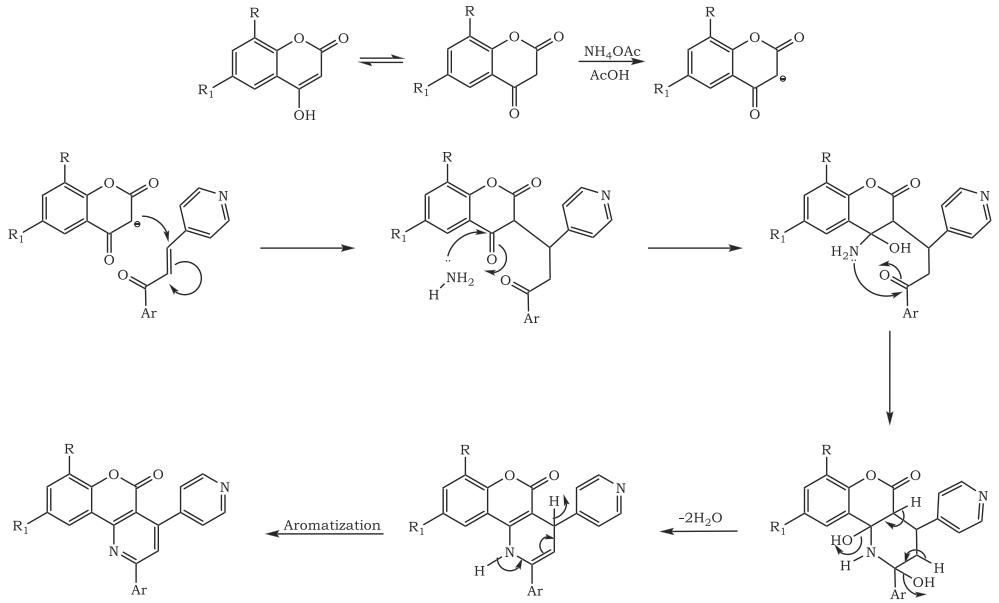


Table 2

Antimicrobial activity data for compounds **3a–l**.

	Minimum Inhibitory Concentration (μg/mL)					
	Gram +ve bacteria		Gram −ve bacteria		Fungi	
Compound	*B.s.*	*S.a.*	*E.c.*	*S.t.*	*A.n.*	*C.a.*
3a	250	100	125	250	>1000	>1000
3b	250	200	200	250	1000	500
3c	125	100	250	200	500	500
3d	250	100	100	100	>1000	500
3e	250	250	100	200	250	1000
3f	100	200	200	100	>1000	1000
3g	200	250	200	250	500	250
3h	250	200	250	250	>1000	>1000
3i	125	125	125	200	500	250
3j	250	200	250	200	1000	>1000
3k	250	200	200	250	250	>1000
3l	200	250	250	250	>1000	>1000
Ampicillin	250	250	100	100	—	—
Chloramphenicol	50	50	50	50	—	—
Ciprofloxacin	50	50	25	25	—	—
Norfloxacin	100	10	10	10	—	—
Gentamicin	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*.

(MIC = 100 μg/mL) and **3i** (MIC = 125 μg/mL) exerted excellent activity against *S. aureus* than did ampicillin (MIC = 250 μg/mL).

Compounds **3g** and **3l** (MIC = 200 μg/mL) showed better activity against *B. subtilis*, whereas compounds **3b**, **3f** and **3h**, and **3j** and **3k** (MIC = 200 μg/mL) were found to be more potent against *S. aureus* than ampicillin (MIC = 250 μg/mL).

Compounds **3a**, **3b**, **3d**, **3e**, **3h**, **3j**, and **3k** (MIC = 250 μg/mL) were found to be equipotent against *B. subtilis*, while compounds **3e**, **3g**, and **3l** (MIC = 250 μg/mL) exhibited equal activity against

S. aureus than ampicillin. Compounds **3d** and **3e** (MIC = 100 μg/mL) and compounds **3d** and **3f** (MIC = 100 μg/mL) were found to be equipotent to ampicillin against *E. coli and Salmonella typhi*, respectively.

Compounds **3g** and **3i** (MIC = 250 μg/mL) depicted better activity than did griseofulvin (MIC = 500 μg/mL), whereas compounds **3b**, **3c**, and **3d** were found to be equipotent to griseofulvin (MIC = 500 μg/mL) against *C. albicans*. None of the tested compounds were found to possess better activity against *A. niger* than were standard drugs.

All compounds **3a–l** possess promising antibacterial activity against Gram-positive bacteria *B. subtilis* and *S. aureus*. By examining the antimicrobial data, it has been observed that the derivatization of the parent molecule altered the antimicrobial potency of the synthesized analogues.

The observation indicates that varying the substitution on coumarin ring did not affect the antibacterial activity to a remarkable extent, but as the substitution on pendant phenyl ring was altered, a drastic change in antibacterial potency was observed. For example, when CH_3 group was replaced with OCH_3 group, the antibacterial potency varied but to a smaller extent. When the CH_3 was replaced by Cl group, the antibacterial potency enhanced markedly. In fact, compounds **3c**, **3f**, and **3i** (R_2 = Cl) emerged as most proficient members of the series. It can be concluded that introducing an electron-withdrawing group in pendant phenyl ring enhances the antibacterial activity against the Gram-positive bacterial stains.

Among all the tested compounds, compounds **3c**, **3f**, and **3i** were found to be the most proficient members of the series.

EXPERIMENTAL SECTION

All the melting points were determined on μThermoCal 10 apparatus. All the IR spectra (KBr disc) were recorded on Shimadzu FT-IR 8400-S spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance 400 spectrometer (Bruker Corp., Billerica, MA) operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. The chemical shift (δ) is reported in ppm using DMSO-d_6 as a solvent and calibrated standard solvent signal. Mass spectra were recorded on Shimadzu QP 2010 spectrometer (Shimadzu Corp., Kyoto, Japan). Elemental analysis was carried out on PerkinElmer 2400 C-H-N-S-O Analyzer Series-II. The precursors 4-hydroxycoumarin [31] **1a–d** and 3-(pyridin-4-yl)-1-aryl-prop-2-ene-1-ones [27] **2a–c** were prepared according to literature procedures.

General procedure for synthesis of 2-aryl-4-(pyridin-4-yl)-5*H*-chromeno[4,3-*b*]pyridin-5-one (3a–l). An appropriate 4-hydroxycoumarin **1a–d** (0.005 mol) was taken in glacial acetic acid (15 mL). To this solution, ammonium acetate (0.05 mol) was added, and then a solution of appropriate 3-(pyridin-4-yl)-1-aryl-prop-2-en-1-one **2a–c** (0.005 mol) in acetic acid (15 mL) was added while stirring at ambient temperature. The reaction mixture was further stirred for 30 min and then refluxed in an oil bath at 140°C. The progress of reaction was monitored by thin-layer chromatography. It was then allowed to come to ambient temperature and poured into ice-cold water (75 mL). The gummy mass obtained was extracted with chloroform (3 × 30 mL). The combined chloroform extract was washed with 10% sodium bicarbonate solution (3 × 20 mL) and then with water (3 × 20 mL). It was then dried over anhydrous sodium sulfate. The removal of chloroform under vacuum gave a solid product. This was further purified by column chromatography using silica gel and ethyl acetate–pet.ether (60:80) (2:8) as an eluent. Thus, 2-aryl-4-(pyridin-4-yl)-5*H*-chromeno[4,3-*b*]pyridin-5-one (**3a–l**) (bipyridyl-fused coumarins) were obtained as white to pale yellow solid, which were recrystallized from chloroform–hexane.

4-(Pyridin-4-yl)-2-p-tolyl-5H-chromeno[4,3-b]pyridin-5-one (3a). IR (KBr) (ν): 3031 (Ar C–H), 2921 (Ali.C–H), 1728 (C═O), 1581 (C═C), 1534 (C═N), 819 (*p*-disubstituted benzene ring) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.49 (s, 3H, CH_3), 7.35–7.71 (m, 8H, Ar–H), 7.86 (dd, 1H, J = 1.6 and 8.0 Hz, C_{10}–H), 8.20 (d, 2H, J = 8.0 Hz, C_2''–H and C_6''–H), 8.76 (d, 2H, J = 4.4 Hz, C_2'–H and C_6'–H); ^{13}C NMR ($CDCl_3$) δ (ppm): 21.48 (CH_3), 112.43 (C), 116.90 (CH), 119.53 (C), 121.77 (CH), 122.79 (CH), 124.64 (CH), 125.60 (CH), 127.73 (CH), 129.86 (CH), 132.44 (CH), 134.45 (C), 141.71 (C), 147.71 (C), 149.45 (CH), 152.03 (C), 152.89 (C), 153.15 (C), 159.35 (C), 161.53 (CO of coumarin); MS *m/z* (%): 364 [M+]. *Anal*. Calcd. for $C_{24}H_{16}N_2O_2$: C (79.11), H (4.43), N (7.69); found: C (79.08), H (4.47), N (7.74).

2-(4-Methoxyphenyl)-4-(pyridin-4-yl)-5H-chromeno[4,3-b] pyridin-5-one (3b). IR (KBr) (ν): 3038 (Ar C–H), 2932 (Ali.C–H), 1730 (C═O), 1589 (C═C), 1536 (C═N), 827 (*p*-disubstituted benzene ring) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ (ppm): 3.93 (3H, s, OCH_3), 7.04–7.68 (8H, m, Ar–H), 7.78 (1H, dd, J = 1.6 and 8.4 Hz, C_{10}–H), 8.19 (2H, d, J = 8.8 Hz, C_2''–H and C_6''–H), 8.76 (2H, concealed d, C_2'–H and C_6'–H); ^{13}C NMR ($CDCl_3$) δ (ppm): 55.52 (OCH_3), 111.32 (C), 114.52 (CH), 118.10 (CH), 119.02 (CH), 122.26 (C), 122.98 (CH), 124.75 (CH), 126.68 (CH), 128.95 (C), 129.44 (CH), 132.44 (C), 135.43 (CH), 141.52 (C), 148.27 (C), 149.08 (CH), 151.81 (C), 155.09 (C), 160.39 (C), 161.27 (CO of coumarin). *Anal*. Calcd.

for $C_{24}H_{16}N_2O_3$: C (75.78), H (4.24), N (7.36); found: C (75.82), H (4.29), N (7.40).

2-(4-Chlorophenyl)-4-(pyridin-4-yl)-5H-chromeno[4,3-b] pyridin-5-one (3c). IR (KBr) (ν): 3041 (Ar C–H), 2931 (Ali.C–H), 1736 (C═O), 1593 (C═C), 1528 (C═N), 832 (*p*-disubstituted benzene ring) cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm): 7.34–7.69 (8H, m, Ar–H), 7.74 (1H, dd, J = 1.6 and 10.0 Hz, C_{10}–H), 8.15 (2H, d, J = 8.0 Hz, C_2''–H and C_6''–H), 8.80 (2H, d, J = 5.6 Hz, C_2'–H and C_6'–H); ^{13}C NMR ($CDCl_3$) δ (ppm): 111.93 (C), 117.03 (CH), 119.76 (C), 121.86 (CH), 122.71 (CH), 124.66 (CH), 126.17 (CH), 127.75 (CH), 130.11 (CH), 132.52 (CH), 135.53 (C), 142.22 (C), 147.89 (C), 149.61 (CH), 151.94 (C), 153.19 (C), 154.47 (C), 160.21 (C), 162.42 (CO of coumarin). *Anal*. Calcd. for $C_{23}H_{13}ClN_2O_2$: C (71.79), H (3.41), N (7.28); found: C (71.83), H (3.38), N (7.33).

9-Methyl-4-(pyridin-4-yl)-2-p-tolyl-5H-chromeno[4,3-b] pyridin-5-one (3d). IR (KBr) (ν): 3043 (Ar C–H), 2939 (Ali.C–H), 1734 (C═O), 1598 (C═C), 1536 (C═N), 824 (*p*-disubstituted benzene ring) cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm): 2.46 and 2.47 (6H, two s, 2 × CH_3), 7.35–8.20 (10H, m, Ar–H), 8.76 (2H, d, J = 5.6 Hz, C_2'–H and C_6'–H); ^{13}C NMR ($CDCl_3$) δ (ppm): 20.88 (CH_3), 21.50 (CH_3), 117.88 (CH), 119.38 (CH), 122.82 (CH), 126.05 (CH), 127.76 (CH), 129.87 (CH), 133.79 (C), 134.38 (C), 134.54 (C), 134.68 (C), 136.71 (CH), 141.65 (C), 141.84 (C), 147.95 (C), 149.33 (CH), 152.01 (C), 152.04 (C), 153.30 (C), 160.61 (CO of coumarin). *Anal*. Calcd. for $C_{25}H_{18}N_2O_2$: C (79.35), H (4.79), N (7.40); found: C (79.40), H (4.83), N (7.37).

2-(4-Methoxyphenyl)-9-methyl-4-(pyridin-4-yl)-5H-chromeno[4,3-b]pyridin-5-one (3e). IR (KBr) (ν): 3041 (Ar C–H), 2931 (Ali.C–H), 1731 (C═O), 1597 (C═C), 1547 (C═N), 832 (*p*-disubstituted benzene ring) cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm): 2.41 (3H, s, CH_3), 3.92 (3H, s, OCH_3), 7.04–8.28 (10H, m, Ar–H), 8.75 (2H, concealed d, C_2'–H and C_6'–H); ^{13}C NMR ($CDCl_3$) δ (ppm): 21.52 (CH_3), 55.36 (OCH_3), 108.14 (CH), 114.62 (CH), 120.03 (CH), 121.49 (C), 122.40 (CH), 123.73 (C), 123.77 (C), 124.02 (C), 128.68 (CH), 131.61 (CH), 136.85 (CH), 141.52 (C), 145.36 (C), 149.51 (C), 150.34 (CH), 151.93 (C), 160.98 (C), 162.11 (C), 162.72 (CO of coumarin). *Anal*. Calcd. for $C_{25}H_{18}N_2O_3$: C (76.13), H (4.60), N (7.10); found: C (76.09), H (4.57), N (7.15).

2-(4-Chlorophenyl)-9-methyl-4-(pyridin-4-yl)-5H-chromeno[4,3-b]pyridin-5-one (3f). IR (KBr) (ν): 3037 (Ar C–H), 2928 (Ali.C–H), 1736 (C═O), 1601 (C═C), 1543 (C═N), 829 (*p*-disubstituted benzene ring) cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm): 2.46 (3H, s, CH_3), 7.13–7.68 (7H, m, Ar–H), 7.92 (1H, dd, J = 1.6 and 7.6 Hz, C_{10}–H), 8.06 (2H, d, J = 8.4 Hz, C_2''–H and C_6''–H), 8.78 (2H, d, J = 6.0 Hz, C_2'–H and C_6'–H); ^{13}C NMR ($CDCl_3$) δ (ppm): 21.61 (CH_3), 107.37 (CH), 118.12 (CH), 121.21 (C), 122.52 (CH), 124.03 (C), 125.14 (C), 128.43 (CH), 129.50 (CH), 130.17 (C), 131.22 (CH), 137.09 (CH), 137.64 (C), 145.31 (C), 148.61 (C), 150.11 (CH), 151.88 (C), 152.48 (C), 161.22 (C), 162.75 (CO of coumarin). *Anal*. Calcd. for $C_{24}H_{15}ClN_2O_2$: C (72.27), H (3.79), N (7.02); found: C (72.32), H (3.82), N (6.97).

7-Methyl-4-(pyridin-4-yl)-2-p-tolyl-5H-chromeno[4,3-b] pyridin-5-one (3g). IR (KBr) (ν): 3042 (Ar C–H), 2932 (Ali.C–H), 1735 (C═O), 1593 (C═C), 1533 (C═N), 820 (*p*-disubstituted benzene ring) cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm): 2.47 and 2.71 (6H, two s, 2 × CH_3), 7.32–7.64 (7H, m, Ar–H), 8.01 (1H, concealed dd, C_{10}–H), 8.12 (2H, d, J = 8.0 Hz, C_2''–H and C_6''–H), 8.76 (2H, d, J = 5.2 Hz, C_2'–H and C_6'–H); ^{13}C NMR ($CDCl_3$) δ (ppm): 15.98 (CH_3), 21.48 (CH_3), 119.46 (CH), 122.18 (C), 122.88 (CH), 124.21 (CH), 124.24 (CH), 127.56 (C), 127.79 (CH), 129.84 (CH), 133.96 (C), 136.39 (CH), 138.28 (C), 141.73 (C), 148.08 (C), 149.25 (CH), 151.89 (C), 153.58 (C), 157.83 (C), 160.72 (C), 161.21 (CO of coumarin). *Anal*. Calcd. for $C_{25}H_{18}N_2O_2$: C (79.35), H (4.79), N (7.40); found: C (79.39), H (4.83), N (7.37).

2-(4-Methoxyphenyl)-7-methyl-4-(pyridin-4-yl)-5H-chromeno[4,3-b]pyridin-5-one (3h). IR (KBr) (ν): 3032 (Ar C–H), 2938 (Ali.C–H), 1737 (C═O), 1599 (C═C), 1537 (C═N), 829 (*p*-disubstituted benzene ring) cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm): 2.71 (3H, s, CH_3), 3.92 (3H, s, OCH_3), 7.05–7.64 (7H, m, Ar–H), 8.01 (1H, concealed dd, C_{10}–H), 8.21 (2H, d, J = 9.2 Hz, C_2''–H and C_6''–H), 8.76 (2H, concealed d, C_2'–H and C_6'–H); ^{13}C NMR ($CDCl_3$) δ (ppm): 15.98 (CH_3), 55.49 (OCH_3), 114.49 (CH), 118.98 (CH), 122.22 (C), 122.88 (CH), 124.16 (CH), 124.23 (CH), 127.49 (C), 129.53 (CH), 134.87 (C), 136.31 (CH), 139.55 (C), 141.02 (C), 145.77 (C), 148.13 (C), 149.27 (CH), 153.58 (C), 160.33 (C), 161.28 (C), 162.30 (CO of coumarin). *Anal*. Calcd. for $C_{25}H_{18}N_2O_3$: C (76.13), H (4.60), N (7.10); found: C (76.10), H (4.56), N (7.15).

2-(4-Chlorophenyl)-7-methyl-4-(pyridin-4-yl)-5H-chromeno[4,3-b]pyridin-5-one (3i). IR (KBr) (ν): 3034 (Ar C–H), 2928 (Ali.C–H), 1738 (C═O), 1596 (C═C), 1534 (C═N), 826 (*p*-disubstituted benzene ring) cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm): 2.75 (3H, s, CH_3), 7.33–7.69 (7H, m, Ar–H), 8.01 (1H, concealed dd, C_{10}–H), 8.24 (2H, d, J = 8.8 Hz, C_2''–H and C_6''–H), 8.76 (2H, concealed d, C_2'–H and C_6'–H); ^{13}C NMR ($CDCl_3$) δ (ppm): 15.60 (CH_3), 112.00 (C), 119.48 (CH), 122.15 (C), 124.36 (CH), 127.58 (C), 129.13 (CH), 129.35 (CH), 131.79 (C), 133.96 (CH), 135.15 (C), 135.35 (CH), 136.56 (CH), 137.55 (C), 147.72 (C), 149.38 (CH), 152.34 (C), 153.56 (C), 159.33 (C), 161.17 (CO of coumarin). *Anal*. Calcd. for $C_{24}H_{15}ClN_2O_2$: C (72.27), H (3.79), N (7.02); found: C (72.31), H (3.84), N (6.97).

9-Chloro-4-(pyridin-4-yl)-2-p-tolyl-5H-chromeno[4,3-b] pyridin-5-one (3j). IR (KBr) (ν): 3033 (Ar C–H), 2925 (Ali.C–H), 1732 (C═O), 1585 (C═C), 1539 (C═N), 825 (*p*-disubstituted benzene ring) cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm): 2.47 (3H, s, CH_3), 7.34–7.74 (7H, m, Ar–H), 7.87 (1H, dd, J = 1.6 and 8.0 Hz, C_{10}–H), 8.11 (2H, d, J = 8.4 Hz, C_2''–H and C_6''–H), 8.78 (2H, d, J = 5.2 Hz, C_2'–H and C_6'–H); ^{13}C NMR ($CDCl_3$) δ (ppm): 21.49 (CH_3), 119.74 (CH), 122.77 (CH), 123.17 (C), 126.06 (CH), 126.97 (CH), 127.79 (CH), 129.91 (CH), 130.62 (C), 133.57 (C), 135.49 (CH), 136.36 (C), 139.37 (C), 142.13 (C), 147.47 (C), 149.39 (CH), 152.15 (C), 153.40 (C), 157.86 (C), 161.05 (CO of coumarin). *Anal*. Calcd. for $C_{24}H_{15}ClN_2O_2$: C (72.27), H (3.79), N (7.02); found: C (72.24), H (3.82), N (7.08).

9-Chloro-2-(4-methoxyphenyl)-4-(pyridin-4-yl)-5H-chromeno[4,3-b]pyridin-5-one (3k). IR (KBr) (ν): 3041 (Ar C–H), 2926 (Ali.C–H), 1731 (C═O), 1588 (C═C), 1529 (C═N), 818 (*p*-disubstituted benzene ring) cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm): 3.87 (3H, s, OCH_3), 6.59–7.28 (7H, m, Ar–H), 7.58 (1H, concealed dd, C_{10}–H), 7.69 (2H, d, J = 8.8 Hz, C_2''–H and C_6''–H), 8.63 (2H, d, J = 4.8 Hz, C_2'–H and C_6'–H); ^{13}C NMR ($CDCl_3$) δ (ppm): 55.47 (OCH_3), 105.24 (CH), 114.51 (CH), 119.84 (CH), 121.33 (C), 122.30 (CH), 123.56 (C), 123.91 (C), 123.94 (C), 128.62 (CH), 131.49 (CH), 136.73 (CH), 141.52 (C), 145.27 (C), 149.45 (C), 150.27 (CH), 151.85 (C), 161.03 (C), 162.02 (C), 162.63 (CO of coumarin). *Anal*. Calcd. for $C_{24}H_{15}ClN_2O_3$: C (69.49), H (3.64), N (6.75); found: C (69.53), H (3.59), N (6.79).

9-Chloro-2-(4-chlorophenyl)-4-(pyridin-4-yl)-5H-chromeno[4,3-b]pyridin-5-one (3l). IR (KBr) (ν): 3036 (Ar C–H), 2924 (Ali.C–H), 1732 (C═O), 1582 (C═C), 1535 (C═N), 814 (*p*-disubstituted benzene ring) cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm): 6.64–7.55 (7H, m, Ar–H), 7.67 (2H, d, J = 8.4 Hz, C_2''–H and C_6''–H), 7.91 (1H, concealed dd, C_{10}–H), 8.64 (2H, d, J = 5.6 Hz, C_2'–H and C_6'–H); ^{13}C NMR ($CDCl_3$) δ (ppm): 106.39 (CH), 120.16 (CH), 121.08 (C), 122.45 (CH), 123.96 (C), 125.07 (C), 128.31 (CH), 129.43 (CH), 130.07 (C), 131.14 (CH), 137.06 (CH), 137.61 (C), 145.20 (C), 148.42 (C), 150.03 (CH), 151.77 (C), 152.36 (C), 161.10 (C), 162.59 (CO of coumarin). *Anal*. Calcd. for $C_{23}H_{12}Cl_2N_2O_2$: C (65.89), H (2.88), N (6.68); found: C (65.94), H (2.92), N (6.73).

***In vitro* evaluation of antimicrobial activity.** The MICs of synthesized compounds were carried out by broth microdilution method. Bacterial strains were primarily inoculated into Mueller–Hinton agar, and after overnight growth, a number of colonies were directly suspended in saline solution until the turbidity matched the turbidity of the McFarland standard [approximately 10^8 CFU/mL (colony-forming unit) per milliliter well]; that is, inoculum size for test strain was adjusted to 10^8 CFU/mL by comparing the turbidity (turbidimetric method). Similarly, fungi were inoculated on Sabouraud dextrose broth; the procedures of inoculum standardization were also similar. Dimethyl sulfoxide (DMSO) was used as diluent to obtain desired concentration of synthesized compounds and standard drugs to test upon standard microbial strains; that is, the compounds were dissolved in DMSO, and the solutions were diluted with a culture medium. Each compound and standard drugs were diluted obtaining 2000 μg/mL concentration, as a stock solution. By further progressive dilutions with the test medium, the required concentrations were obtained for primary and secondary screening. In the primary screening 1000, 500, and 250 μg/mL concentrations of the synthesized compounds were taken. The active compounds found in this primary screening were further diluted to obtain 200, 100, 62.5, 50, 25, 12.5, and 6.25 μg/mL concentrations for secondary screening to test in a second set of dilution against all microorganisms. Briefly, the control tube containing no antibiotic is immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of a plate of medium suitable for the growth of the test organism. The tubes are then put for incubation at 37°C for 24 h for bacteria and 48 h for fungi. Growth or a lack of growth in the tubes containing the antimicrobial agent was determined by comparison with the growth control, indicated by turbidity. The lowest concentration that completely inhibited visible growth of the organism was recorded as the MIC (μg/mL); that is, the amount of growth from the control tube before incubation (which represents the original inoculum) is compared. A set of tubes containing only seeded broth and the solvent controls were maintained under identical conditions so as to make sure that the solvent had no influence on strain growth. The protocols were summarized in Table 2 as the MIC (μg/mL).

CONCLUSION

In conclusion, we reported convenient and efficient synthesis of some new bipyridyl-fused coumarins. The adopted strategy gave the target compounds in better yields than did earlier reported methods. The antimicrobial assay of the synthesized compounds showed good-to-moderate activity. The compounds not only can serve as lead for novel biologically potent agents with varied mechanism of action but also can find application in other fields as well, such as photochemistry and nonlinear optical chromophores.

Acknowledgment. The authors are thankful to the Head of the Department of Chemistry, Sardar Patel University, for providing basic research amenities.

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