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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of a new series of 2-(2-oxo-2*H*-chromen-3-yl)-5*H*-chromeno[4,3-*b*]pyridin-5-ones by two facile methods and evaluation of their antimicrobial activity

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Abstract A series of novel 2-(2-oxo-2*H*-chromen-3-yl)-5*H*-chromeno[4,3-*b*]pyridin-5-ones **4a**–**4l** have been synthesized using two methodologies (using Mannich base of 4-hydroxy coumarin and 4-chloro-3-formyl coumarin) (Patel *et al.*, 2012) and assayed for their antibacterial activity against gram-positive bacteria viz. *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96) and gram-negative bacteria viz. *Escherichia coli* (MTCC 443), *Salmonella typhimurium* (MTCC 98). The compounds **4a**–**4l** were also assayed for their antifungal activity against *Aspergillus niger* (MTCC 282) and *Candida albicans* (MTCC 227). All the compounds **4a**–**4l** exhibited potent inhibitory activity against gram-positive bacteria compared to standard drugs at the tested concentrations. The compounds also showed appreciable activity against gram-negative bacteria as well as fungi. The compounds **4e**, **4f**, **4i**, **4k**, and **4l** are found to be the most proficient members of the series and emerged as potential molecules for further development.

Keywords Krohnke's reaction · 3-[(Ethylamino)methyl]-4-hydroxy coumarin · 4-Chloro-2-oxo-2*H*-chromene-3-carbaldehyde · 1-[2-Oxo-2-(2-oxo-2*H*-chromen-3-yl)ethyl]pyridinium salt · Antimicrobial activity

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Introduction

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens with particular relevance for gram-positive bacteria (Tenover and McDonald, 2005; Pfeltz and Wilkinson, 2004; Roberts, 2004; Kratky *et al.*, 2012; Muroi *et al.*, 2004). On the other hand, a recent survey of novel small molecule therapeutics revealed that the majority of them result from an analog-based approach and that their market value represents two-thirds of all drug sales (Wermuth, 2006). 2*H*-Chromen-2-ones (coumarins) are important oxygen containing heterocycles which possess variety of biological activities such as anti-inflammatory (Timonen *et al.*, 2011), antibacterial (Song *et al.*, 2012), antioxidant (Xiao *et al.*, 2012), anticoagulant (Mladenovic *et al.*, 2012), anti-HIV (Olmedo *et al.*, 2012), antihyperlipidemic (Sashidhara *et al.*, 2010), and as antitumour (Shen *et al.*, 2012). Besides, 2*H*-chromen-2-one nucleus is also present in promising drug candidates as topoisomerase II (Rappa *et al.*, 2000) and tyrosine kinase (Yang *et al.*, 1999) inhibitors. 2*H*-Chromen-2-ones having pyridyl substitution possess diverse physiological actions viz. CNS depressant activity (Moffett, 1964), antifungal (Sardari *et al.*, 1999), and antibacterial (Modranka *et al.*, 2006) activities. Also, 2*H*-chromen-2-ones fused with pyridines have been reported to possess antiallergic (Ukawa *et al.*, 1986), antimicrobial (Patel *et al.*, 2012), analgesic properties (Heber and Berghaus, 1994) and even antidiabetic activities (Heber, 1987). Owing to such interesting biological properties of both, pyridyl-substituted and pyrido-fused 2*H*-chromen-2-ones, it was thought worthwhile to synthesize molecules which incorporate both of these structural features. Therefore, the simple and efficient method for

synthesis of such compounds would be a favorable step towards further investigation in drug discovery.

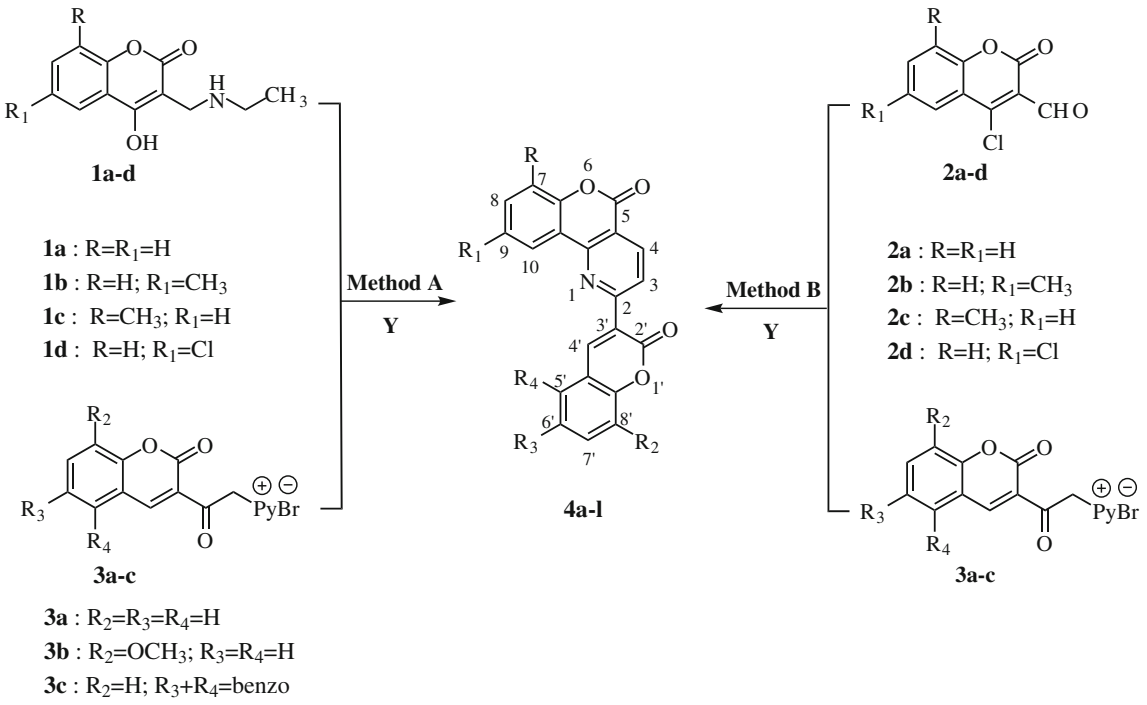
Hence, in a view of the biological significance of pyridyl-substituted 2*H*-chromen-2-ones, pyrido-fused 2*H*-chromen-2-ones, and in continuation of our research work on the synthesis of such biologically active 2*H*-chromen-2-one derivatives (Brahmbhatt *et al*., 2011), we herein, report the synthesis of a series of 2-(2-oxo-2*H*-chromen-3-yl)-5*H*-chromeno[4,3-*b*]pyridin-5-ones **4a–4l** by two facile methods (**A** and **B**), employing different precursors, in one-pot three-component system (Scheme 1). In method **A**, 3-[(ethylamino)methyl]-4-hydroxy-2*H*-chromen-2-ones **1a–1d** were reacted with 1-[2-oxo-2-(2-oxo-2*H*-chromen-3-yl)ethyl]-pyridinium salts **3a–3c** in the presence of ammonium acetate and acetic acid, while in method **B**, 4-chloro-2-oxo-2*H*-chromene-3-carbaldehydes **2a–2d** were reacted with the salts **3a–3c** in the presence of ammonium acetate for building up the pyridine ring. Further, all the newly synthesized compounds were evaluated for their in vitro antimicrobial activity by broth micro dilution method (NCCLS, 2002) against different pathogenic strains.

Results and discussion

The synthetic routes adopted to obtain various 2-(2-oxo-2*H*-chromen-3-yl)-5*H*-chromeno[4,3-*b*]pyridin-5-ones **4a–4l** are shown in Scheme 1. The subsequent one-pot, three-component reaction of precursors **1a–1d** or **2a–2d** with appropriate salt **3a–3c** in the presence of ammonium acetate in refluxing glacial acetic acid solvent, resulted the new series of 2-(2-oxo-2*H*-chromen-3-yl)-5*H*-chromeno[4,3-*b*]-pyridin-5-ones **4a–4l** in good yields (Table 1).

The reaction pathways for methods **A** and **B** are shown in Scheme 2. Here, the starting material Mannich base **[1]** undergoes decomposition and results in the formation of coumarin methide **[C]**. This coumarin methide **[C]** then reacts with salt **[3]** in the presence of ammonium acetate and acetic acid, and results in a formation of a 1,5-dicarbonyl intermediate **[D]** which finally gets converted into expected product **[4]** by Krohnke's reaction (Krohnke, 1976). The coumarin methide **[C]** also undergoes a side reaction and gives a dicoumarol **[E]** as a byproduct. The formation of dicoumarol **[E]** as a byproduct may be responsible for the low yield in method **A** compared to method **B**. Similarly in method **B**, **[2]** and **[3]** react to give the intermediate **[F]** which finally gets converted into expected product **[4].**

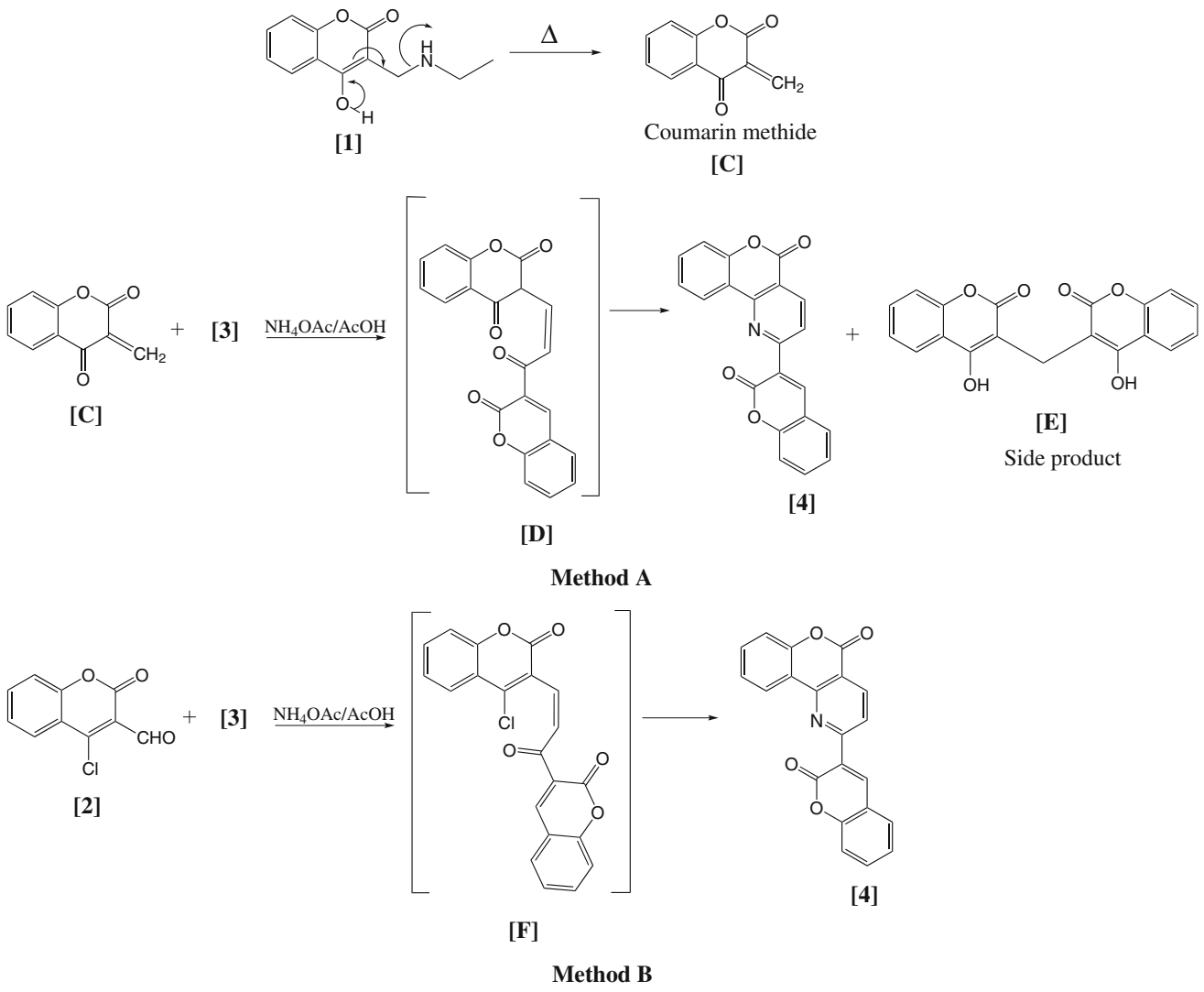
Here, we observed that the 4-chloro-3-formyl coumarins **2a–2d** reacted much smoothly than Mannich bases of 4-hydroxy coumarins **1a–1d**, with salts **3a–3c** to afford target compounds in good yield. In our earlier work (Patel *et al*., 2012) also similar observations were made. There also 4-chloro-3-formyl coumarins reacted smoothly to give fused chromenone derivatives in appreciable yield. Thus, it can be said that 4-chloro-3-formyl coumarins are preferable precursors for the synthesis of pyrido-fused chromenone derivatives.



Scheme 1 Reagent and conditions: (**Y**) NH_4OAc, AcOH, reflux

Table 1 Isolated yields from methods **A** and **B** of compounds **4a–4l**

Compounds	R	R_1	R_2	R_3	R_4	Isolated yields (%)	
						Method **A**	Method **B**
4a	H	H	H	H	H	47	62
4b	H	H	OCH_3	H	H	38	54
4c	H	H	H	Benzo		43	64
4d	H	CH_3	H	H	H	40	59
4e	H	CH_3	OCH_3	H	H	44	61
4f	H	CH_3	H	Benzo		46	58
4g	CH_3	H	H	H	H	50	69
4h	CH_3	H	OCH_3	H	H	39	56
4i	CH_3	H	H	Benzo		54	73
4j	H	Cl	H	H	H	56	78
4k	H	Cl	OCH_3	H	H	57	79
4l	H	Cl	H	Benzo		52	76



Scheme 2 Reaction pathways for methods **A** and **B**

The structures of all the synthesized compounds **1a–1d** and **4a–4l** were established on the basis of elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR, APT, and selected mass spectral data.

In IR spectra, compounds **4a–4l** showed a very strong band between 1,689 and 1,708 cm^{-1} for the carbonyl (C=O) stretching of δ-lactone ring present in coumarin nucleus. The strong bands for aromatic C=C and C=N stretching vibrations, were observed between 1,606–1,632 and 1,479–1,505 cm^{-1}, respectively. The aromatic C–H stretching vibrations were observed between 3,093 and 3,144 cm^{-1}. The δ-lactone carbonyl stretching frequency was observed somewhat lower than the expected frequency ($\sim$1,720 cm^{-1}). This may be due to the reduction of carbonyl character of δ-lactone ring by the nitrogen of pyridine ring. Such type of reduction in carbonyl frequency is observed in coumarin derivatives having nitrogen attached at 4-position (Stamboliyska *et al*., 2010).

In the ^{1}H-NMR spectra of all the compounds **4a–4l**, the aromatic proton signals except C_{10}-H were observed in the region of 6.50δ–8.50δ. In all the compounds the C_{10}-H and C_4'-H signals were observed in the downfield region between 9.50δ and 10.80δ. These two proton signals shift in the downfield region due to the peri effect of the nitrogen atom of the pyridine ring.

All the compounds showed signals at expected δ values in the ^{13}C-NMR spectra. The aromatic carbon signals were observed between 95.0δ and 176.0δ while the two δ-lactone carbonyl carbons appeared between 160.0δ and 181.0δ. The differentiation of quaternary and tertiary carbons was done by the help of DEPT-135 spectra. The NMR spectral data fully supported the structure of the products. The structure of one representative compound **4a** was further supported by the help of mass spectral data.

Evaluation of antimicrobial activity

All the compounds **4a–4l** were assayed for their in vitro antimicrobial activity against gram-positive bacteria viz. *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), gram-negative bacteria viz. *Escherichia coli* (MTCC 443), *Salmonella typhimurium* (MTCC 98), and antifungal activity against *Aspergillus niger* (MTCC 282) and *Candida albicans* (MTCC 227) by broth dilution method (NCCLS, 2002).

It is perceived by examining the antimicrobial activity data (Table 2) that compounds **4e** and **4k** (MIC = 62.5 μg/mL) and compounds **4c**, **4h**, and **4i** (MIC = 125 μg/mL) showed excellent activity against gram-positive bacteria *B. subtilis* as compared to ampicillin (MIC = 250 μg/mL) where as compounds **4f** and **4l** (MIC = 62.5 μg/mL), compounds **4b**, **4c**, **4e**, and **4i** (MIC = 100 μg/mL), and compound **4j** (MIC = 125 μg/mL) showed excellent activity against gram-positive bacteria *S. aureus* as compared to ampicillin (MIC = 250 μg/mL). Compounds **4i** and **4l** (MIC = 62.5 μg/mL) have shown excellent activity against *E. coli* as compared to ampicillin (MIC = 100 μg/mL).

As compared to ampicillin (MIC = 250 μg/mL) compounds **4b**, **4d**, **4f**, **4j**, and **4l** (MIC = 200 μg/mL) showed better activity against *B. subtilis*, whereas compounds **4a**, **4g**, and **4k** (MIC = 200 μg/mL) were found to be more potent against *S. aureus* as compared to ampicillin (MIC = 250 μg/mL).

Compounds **4a** and **4g** (MIC = 250 μg/mL) were found to be equally active against gram-positive bacteria *B. subtilis* while compounds **4d** and **4h** (MIC = 250 μg/mL) showed equal activity against *S. aureus* compared to ampicillin (MIC = 250 μg/mL). Compounds **4i**, **4j**, and **4l** (MIC = 100 μg/mL) have been found to be equipotent as compared to ampicillin (MIC = 100 μg/mL) against *S. typhi*.

Compounds **4h** (MIC = 200 μg/mL) and **4c** (MIC = 250 μg/mL) showed better activity than griseofulvin (MIC = 500 μg/mL) against fungal pathogen *C. albicans*. Compounds **4d**, **4g**, and **4l** have been found to be equipotent as compared to griseofulvin (MIC = 500 μg/mL) against *C. albicans*. None of the tested compounds showed better activity against *A. niger*.

Structure–activity relationship

All the compounds **4a–4l** possess promising antibacterial activity against gram-positive bacteria *B. subtilis* and *S. aureus*. Examining the antimicrobial data, it has been revealed that the derivatization of the parent molecule increased the antimicrobial potency of the synthesized analogs.

The observation indicates that the compounds **4b**, **4e**, **4h**, and **4k** bearing electron releasing group, i.e., methoxy ($R_2 = OCH_3$) group in the pendent 2-oxo-2*H*-chromenyl nucleus showed significant inhibitory activity against gram-positive bacteria than the parent analogs. Compounds **4d**, **4e**, and **4f** ($R_1 = CH_3$) and compounds **4g**, **4h**, and **4i** ($R = CH_3$) having lipophilic methyl group showed moderate to good antimicrobial activity.

Among all the compounds **4a–4l**, compounds bearing additional benzene ring, i.e., **4c**, **4f**, **4i**, and **4l** in the pendent 2-oxo-2*H*-chromenyl nucleus have been found to be more active against gram-positive bacteria than their other analogs. Compounds **4j**, **4k**, and **4l** ($R_1 = Cl$) having electron withdrawing group showed good antimicrobial activity.

Compound **4l** having both chloro substitution and benzene ring fusion in the pendent 2-oxo-2*H*-chromenyl nucleus possesses the highest antibacterial effectiveness against both gram-positive and gram-negative bacteria.

Table 2 Antimicrobial activity of compounds **4a–4l**

Compounds	Minimum inhibitory concentration (MIC, μg/mL)					
	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	B.s.	*S.a.*	*E.c.*	*S.t.*	*A.n.*	*C.a.*
4a	250	**200**	125	200	500	1,000
4b	**200**	**100**	200	250	1,000	1,000
4c	**125**	**100**	250	200	>1,000	**250**
4d	**200**	250	125	125	>1,000	500
4e	**62.5**	**100**	250	200	500	1,000
4f	**200**	**62.5**	250	250	500	1,000
4g	250	**200**	200	250	>1,000	500
4h	**125**	250	250	250	>1,000	**200**
4i	**125**	**100**	**62.5**	100	>1,000	1,000
4j	**200**	**125**	125	100	>1,000	>1,000
4k	**62.5**	**200**	200	250	500	>1,000
4l	**200**	**62.5**	**62.5**	100	1,000	500
Ampicillin	250	250	100	100	NT	NT
Norfloxacin	100	10	10	10	NT	NT
Griseofulvin	NT	NT	NT	NT	100	500
Nystatin	NT	NT	NT	NT	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*; NT, not tested

Bold values indicate that the particular compound possesses better activity than the standard drug

Among all the tested compounds, the compounds **4e**, **4f**, **4i**, **4k**, and **4l** are found to be the most proficient members of the series.

It is interesting to note that the antimicrobial data of compounds **4a–4l** are found to exhibit better activity than our earlier reported compounds (Patel *et al*., 2012). The compounds **4a–4l** showed better activity against gram-positive and gram-negative bacteria than the earlier reported compounds. No significant change in antifungal activity was observed. It is noteworthy that here also the compounds bearing methyl and chloro substitution exhibited better activity than the other analogs.

Experimental section

All the reagents used were of commercial grade. Melting points were determined through a Thiele apparatus and are uncorrected. IR spectra were recorded using KBr on Perkin Elmer Spectrum 100 FT-IR spectrometer. The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on Bruker Avance 400 spectrometer. Mass spectrum was recorded on Shimadzu QP 2010 spectrometer. Elemental analyzes (C, H, N) were carried out by means of Perkin Elmer 2400 C–H–N–S–O Analyzer Series II, and the data found were within ±0.4 % of theoretical values.

Various 1-[2-oxo-2-(2-oxo-2*H*-chromen-3-yl)ethyl]pyridinium salts (Koelsch, 1950; Rao and Rao, 1986) **3a–3c** and various 4-chloro-2-oxo-2*H*-chromene-3-carbaldehydes (Moorty *et al*., 1973) **2a–2d** were synthesized according to the literature procedures.

General procedure for the synthesis of 3-[(ethylamino)methyl]-4-hydroxy coumarins (**1a–1d**)

In a round bottom flask formaldehyde (37 % formalin) (0.025 mol), ethyl amine (0.03 mol), and ethanol (25 mL) were taken and stirred for 30 min at room temperature. Then a solution of appropriate 4-hydroxy coumarin (0.025 mol) in ethanol (15 mL) was added dropwise to the above solution during 30 min at room temperature with stirring. The stirring was further continued overnight at room temperature. The solid product thus obtained was filtered out, washed with diethyl ether and dried to yield **1a–1d** which were pure enough (checked by thin layer chromatography [TLC]) to use for further reaction. The purity of compounds was confirmed by TLC.

*3-((Ethylamino)methyl)-4-hydroxy-2H-chromen-2-one (**1a**)*

White solid; Yield: 67 %; m.p. 185 °C (lit. 186 °C) (Robertson and Link, 1953).

*3-((Ethylamino)methyl)-4-hydroxy-6-methyl-2H-chromen-2-one (**1b**)*

White solid; Yield: 69 %; m.p. 164 °C; IR (KBr, cm^{-1}): 3365 (O–H), 3325 (N–H), 3045 (Ar C–H), 2960 (aliphatic

C–H), 1720 (C=O), 1605 (Ar C=C); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 1.19 (3H, t, $J = 7.2$ *Hz*, –NHCH$_2$CH$_3$), 2.36 (3H, s, CH$_3$), 2.89 (2H, q, $J = 7.2$ *Hz*, –NHCH$_2$CH$_3$), 3.72 (2H, s, –CH$_2$–NH–), 7.17–7.68 (3H, m, Ar–H), 8.33 (2H, br, –NH– and –OH); ^{13}C-NMR (100 MHz, CD$_3$OD-d_4, δ ppm): 20.86 (CH$_3$), 30.48 (CH$_3$), 42.69 (CH$_2$), 43.40 (CH$_2$), 113.32 (C), 116.96 (C), 117.37 (CH), 124.18 (CH), 134.90 (CH), 135.36 (C), 153.36 (C), 166.35 (C), 168.44 (C=O); Anal. Calc. for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48; N, 6.00. Found: C, 67.18; H, 6.56; N, 5.89 %.

*3-((Ethylamino)methyl)-4-hydroxy-8-methyl-2H -chromen-2-one (**1c**)*

Yellow solid; Yield: 72 %; m.p. 157 °C; IR (KBr, cm^{-1}): 3360 (O–H), 3340 (N–H), 3055 (Ar C–H), 2935 (aliphatic C–H), 1710 (C=O), 1600 (Ar C=C); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 1.19 (3H, t, $J = 7.2$ *Hz*, –NHCH$_2$CH$_3$), 2.30 (3H, s, CH$_3$), 2.89 (2H, q, $J = 7.2$ *Hz*, –NHCH$_2$CH$_3$), 3.89 (2H, s, –CH$_2$–NH–), 7.01–7.69 (3H, m, Ar–H), 8.16 (2H, br, –NH– and –OH); ^{13}C-NMR (100 MHz, CD$_3$OD-d_4, δ ppm): 11.50 (CH$_3$), 15.74 (CH$_3$), 42.80 (CH$_2$), 43.63 (CH$_2$), 123.50 (CH), 123.90 (C), 124.32 (CH), 126.77 (C), 133.86 (CH), 152.49 (C), 153.62 (C), 167.66 (C), 168.38 (C=O); Anal. Calc. for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48; N, 6.00. Found: C, 66.73; H, 6.41; N, 5.91 %.

*6-Chloro-3-((ethylamino)methyl)-4-hydroxy-2H -chromen-2-one (**1d**)*

Yellow solid; Yield: 68 %; m.p. 174 °C; IR (KBr, cm^{-1}): 3370 (O–H), 3315 (N–H), 3040 (Ar C–H), 2950 (aliphatic C–H), 1715 (C=O), 1610 (Ar C=C); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 1.19 (3H, t, $J = 7.2$ *Hz*, –NHCH$_2$CH$_3$), 2.90 (2H, q, $J = 7.2$ *Hz*, –NHCH$_2$CH$_3$), 3.87 (2H, s, –CH$_2$–NH–), 7.18–7.76 (3H, m, Ar–H), 8.14 (2H, br, –NH– and –OH); ^{13}C-NMR (100 MHz, CD$_3$OD-d_4, δ ppm): 11.53 (CH$_3$), 43.00 (CH$_2$), 43.49 (CH$_2$), 119.29 (CH), 122.56 (C), 124.48 (C), 125.21 (CH), 129.77 (C), 132.44 (CH), 152.80 (C), 153.70 (C), 167.91 (C=O); Anal. Calc. for $C_{12}H_{12}ClNO_3$: C, 56.81; H, 4.77; N, 5.52. Found: C, 56.53; H, 4.71; N, 5.43 %.

Synthesis of 2-(2-oxo-2*H*-chromen-3-yl)-5*H*-chromeno[4,3-*b*]pyridin-5-one (**4a**)

*Method **A***

To a solution of **3a** (0.005 mol) in glacial acetic acid (15 mL), ammonium acetate (0.05 mol) was added with stirring at room temperature. Then a solution of **1a** (0.005 mol) in glacial acetic acid (15 mL) was added with stirring at room temperature during 15 min. The reaction mixture was further stirred for 45 min at room temperature, and then refluxed in an oil bath at 140 °C for 8 h. It was then allowed to cool to ambient temperature and poured into ice cold water (75 mL). The gummy mass thus 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 dried over anhydrous sodium sulfate. The removal of chloroform under vacuum gave a crude solid product. The TLC of the crude product showed two major spots. The crude product was subjected to column chromatography using silica gel and ethyl acetate–petroleum ether as an eluent. By eluting the column using (1:9) ethyl acetate–petroleum ether as a eluent, the product obtained, was found to be dicoumarol **(E)**. Then eluting the column using ethyl acetate–petroleum ether (3:7) as an eluent, the expected compound **4a** was obtained.

Dicoumarol **(E)**: Yield: 20 %; m.p. 289 °C (lit. 288–289 °C Stahmann *et al*., 1941).

Mixed melting point did not change.

The spectral data of the compound **4a** are given below.

*2-(2-Oxo-2H-chromen-3-yl)-5H-chromeno[4,3-b] pyridin-5-one (**4a**)*

Brown solid; Yield 47 %; m.p. 218–220 °C; IR (KBr, cm^{-1}): 3123 (Ar C–H), 1689 (C=O), 1624 (Ar C=C), 1501 (Ar C=N); ^{1}H-NMR (400 MHz, CDCl$_3$ + TFA-d_1, δ ppm): 7.35–8.12 (9H, m, Ar–H), 8.74 (1H, brs, C$_{10}$-H), 9.03 (1H, s, C$_4$′-H); ^{13}C-NMR (100 MHz, CDCl$_3$ + TFA-d_1, δ ppm): 110.00 (C), 112.82 (C), 115.63 (C), 117.62 (CH), 117.77 (CH), 118.48 (CH), 119.46 (CH), 125.58 (CH), 125.75 (CH), 126.64 (CH), 131.44 (C), 133.15 (C), 136.24 (CH), 136.51 (CH), 139.18 (C), 142.86 (CH), 147.78 (CH), 154.52 (C), 163.22 (C=O), 163.96 (C=O); Mass (*m/z*): 285 (M$^+$-56), 189 (100 %), 172 (17 %), 162 (21 %), 144 (15 %), 133 (36 %), 121 (93 %), 105 (11 %), 92 (68 %), 77 (10 %), 63 (31 %); Anal. Calc. for $C_{21}H_{11}NO_4$: C, 73.90; H, 3.25; N, 4.10. Found: C, 74.17; H, 3.19; N, 4.18 %.

Synthesis of 2-(2-oxo-2*H*-chromen-3-yl)-5*H*-chromeno[4,3-*b*]pyridin-5-one (**4a**)

*Method **B***

To a solution of **3a** (0.005 mol) in glacial acetic acid (15 mL), ammonium acetate (0.05 mol) was added with stirring at room temperature. Then a solution of **2a** (0.005 mol) in glacial acetic acid (15 mL) was added with stirring at room temperature during 15 min. The reaction mixture was further stirred for 45 min at room temperature and then refluxed in an oil bath at 140 °C for 8 h. It was

then allowed to cool 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 dried over anhydrous sodium sulfate. The removal of chloroform under vacuum gave a crude solid product. This was further purified by column chromatography using silica gel and ethyl acetate–petroleum ether (3:7) as an eluent, it gave brown solid product **4a** in 62 % yield. The product **4a** was identical in all aspects with that obtained by method **A**.

Similarly, the compounds **4b**–**4l** were synthesized using above two methods.

The spectral data of compounds **4b**–**4l** are given below.

*2-(8-Methoxy-2-oxo-2H-chromen-3-yl)-5H-chromeno[4,3-b]pyridin-5-one (**4b**)*

Brown solid; m.p. 211–213 °C; IR (KBr, cm^{-1}): 3137 (Ar C–H), 1696 (C=O), 1631 (Ar C=C), 1502 (Ar C=N); ^{1}H-NMR (400 MHz, $CDCl_3$ + TFA-d_1, δ ppm): 3.97 (3H, s, OCH_3), 6.98–8.11 (8H, m, Ar–H), 8.73 (1H, brs, C_{10}-H), 9.01 (1H, s, C_4'-H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 56.45 (OCH_3), 96.69 (C), 96.81 (C), 115.65 (C), 117.40 (CH), 117.52 (CH), 120.32 (C), 120.88 (C), 120.94 (C), 124.38 (CH), 124.48 (CH), 125.82 (CH), 126.23 (CH), 134.83 (CH), 134.92 (CH), 154.77 (C), 160.80 (CH), 162.50 (CH), 163.37 (C), 163.56 (C), 178.03 (C=O), 180.28 (C=O); Anal. Calc. for $C_{22}H_{13}NO_5$: C, 71.16; H, 3.53; N, 3.77. Found: C, 70.88; H, 3.48; N, 3.70 %.

*2-(3-Oxo-3H-benzo[f]chromen-2-yl)-5H-chromeno [4,3-b]pyridin-5-one (**4c**)*

Brown solid; m.p. 222–224 °C; IR (KBr, cm^{-1}): 3137 (Ar C–H), 1692 (C=O), 1627 (Ar C=C), 1505 (Ar C=N); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 7.26–8.48 (9H, m, Ar–H), 9.71–9.92 (3H, m, C_4-H, C_{10}-H, and C_5'-H), 10.83 (1H, s, C_4'-H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 96.69 (C), 96.82 (C), 117.39 (CH), 117.49 (CH), 120.88 (C), 120.93 (CH), 124.40 (CH), 124.48 (CH), 125.82 (CH), 126.20 (CH), 134.83 (CH), 134.94 (CH), 152.04 (CH), 154.78 (C), 160.78 (CH), 162.49 (CH), 163.41 (C), 163.53 (C), 163.81 (C), 178.05 (C=O), 180.29 (C=O); Anal. Calc. for $C_{25}H_{13}NO_4$: C, 76.72; H, 3.35; N, 3.58. Found: C, 76.46; H, 3.28; N, 3.51 %.

*9-Methyl-2-(2-oxo-2H-chromen-3-yl)-5H-chromeno [4,3-b]pyridin-5-one (**4d**)*

Brown solid; m.p. 225–227 °C; IR (KBr, cm^{-1}): 3141 (Ar C–H), 1701 (C=O), 1620 (Ar C=C), 1497 (Ar C=N); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 2.34 (3H, s, CH_3), 7.14–8.47 (7H, m, Ar–H), 9.69–9.87 (2H, m, C_4-H and C_{10}-H), 10.82 (1H, s, C_4'-H); ^{13}C-NMR (100 MHz,DMSO-d_6, δ ppm): 20.70 (CH_3), 96.73 (C), 96.87 (C), 117.17 (CH), 117.31 (CH), 120.49 (CH), 120.61 (CH), 125.40 (CH), 125.83 (CH), 133.58 (C), 133.66 (C), 135.54 (CH), 135.65 (CH), 152.85 (C), 158.60 (C), 160.77 (CH), 162.41 (CH), 162.65 (C), 163.55 (C), 163.65 (C), 178.10 (C=O), 180.39 (C=O); Anal. Calc. for $C_{22}H_{13}NO_4$: C, 74.36; H, 3.69; N, 3.94. Found: C, 74.62; H, 3.65; N, 3.87 %.

*2-(8-Methoxy-2-oxo-2H-chromen-3-yl)-9-methyl-5H-chromeno[4,3-b]pyridin-5-one (**4e**)*

Brown solid; m.p. 219–221 °C; IR (KBr, cm^{-1}): 3144 (Ar C–H), 1703 (C=O), 1620 (Ar C=C), 1490 (Ar C=N); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 2.35 (3H, s, CH_3), 3.92 (3H, s, OCH_3), 7.10–8.47 (6H, m, Ar–H), 9.69–9.87 (2H, m, C_4-H and C_{10}-H), 10.81 (1H, s, C_4'-H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 20.71 (CH_3), 56.76 (OCH_3), 96.68 (C), 96.84 (C), 117.20 (CH), 117.29 (CH), 120.50 (C), 120.58 (C), 125.40 (CH), 125.82 (CH), 133.57 (C), 133.67 (C), 135.56 (CH), 135.65 (CH), 152.86 (C), 160.75 (CH), 162.41 (CH), 163.56 (C), 163.64 (C), 178.08 (C=O), 180.36 (C=O); Anal. Calc. for $C_{23}H_{15}NO_5$: C, 71.68; H, 3.92; N, 3.63. Found: C, 71.45; H, 3.98; N, 3.71 %.

*9-Methyl-2-(3-oxo-3H-benzo[f]chromen-2-yl)-5H-chromeno[4,3-b]pyridin-5-one (**4f**)*

Brown solid; m.p. 214–216 °C; IR (KBr, cm^{-1}): 3144 (Ar C–H), 1696 (C=O), 1620 (Ar C=C), 1490 (Ar C=N); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 2.35 (3H, s, CH_3), 7.14–8.47 (8H, m, Ar–H), 9.69–9.87 (3H, m, C_4-H, C_{10}-H, and C_5'-H), 10.82 (1H, s, C_4'-H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 20.71 (CH_3), 96.70 (C), 96.84 (C), 117.20 (CH), 117.29 (CH), 120.50 (C), 120.58 (C), 124.40 (CH), 124.48 (CH), 125.40 (CH), 125.82 (CH), 126.20 (CH), 133.56 (C), 133.66 (C), 135.55 (CH), 135.64 (CH), 152.86 (C), 154.78 (C), 160.74 (CH), 162.41 (CH), 163.57 (C), 163.64 (C), 178.08 (C=O), 180.36 (C=O); Anal. Calc. for $C_{26}H_{15}NO_4$: C, 77.03; H, 3.73; N, 3.46. Found: C, 77.28; H, 3.67; N, 3.39 %.

*7-Methyl-2-(2-oxo-2H-chromen-3-yl)-5H-chromeno [4,3-b]pyridin-5-one (**4g**)*

Brown solid; m.p. 253–255 °C; IR (KBr, cm^{-1}): 3123 (Ar C–H), 1704 (C=O), 1621 (Ar C=C), 1494 (Ar C=N); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 2.32 (3H, s, CH_3), 7.17–8.48 (7H, m, Ar–H), 9.70–9.92 (2H, m, C_4-H, and C_{10}-H), 10.83 (1H, s, C_4'-H); ^{13}C-NMR (100 MHz,

DMSO-d_6, δ ppm): 15.77 (CH_3), 96.52 (C), 96.71 (C), 117.87 (CH), 120.69 (C), 120.75 (C), 121.05 (C), 123.45 (CH), 123.77 (CH), 123.87 (CH), 126.12 (C), 126.26 (CH), 135.69 (CH), 135.81 (CH), 153.05 (CH), 160.82 (CH), 161.64 (C), 162.46 (CH), 163.29 (C), 163.41 (C), 178.29 (C=O), 180.58 (C=O); Anal. Calc. for $C_{22}H_{13}NO_4$: C, 74.36; H, 3.69; N, 3.94. Found: C, 74.09; H, 3.63; N, 3.84 %.

*2-(8-Methoxy-2-oxo-2H-chromen-3-yl)-7-methyl-5H-chromeno[4,3-b]pyridin-5-one (**4h**)*

Brown solid; m.p. 240–242 °C; IR (KBr, cm^{-1}): 3093 (Ar C–H), 1708 (C=O), 1606 (Ar C=C), 1479 (Ar C=N); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 2.50 (3H, s, CH_3), 3.99 (3H, s, OCH_3), 6.45–7.36 (7H, m, Ar–H), 7.63 (1H, poorly resolved dd, C_{10}-H), 7.94 (1H, s, C_4'-H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 16.07 (CH_3), 56.33 (OCH_3), 105.71 (CH), 111.18 (C), 112.21 (C), 114.31 (CH), 118.55 (CH), 119.28 (C), 119.72 (CH), 124.06 (CH), 124.46 (C), 124.95 (CH), 126.90 (CH), 132.19 (CH), 142.44 (CH), 143.33 (C), 147.26 (C), 151.02 (C), 154.88 (C), 157.80 (C), 157.97 (C), 160.03 (C=O), 169.59 (C=O); Anal. Calc. for $C_{23}H_{15}NO_5$: C, 71.68; H, 3.92; N, 3.63. Found: C, 71.44; H, 3.99; N, 3.70 %.

*7-Methyl-2-(3-oxo-3H-benzo[f]chromen-2-yl)-5H-chromeno[4,3-b]pyridin-5-one (**4i**)*

Brown solid; m.p. 256–258 °C; IR (KBr, cm^{-1}): 3132 (Ar C–H), 1704 (C=O), 1621 (Ar C=C), 1495 (Ar C=N); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 2.32 (3H, s, CH_3), 7.17–8.36 (8H, m, Ar–H), 9.70–9.92 (3H, m, C_4-H, C_{10}-H, and C_5'-H), 10.83 (1H, s, C_4'-H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 15.73 (CH_3), 96.50 (C), 96.64 (C), 116.94 (CH), 120.39 (C), 120.68 (C), 120.79 (C), 121.90 (C), 123.48 (CH), 123.80 (CH), 123.86 (CH), 126.15 (C), 126.29 (C), 129.14 (C), 135.73 (CH), 135.84 (CH), 153.04 (C), 160.82 (CH), 161.21 (CH), 161.98 (C), 162.45 (CH), 162.83 (C), 163.30 (C), 163.44 (C), 178.32 (C=O), 180.61 (C=O); Anal. Calc. for $C_{26}H_{15}NO_4$: C, 77.03; H, 3.73; N, 3.46. Found: C, 76.79; H, 3.67; N, 3.38 %.

*9-Chloro-2-(2-oxo-2H-chromen-3-yl)-5H-chromeno[4,3-b]pyridin-5-one (**4j**)*

Brown solid; m.p. 265 °C; IR (KBr, cm^{-1}): 3115 (Ar C–H), 1696 (C=O), 1632 (Ar C=C), 1502 (Ar C=N); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 7.32–8.48 (7H, m, Ar–H), 9.77–10.03 (2H, m, C_4-H, and C_{10}-H), 10.81 (1H, s, C_4'-H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 96.43 (C), 96.64 (C), 119.74 (CH), 119.86 (CH), 122.27 (C), 124.84 (CH), 125.23 (CH), 128.57 (C), 128.69 (CH), 134.39 (CH), 134.49 (CH), 153.39 (C), 155.41 (C), 161.03 (CH), 161.72 (CH), 162.70 (CH), 162.92 (C), 163.08 (C), 175.62 (C), 176.84 (C=O), 178.82 (C=O), Anal. Calc. for $C_{21}H_{10}ClNO_4$: C, 67.12; H, 2.68; N, 3.73. Found: C, 67.41; H, 2.63; N, 3.66 %.

*9-Chloro-2-(8-methoxy-2-oxo-2H-chromen-3-yl)-5H-chromeno[4,3-b]pyridin-5-one (**4k**)*

Brown solid; m.p. 261 °C; IR (KBr, cm^{-1}): 3123 (Ar C–H), 1695 (C=O), 1627 (Ar C=C), 1501 (Ar C=N); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 3.93 (3H, s, OCH_3), 7.31–8.48 (6H, m, Ar–H), 9.77–10.06 (2H, m, C_4-H and C_{10}-H), 10.81 (1H, s, C_4'-H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 56.15 (OCH_3), 96.42 (C), 96.65 (C), 119.73 (CH), 119.84 (CH), 122.28 (C), 124.83 (CH), 125.22 (CH), 128.60 (C), 128.69 (C), 134.37 (CH), 134.48 (CH), 153.38 (C), 156.92 (C), 160.99 (CH), 162.70 (CH), 162.92 (C), 163.09 (C), 176.83 (C=O), 178.84 (C=O); Anal. Calc. for $C_{22}H_{12}ClNO_5$: C, 65.12; H, 2.98; N, 3.45. Found: C, 65.38; H, 2.92; N, 3.36 %.

*9-Chloro-2-(3-oxo-3H-benzo[f]chromen-2-yl)-5H-chromeno[4,3-b]pyridin-5-one (**4l**)*

Brown solid; m.p. 258–260 °C; IR (KBr, cm^{-1}): 3123 (Ar C–H), 1695 (C=O), 1631 (Ar C=C), 1501 (Ar C=N); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 7.31–8.47 (8H, m, Ar–H), 9.80–10.05 (3H, m, C_4-H, C_{10}-H, and C_5'-H), 10.81 (1H, s, C_4'-H); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 96.45 (C), 96.64 (C), 119.75 (CH), 119.86 (CH), 122.26 (C), 122.53 (C), 124.84 (CH), 125.21 (CH), 128.58 (C), 128.68 (C), 133.77 (CH), 134.37 (CH), 134.46 (CH), 152.45 (C), 153.37 (C), 158.64 (CH), 161.02 (CH), 162.68 (CH), 162.92 (C), 163.07 (C), 176.83 (C=O), 178.80 (C=O); Anal. Calc. for $C_{25}H_{12}ClNO_5$: C, 70.52; H, 2.84; N, 3.29. Found: C, 70.32; H, 2.80; N, 3.22 %.

Minimum inhibitory concentration (MIC) measurement

Antibacterial assay

For the determination of MIC, bacteria were grown over night in Mueller–Hinton broth as a nutrient medium at 37 °C. DMSO was used as diluents/vehicle to get desired concentration of synthesized compounds and standard drugs to test upon standard microbial strains. Serial dilutions were prepared in primary and secondary screening. Each synthesized compound and standard drugs were diluted obtaining 2,000 μg/mL concentration, as a stock solution. In primary screening 1,000, 500, and 250 μg/mL concentrations of the synthesized drugs were taken. The active synthesized compounds found in this primary

screening were further diluted to obtain 200, 125, 100, 62.5, 50, 25, 12.5, and 6.250 μg/mL concentrations for secondary screening. Inoculum's size for test strain was adjusted to 10^8 colony forming unit (CFU) per milliliter by comparing the turbidity. The MIC (μg/mL) was determined and compared with the standard drugs, ampicillin and norfloxacin. The MIC data are presented in Table 2.

Antifungal assay

For the antifungal assay, each test compound was dissolved in DMSO and Sabouraud Dextrose broth was used for fungal nutrition. Inoculum's size for test strain was adjusted to 10^8 CFU/mL by comparing the turbidity. MIC (μg/mL) was determined and compared with the standard drugs, griseofulvin and nystatin. The MIC data are presented in Table 2.

Conclusion

In conclusion, we have developed a highly efficient and practical route for the syntheses of 2-(2-oxo-2*H*-chromen-3-yl)-5*H*-chromeno[4,3-*b*]pyridin-5-ones **4a**–**4l** by two different methodologies. Our interest in the synthesis of title compounds was to focus on their study as antimicrobial agents as a part of our research work which is aimed at the development of new heterocyclic compounds as more potent antimicrobial agents. Compounds **4e**, **4f**, **4i**, **4k**, and **4l** were found to be the most efficient members of the series. These compounds were structurally different from already available inhibitors and we suggest that these compounds could serve as potential leads for further development of new antimicrobial agents.

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