Synthesis, characterization and antimicrobial activity of some 4-aryl-2,6-di(coumarin-3-yl)pyridines

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Abstract

Some 4-aryl-2,6-di(coumarin-3-yl)pyridines **3a-r** are synthesized by the reaction of 3-coumarinoyl methyl pyridinium salts **1a-c** with appropriate 1-[2*H*-1-benzopyran-2-on-3-yl]-3-aryl-prop-2-en-1-ones **2a-f** in the presence of ammonium acetate and acetic acid under the Kröhnke reaction conditions. All the synthesized compounds are screened for antimicrobial activity.

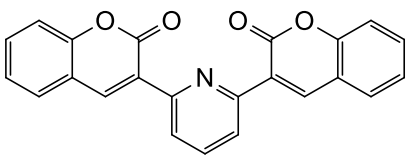
Keywords: Coumarins, dicoumarinyl pyridines, pyridylcoumarins, Kröhnke reaction, antimicrobial activity

Introduction

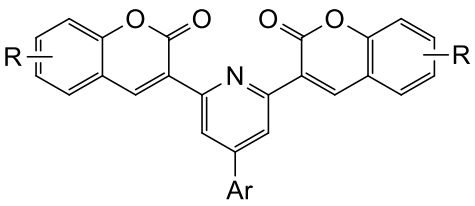
Coumarins constitute an important class of benzopyrones, exhibiting a broad range of biological activities such as anticoagulants,[1] antimicrobial,[2] antibacterial,[3] anticancer,[4] and anti-HIV activity.[5] The interesting biological activities of the coumarins make them attractive targets in organic synthesis. Coumarins having pyridine substitution at C-3 are reported to have interesting biological activity. Many 3-(2-pyridyl)- and 3-(3-pyridyl)coumarins are known for their useful bioactivities viz. antifungal,[6,9] bactericidal,[7] fish toxicity[7] and moth proofing activity.[8] Some of them are also known for their CNS depressant activity.[9] Considering their biological importance, a variety of 3-pyridyl substituted coumarins were earlier synthesized from our laboratory.[10] In continuation of this interest, we now report the synthesis of 4-aryl-2,6-di(coumarin-3-yl)pyridines **3**. The pyridine nucleus in these compounds is flanked by two coumarin moieties at C-2 and C-6 and the structure seems as if two coumarins have 3-(2-pyridyl) substitution. One can expect improved biological potency for such compounds.

During our literature search on the synthesis of 2,6-dicoumarinyl pyridine derivatives, we came across to two reports in which this type of compound had been synthesized. El-Taweel *et al*.[11] synthesized 2,6-di(coumarin-3-yl)pyridine (**A**) by reacting enaminones of 3-acetylcoumarin

with 3-acetylcoumarin in the presence of ammonium acetate in refluxing acetic acid. However, the main objective of these authors was not to synthesize dicoumarinyl pyridines, but to study the reaction of enaminones of 3-acetylcoumarin with variety of reagents and to prepare various heterocyclic substituted coumarins. Only one dicoumarinyl pyridine (**A**) was prepared in this work. Verma *et al*.[12] synthesized various 4-aryl-2,6-dicoumarinyl pyridines (**B**) by the condensation of 1-[2*H*-1-benzopyran-2-on-3-yl]-3-aryl-prop-2-en-1-ones with urea or amide derivatives using bismuth(III) nitrate–Al_2O_3 as a catalyst. However though the method is simple and convenient, the main drawback of the method is that only symmetrically substituted 2,6-dicoumarinyl compounds can be prepared, i.e. the product will have identical coumarin moieties at C-2 and C-6 of the pyridine.



[A]

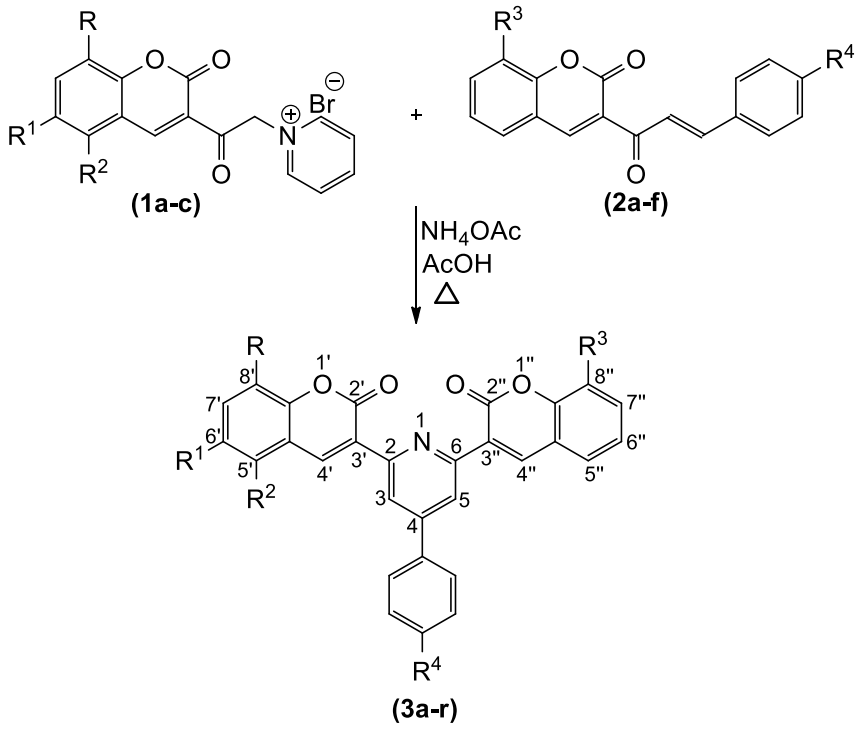


[B]

Considering the above facts, in the present work we have developed a simple, convenient and general method for the synthesis of 4-aryl-2,6-dicoumarin substituted pyridines utilizing easily accessible starting materials. The method involves the condensation of 3-coumarinoyl methyl pyridinium salts with 1-[2*H*-1-benzopyran-2-on-3-yl]-3-aryl-prop-2-en-1-ones under Kröhnke reaction conditions. By this method, symmetrically as well as asymmetrically 2,6-dicoumarinyl substituted pyridines can be synthesized.

Results and Discussion

In the present work, various 4-aryl-2,6-di(coumarin-3-yl)pyridines **3a-r** have been synthesized by reacting 3-coumarinoyl methyl pyridinium salts **1a-c** with appropriate 1-[2*H*-1-benzopyran-2-on-3-yl]-3-aryl-prop-2-en-1-ones **2a-f** in the presence of ammonium acetate and acetic acid under Kröhnke reaction conditions[13] (Scheme 1).



	R	R^1	R^2	R^3	R^4		R	R^1	R^2	R^3	R^4
3a	H	H	H	H	H	**3j**	OCH_3	H	H	OCH_3	H
3b	H	H	H	H	CH_3	**3k**	OCH_3	H	H	OCH_3	CH_3
3c	H	H	H	H	OCH_3	**3l**	OCH_3	H	H	OCH_3	OCH_3
3d	H	H	H	OCH_3	H	**3m**	H	Benzo		H	H
3e	H	H	H	OCH_3	CH_3	**3n**	H	Benzo		H	CH_3
3f	H	H	H	OCH_3	OCH_3	**3o**	H	Benzo		H	OCH_3
3g	OCH_3	H	H	H	H	**3p**	H	Benzo		OCH_3	H
3h	OCH_3	H	H	H	CH_3	**3q**	H	Benzo		OCH_3	CH_3
3i	OCH_3	H	H	H	OCH_3	**3r**	H	Benzo		OCH_3	OCH_3

Scheme 1. Synthetic scheme for 4-aryl-2,6-di(coumarin-3-yl)pyridines **3a-r**.

The reactions proceeded smoothly and gave the expected products **3a-r** in moderate yields (55-66%). The structures of the compounds **3a-r** were established on the basis of IR, ^1H, ^{13}C, DEPT NMR and elemental analysis.

In the IR spectra, compounds **3a-r** showed a very strong band between 1713-1730 cm^{-1} for δ-lactone carbonyl (C=O) stretching vibrations. The strong bands for aromatic C=C and C=N stretching vibrations, were observed between 1595-1610 and 1495-1560 cm^{-1} respectively. The aromatic C-H stretching vibrations were observed between 3050-3060 cm^{-1} in the form of a medium band.

In the ^{1}H NMR spectra of compounds **3a-r**, the aromatic protons appeared in the form of multiplets between δ 7.02-8.59. The pyridine 3-H and 5-H protons showed signals in the downfield region compared to the benzenoid protons, with the 4'-H and 4''-H signals the most downfield. In the compounds having identical coumarin substitutions at 2- and 6-positions of the pyridine (compounds **3a-c** and **3j-l**), the 3-H and 5-H appeared as a singlet between δ 8.62-8.79. Similarly in these compounds, the 4'-H and 4''-H also gave a singlet in the region δ 8.86-9.05. In the case of compounds **3d-i**, the 3-H and 5-H gave two singlets in the region δ 8.55-8.64, while 4'-H and 4''-H gave two singlets between δ 9.06-9.13. In the case of compounds **3m-r**, the 3-H appeared as singlet at around δ 8.70, while the 5-H appeared as singlet at around δ 8.60. Similarly in these compounds the 4'-H appeared as singlet between δ 9.65-9.83, while 4''-H appeared as a singlet between δ 8.90-9.10. It was possible to differentiate the 3-H, 5-H and 4'-H, 4''-H signals in these compounds **3m-r** because of the presence of an additional fused benzene ring in the coumarin moiety at C-2, which causes diamagnetic anisotropy and deshields the 3-H and 4'-H compared to the 5-H and 4''-H.

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

The selected mass spectrum of compound **3a** showed M^{+} peak at *m/z* 443 (100%) along with other fragment peaks. The appearance of a molecular ion peak at 443 mass units supports the structure of compound **3a**.

Antimicrobial activity

All the synthesized compounds **3a-r** were screened for their antibacterial and antifungal activity. Compounds **3a-r** were screened against *Escherichia coli* (Gram-negative bacteria) and *Bacillus subtilis* (Gram-positive bacteria) and antifungal activity against *Aspergillus niger* (Fungi). The evaluation of antimicrobial activity was carried out using the agar cup diffusion method. The results are summarized in Table 1. None of the compounds showed antifungal activity against fungi *A. niger*. All the compounds **3a-r** showed moderate activity against the Gram-positive bacteria *B. subtilis*. The results towards this bacteria reveal that the incorporation of the substituent groups like CH_3 or OCH_3 either in the coumarin nucleus or in a phenyl ring does not affect the antibacterial activity much more and all the compounds have almost same activity. Compounds **3a-l** show moderate activity towards Gram-negative bacteria *E. coli*, but compounds **3m-r** are inactive. Thus the presence of an additional fused benzene ring between the C-5' and C-6' positions inhibits the antibacterial activity towards *E. coli*.

Table 1. Antimicrobial activity of 4-aryl-2,6-di(coumarin-3-yl)pyridines **3a-r**

Compd.	Inhibition zone in mm

	Bacteria		Fungi
	E. coli	*B. subtilis*	*A. niger*
3a	14	11	-
3b	15	12	-
3c	12	12	-
3d	14	10	-
3e	14	12	-
3f	15	11	-
3g	14	10	-
3h	14	12	-
3i	15	11	-
3j	15	12	-
3k	13	12	-
3l	14	13	-
3m	-	12	-
3n	-	11	-
3o	-	12	-
3p	-	13	-
3q	-	10	-
3r	-	12	-
Ciprofloxacin	18	25	NT
Ampicillin	20	27	NT
Griseofulvin	NT	NT	25

- denotes: inhibition zone was not observed.
NT denotes: not tested.

Conclusions

We have developed a simple and convenient method for the synthesis of 4-aryl-2,6-dicoumarinyl pyridine derivatives utilizing the Kröhnke reaction. The method allows incorporation of identical or non-identical coumarin moieties at 2- and 6-positions of pyridine.

Experimental Section

General. The melting points reported are uncorrected. 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 $CDCl_3$ as a solvent and a calibrated standard solvent signal. The mass spectrum of compound **3a** was recorded on a Shimadzu QP 2010 spectrometer. 3-Coumarinoyl methyl pyridinium salts **1a-c** and 1-[2*H*-1-benzopyran-2-on-3-yl]-3-aryl-prop-2-en-1-ones **2a-f** were prepared according literature procedures.[14,15]

General procedure for the synthesis of 4-aryl-2,6-di(coumarin-3-yl)pyridines (3a-r)

In a 100 mL three necked round bottom flask equipped with a dropping funnel, condenser, guard tube and magnetic needle, an appropriate 3-coumarinoyl methyl pyridinium salt **1a-c** (0.003 mol) in glacial acetic acid (15 mL) was taken. To this, NH_4OAc (0.03 mol) was added with stirring at room temperature. Then a solution of an appropriate 1-[2*H*-1-benzopyran-2-on-3-yl]-3-aryl-prop-2-en-1-one **2a-f** (0.003 mol) in glacial acetic acid (15 mL) was added with stirring at rt during 15 min. The reaction mixture was further stirred for 1 h and then heated for 8 h at 140 ˚C. It was then allowed to come to rt and 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% $NaHCO_3$ solution (3 x 20 mL), water (2 x 20 mL) and dried over anhydrous Na_2SO_4. The removal of chloroform under reduced pressure gave crude material which was subjected to column chromatography using silica gel and $CHCl_3$-petroleum ether (60-80) (9:1) as an eluent to give appropriate product **3a-r**. The compounds were recrystallized from $CHCl_3$-hexane.

4-Phenyl-2,6-di(coumarin-3-yl)pyridine (3a). Yield 57%; mp 253-254 ºC; white solid; IR (KBr): 3059 (m), 1713 (vs), 1607 (s), 1543 (s), 749 (s), 688 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.36-7.84 (13H, m, Ar-H), 8.66 (2H, s, 3-H and 5-H of pyridine ring), 8.89 (2H, s, 4'-H and 4''-H of coumarin rings); ^{13}C NMR: δ 116.4 (CH), 119.5 (C), 121.8 (CH), 124.6 (CH), 125.3 (C), 127.4 (CH), 128.9 (CH), 129.1 (CH), 129.2 (CH), 132.3 (CH), 138.0 (C), 142.8 (CH), 149.8 (C), 151.4 (C), 154.0 (C), 160.3 (C). Anal. Calcd. for $C_{29}H_{17}NO_4$: C, 78.55; H, 3.86; N, 3.16%. Found: C, 78.46; H, 3.94; N, 3.25%.

4-(4-Methylphenyl)-2,6-di(coumarin-3-yl)pyridine (3b). Yield 64%; mp 269-270 °C; light yellow solid; IR (KBr): 3055 (m), 1720 (vs), 1605 (s), 1530 (s), 825 (s) cm^{-1}; ^1H NMR: δ 2.43 (3H, s, CH_3), 7.30-7.76 (12H, m, Ar-H), 8.62 (2H, s, 3-H and 5-H of pyridine ring), 8.86 (2H, s, 4'-H and 4''-H of coumarin rings); ^{13}C NMR: δ 21.3 (CH_3), 116.4 (CH), 119.5 (CH), 121.5 (C), 124.6 (CH), 125.4 (C), 127.2 (CH), 128.9 (CH), 129.8 (CH), 132.3 (CH), 135.1 (C), 139.4 (C), 142.8 (CH), 149.8 (C), 151.4 (C), 154.0 (C), 160.3 (C). Anal. Calcd. for $C_{30}H_{19}NO_4$: C, 78.76; H, 4.19; N, 3.06%. Found: C, 78.85; H, 4.12; N, 3.01%.

4-(4-Methoxyphenyl)-2,6-di(coumarin-3-ylpyridine (3c). Yield 59%; mp 277-278 ºC; light yellow solid; IR (KBr): 3060 (m), 1715 (vs), 1610 (s), 1510 (s), 825 (s) cm^{-1}; ^1H NMR: δ 3.90 (3H, s, OCH_3), 7.02-7.80 (12H, m, Ar-H), 8.62 (2H, s, 3-H and 5-H of pyridine ring), 8.88 (2H, s, 4'-H and 4''-H of coumarin rings); ^{13}C NMR: δ 55.5 (OCH_3), 113.9 (C), 114.5 (CH), 116.4 (CH), 119.5 (C), 121.1 (CH), 124.6 (CH), 125.4 (C), 128.6 (CH), 128.9 (CH), 130.2 (C), 132.2 (CH), 142.8 (CH), 151.3 (C), 154.0 (C), 160.3 (C), 160.7 (C). Anal. Calcd. for $C_{30}H_{19}NO_5$: C, 76.10; H, 4.04; N, 2.96%. Found: C, 76.01 H, 4.13; N, 2.96%.

4-Phenyl-2-(coumarin-3-yl)-6-(8-methoxycoumarin-3-yl)pyridine (3d). Yield 55%; mp >300 ºC; white solid; IR (KBr): 3055 (m), 1720 (vs), 1595 (s), 1495 (s), 755 (s), 695 (s) cm^{-1}; ^1H NMR: δ 4.06 (3H, s, OCH_3), 7.41-7.91 (12H, m, Ar-H), 8.62 and 8.63 (2H, two s, 3-H and 5-H of pyridine ring), 9.07 and 9.11 (2H, two s, 4'-H and 4''-H of coumarin rings); ^{13}C NMR: δ 56.6 (OCH_3), 114.2 (C), 114.4 (C), 117.1 (CH), 118.2 (CH), 118.6 (C), 118.9 (C), 121.1 (CH), 121.5 (CH), 126.8 (CH), 127.8 (CH), 130.1 (CH), 130.6 (CH), 132.9 (CH), 136.7 (CH), 145.7 (C), 145.8 (C), 147.2 (C), 148.3 (CH), 148.6 (CH), 154.1 (C), 158.9 (C), 161.3 (C). Anal. Calcd. for $C_{30}H_{19}NO_5$: C, 76.10; H, 4.04; N, 2.96%. Found: C, 76.01; H, 4.16; N, 2.84%.

4-(4-Methylphenyl)-2-(coumarin-3-yl)-6-(8-methoxycoumarin-3-yl)pyridine (3e). Yield 61%; mp 283-284 ºC; light yellow solid; IR (KBr): 3060 (m), 1715 (vs), 1595 (s), 1525 (s), 825 (s) cm^{-1}; ^1H NMR: δ 2.45 (3H, s, CH_3), 4.04 (3H, s, OCH_3), 7.35-7.88 (11H, m, Ar-H), 8.61 and 8.64 (2H, two s, 3-H and 5-H of pyridine ring), 9.07 and 9.13 (2H, two s, 4'-H and 4''-H of coumarin rings); ^{13}C NMR: δ 21.2 (CH_3), 56.6 (OCH_3), 116.0 (CH), 118.0 (CH), 118.9 (C), 120.4 (CH), 120.5 (CH), 126.7 (CH), 127.9 (CH), 129.0 (C), 129.6 (CH), 130.2 (CH), 131.0 (C), 139.1 (CH), 143.2 (CH), 143.6 (C), 144.5 (C), 145.4 (C), 146.0 (C), 147.3 (C), 148.5 (CH), 155.4 (C), 158.5 (C), 161.2 (C). Anal. Calcd. for $C_{31}H_{21}NO_5$: C, 76.38; H, 4.34; N, 2.87%. Found: C, 76.48; H, 4.23; N, 2.79%.

4-(4-Methoxyphenyl)-2-(coumarin-3-yl)-6-(8-methoxycoumarin-3-yl)pyridine (3f). Yield 56%; mp 269-270 ºC; yellow solid; IR (KBr): 3055 (m), 1715 (vs), 1610 (s), 1515 (s), 830 (s) cm^{-1}; ^1H NMR: δ 3.92 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 7.11-7.94 (11H, m, Ar-H), 8.55 and 8.56 (2H, two s, 3-H and 5-H of pyridine ring), 9.06 and 9.09 (2H, two s, 4'-H and 4''-H of coumarin rings); ^{13}C NMR: δ 55.7 (OCH_3), 56.6 (OCH_3), 114.5 (C), 114.7 (C), 115.7 (CH), 117.0 (CH), 118.0 (CH), 118.2 (C), 118.9 (C), 119.7 (CH), 121.5 (CH), 125.7 (C), 126.7 (CH), 129.8 (CH), 130.5 (CH), 136.5 (CH), 143.6 (C), 145.3 (C), 145.4 (C), 147.2 (C), 148.0 (CH), 148.2 (CH), 154.1 (C), 157.7 (C), 163.9 (C). Anal. Calcd. for $C_{31}H_{21}NO_6$: C, 73.95; H, 4.20; N, 2.78%. Found: C, 73.87; H, 4.13; N, 2.78%.

4-Phenyl-2-(8-methoxycoumarin-3-yl)-6-(coumarin-3-yl)pyridine (3g). Yield 64%; mp >300 ºC; white solid; IR (KBr): 3055 (m), 1720 (vs), 1595 (s), 1550 (s), 755 (s), 690 (s) cm^{-1}; ^1H NMR: δ 4.06 (3H, s, OCH_3), 7.41-7.91 (12H, m, Ar-H), 8.62 and 8.63 (2H, two s, 3-H and 5-H of pyridine ring), 9.07 and 9.11 (2H, two s, 4'-H and 4''-H of coumarin rings); ^{13}C NMR: δ 56.6 (OCH_3), 114.2 (C), 114.4 (C), 117.1 (CH), 118.2 (CH), 118.6 (C), 118.9 (C), 121.1 (CH), 121.5 (CH), 126.8 (CH), 127.8 (CH), 130.1 (CH), 130.6 (CH), 132.9 (CH), 136.7 (CH), 145.7 (C), 145.8 (C), 147.2 (C), 148.3 (CH), 148.6 (CH), 154.1 (C), 158.7 (C), 161.3 (C). Anal. Calcd. for $C_{30}H_{19}NO_5$: C, 76.10; H, 4.04; N, 2.96%. Found: C, 76.01; H, 4.16; N, 2.84%.

4-(4-Methylphenyl)-2-(8-methoxycoumarin-3-yl)-6-(coumarin-3-yl)pyridine (3h). Yield 59%; mp 283-284 ºC; light yellow solid; IR (KBr): 3060 (m), 1715 (vs), 1595 (s), 1525 (s), 825 (s) cm^{-1}; ^1H NMR: δ 2.45 (3H, s, CH_3), 4.04 (3H, s, OCH_3), 7.35-7.88 (11H, m, Ar-H), 8.61 and 8.64 (2H, two s, 3-H and 5-H of pyridine ring), 9.07 and 9.13 (2H, two s, 4'-H and 4''-H of coumarin rings); ^{13}C NMR: δ 21.2 (CH_3), 56.6 (OCH_3), 116.0 (CH), 118.0 (CH), 118.9 (C), 120.4 (CH), 120.5 (CH), 126.7 (CH), 127.9 (CH), 129.0 (C), 129.6 (CH), 130.2 (CH), 131.0 (C),

139.1 (CH), 143.2 (CH), 143.6 (C), 144.5 (C), 145.4 (C), 146.0 (C), 147.3 (C), 148.5 (CH), 155.4 (C), 158.5 (C), 161.2 (C). Anal. Calcd. for $C_{31}H_{21}NO_5$: C, 76.38; H, 4.34; N, 2.87%. Found: C, 76.29; H, 4.26; N, 2.81%.

(4-Methoxyphenyl)-2-(8-methoxycoumarin-3-yl)-6-(coumarin-3-yl)pyridine (3i). Yield 57%; mp 269-270 ºC; yellow solid; IR (KBr): 3055 (m), 1715 (vs), 1610 (s), 1515 (s), 830 (s) cm^{-1}; ^1H NMR: δ 3.92 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 7.11-7.94 (11H, m, Ar-H), 8.55 and 8.56 (2H, two s, 3-H and 5-H of pyridine ring), 9.06 and 9.09 (2H, two s, 4'-H and 4"-H of coumarin rings); ^{13}C NMR: δ 55.7 (OCH_3), 56.6 (OCH_3), 114.5 (C), 114.7 (C), 115.9 (CH), 117.0 (CH), 118.0 (CH), 118.2 (C), 118.9 (C), 119.7 (CH), 121.5 (CH), 125.7 (C), 126.7 (CH), 129.8 (CH), 130.5 (CH), 136.5 (CH), 143.6 (C), 145.3 (C), 145.4 (C), 147.2 (C), 148.0 (CH), 148.2 (CH), 154.1 (C), 157.7 (C), 163.9 (C). Anal. Calcd. for $C_{31}H_{21}NO_6$: C, 73.95; H, 4.20; N, 2.78%. Found: C, 73.87; H, 4.13; N, 2.87%.

4-Phenyl-2,6-di(8-methoxycoumarin-3-yl)pyridine (3j). Yield 56%; mp 280-281 ºC; light yellow solid; IR(KBr): 3055 (m), 1720 (vs), 1610 (s), 1550 (s), 755 (s), 700 (s) cm^{-1}; ^1H NMR: δ 4.06 (6H, s, 2 x OCH_3), 7.51-7.94 (11H, m, Ar-H), 8.79 (2H, s, 3-H and 5-H of pyridine ring), 9.05 (2H, s, 4'-H and 4"-H of coumarin rings); ^{13}C NMR: δ 56.5 (OCH_3), 116.5 (CH), 119.4 (C), 121.9 (CH), 124.6 (CH), 125.5 (C), 127.4 (CH), 129.0 (CH), 129.1 (CH), 132.4 (CH), 138.0 (C), 142.8 (CH), 147.1 (C), 149.8 (C), 151.3 (C), 154.0(C), 160.3 (C). Anal. Calcd. for $C_{31}H_{21}NO_6$: C, 73.95; H, 4.20; N, 2.78%. Found: C, 73.85; H, 4.32; N, 2.68%.

4-(4-Methylphenyl)-2,6-di(8-methoxycoumarin-3-yl)pyridine (3k). Yield 58%; mp 282-283 ºC; yellow solid; IR (KBr): 3060 (m), 1715 (vs), 1610 (s), 1540 (s), 825 (s) cm^{-1}; ^1H NMR: δ 2.46 (3H, s, CH_3), 3.91 (6H, s, 2 x OCH_3), 7.41-7.92 (10H, m, Ar-H), 8.77 (2H, s, 3-H and 5-H of pyridine ring), 9.04 (2H, s, 4'-H and 4"-H of coumarin rings); ^{13}C NMR: 21.3 (CH_3), 56.6 (OCH_3), 116.5 (CH), 121.6 (C), 124.5 (CH), 125.5 (C), 127.3 (CH), 129.0 (CH), 129.9 (CH), 132.2 (CH), 135.0 (C), 139.4 (C), 142.8 (CH), 147.0 (C), 149.9 (C), 151.5 (C), 154.0 (C), 160.30 (C). Anal. Calcd. for $C_{32}H_{23}NO_6$: C, 74.27; H, 4.48; N, 2.71%. Found: C, 74.20; H, 4.54; N, 2.84%.

4-(4-Methoxyphenyl)-2,6-di(8-methoxycoumarin-3-yl)pyridine (3l). Yield 59%; mp 274-275 ºC; light yellow solid; IR (KBr): 3050 (m), 1715 (vs), 1605 (s), 1555 (s), 830 (s) cm^{-1}; ^1H NMR: δ 3.91 (3H, s, OCH_3), 4.04 (6H, s, 2 x OCH_3), 7.10-7.91 (10H, m, Ar-H), 8.78 (2H, s, 3-H and 5-H of pyridine ring), 9.04 (2H, s, 4'-H and 4"-H of coumarin rings); ^{13}C NMR: δ 55.5 (OCH_3), 56.6 (OCH_3), 114.0 (C), 114.6 (CH), 119.6 (C), 121.2 (CH), 124.7 (CH), 125.4 (C), 128.6 (CH), 129.0 (CH), 130.1 (C), 132.3 (CH), 142.8 (CH), 147.3 (C), 151.2 (C), 153.9 (C), 160.3 (C), 160.7 (C). Anal. Calcd. for $C_{32}H_{23}NO_7$: C, 72.04; H, 4.35; N, 2.63%. Found: C, 72.18; H, 4.48; N, 2.75%.

4-Phenyl-2-(benzo[*f*]coumarin-3-yl)-6-(coumarin-3-yl)pyridine (3m). Yield 65%; mp 265-266 ºC; yellow solid; IR (KBr): 3055(m), 1725 (vs), 1610 (s), 1560 (s), 755(s), 695 (s) cm^{-1}; ^1H NMR: δ 7.36-8.50 (15H, m, Ar-H), 8.64 (1H, s, 5-H of pyridine ring), 8.72 (1H, s, 3-H of pyridine ring), 8.90 (1H, s, 4"-H of coumarin ring), 9.65 (1H, s, 4'-H of coumarin ring); ^{13}C NMR: δ 113.8 (C), 116.5 (CH), 116.6 (CH), 119.6 (C), 121.7 (CH), 121.8 (CH), 121.8 (CH),

124.2 (C), 124.6 (CH), 125.6 (C), 126.2 (CH), 127.4 (CH), 128.7 (CH), 128.9 (CH), 129.0 (CH), 129.1 (CH), 129.2 (CH), 129.6 (C), 130.4 (C), 132.3 (CH), 133.7 (CH), 138.1 (C), 138.4 (CH), 142.7 (CH), 149.8 (C), 151.6 (C), 151.8 (C), 154.0 (C), 154.1 (C), 160.3 (C), 160.4 (C). Anal. Calcd. for $C_{33}H_{19}NO_4$: C, 80.31; H, 3.88; N, 2.84%. Found: C, 80.25; H, 3.75; N, 2.96%.

4-(4-Methylphenyl)-2-(benzo[*f*]coumarin-3-yl)-6-(coumarin-3-yl)pyridine (3n). Yield 66%; mp 287-288 ºC; yellow solid; IR(KBr): 3050 (m), 1730 (vs), 1610 (s), 1520 (s), 825 (s) cm^{-1}; ^1H NMR: 2.44 (3H, s, CH_3), 7.31-8.51 (14H, m, Ar-H), 8.63 (1H, s, 5-H of pyridine ring), 8.71 (1H, s, 3-H of pyridine ring), 8.90 (1H, s, 4"-H of coumarin ring), 9.65 (1H, s, 4'-H of coumarin ring); ^{13}C NMR: δ 21.3 (CH_3), 113.8 (C), 116.5 (CH), 116.7(CH), 119.6 (C), 121.5 (CH), 121.6 (CH), 121.9 (CH), 124.3 (C), 124.6 (CH), 125.7 (C), 126.2 (CH), 127.3 (CH), 128.5 (CH), 128.9 (CH), 129.1 (CH), 129.6 (C), 129.8 (CH), 130.4 (C), 132.2 (CH), 133.7 (CH), 135.2 (C), 138.5 (CH), 139.4 (C), 142.7 (CH), 149.8 (C), 151.5 (C), 151.7 (C), 154.0 (C), 154.0 (C), 160.3 (C), 160.4 (C). Anal. Calcd. for $C_{34}H_{21}NO_4$: C, 80.46; H, 4.17; N, 2.76%. Found: C, 80.33; H, 4.07; N, 2.84%.

4-(4-Methoxyphenyl)-2-(benzo[*f*]coumarin-3-yl)-6-(coumarin-3-yl)pyridine (3o). Yield 62%; mp 281-282 ºC; yellow solid; IR (KBr): 3055 (m), 1720 (vs), 1605 (s), 1540 (s), 830 (s) cm^{-1}; ^1H NMR: δ 3.91 (3H, s, OCH_3), 7.04-8.51 (14H, m, Ar-H), 8.62 (1H, s, 5-H of pyridine ring), 8.70 (1H, s, 3-H of pyridine ring), 8.91 (1H, s, 4"-H of coumarin ring), 9.66 (1H, s, 4'-H of coumarin ring); ^{13}C NMR: δ 55.7 (OCH_3), 110.0 (C), 112.8 (C), 115.6 (C), 115.7 (CH), 116.1 (CH), 117.2 (CH), 118.3 (C), 118.5 (C), 119.5 (C), 119.6 (C), 119.8 (CH), 121.6 (CH), 126.9 (CH), 128.0 (CH), 129.0 (C), 129.8 (CH), 129.9 (CH), 130.1 (CH), 130.6 (CH), 130.9 (C), 134.3 (C), 136.7 (CH), 141.6 (CH), 143.4 (CH), 144.7 (C), 145.8 (C), 154.1 (C), 155.4 (C), 160.2 (C), 160.4 (C). Anal. Calcd. for $C_{34}H_{21}NO_5$: C, 78.00; H, 4.04; N, 2.68%. Found: C, 78.11; H, 4.17; N, 2.59%.

4-Phenyl-2-(benzo[*f*]coumarin-3-yl)-6-(8-methoxycoumarin-3-yl)pyridine (3p). Yield 63%; mp >300 ºC; yellow solid; IR(KBr): 3050 (m), 1725 (vs), 1610 (s), 1545 (s), 750 (s), 700 (s), cm^{-1}; ^1H NMR: δ 4.08 (3H, s, OCH_3), 7.39-8.58 (14H, m, Ar-H), 8.63 (1H, s, 5-H of pyridine ring), 8.82 (1H, s, 3-H of pyridine ring), 9.10 (1H, s, 4"-H of coumarin ring), 9.83 (1H, s, 4'-H of coumarin ring); ^{13}C NMR: δ 56.5 (OCH_3), 112.2 (C), 113.7 (C), 114.4 (C), 116.0 (CH), 118.4 (CH), 118.9 (C), 120.9 (CH), 120.9 (CH), 121.3 (CH), 121.5 (CH), 127.0 (CH), 127.8 (CH), 128.0 (CH), 128.9 (C), 129.8 (CH), 130.1 (CH), 130.4 (CH), 130.9 (C), 133.0 (CH), 134.0 (C), 139.6 (CH), 143.5 (CH), 145.6 (C), 146.1 (C), 147.1 (C), 148.7 (CH), 155.5 (C), 159.1 (C), 161.8 (C). Anal. Calcd. for $C_{34}H_{21}NO_5$: C, 78.00; H, 4.04; N, 2.68%. Found: C, 78.12; H, 4.15; N, 2.58%.

4-(4-Methylphenyl)-2-(benzo[*f*]coumarin-3-yl)-6-(8-methoxycoumarin-3-yl)pyridine (3q). Yield 55%; mp >300 ºC; yellow solid; IR (KBr): 3060 (m) , 1715 (vs), 1600 (s), 1550 (s), 820 (s) cm^{-1}; ^1H NMR: δ 2.46 (3H, s, CH_3), 4.05 (3H, s, OCH_3), 7.37-8.59 (14H, m, Ar-H + 5-H of pyridine ring), 8.78 (1H, s, 3-H of pyridine ring), 9.06 (1H, s, 4"-H of coumarin ring), 9.78 (1H, s, 4'-H of coumarin ring); ^{13}C NMR: δ 21.3 (CH_3), 56.6 (OCH_3), 112.7 (C), 113.6 (C), 114.6 (C), 116.0 (CH), 118.0 (CH), 118.9 (C), 120.4 (CH), 120.6 (CH), 121.6 (CH), 126.7 (CH), 127.8 (CH), 129.0 (C), 129.7 (CH), 130.3 (CH), 130.8 (CH), 131.0 (C), 139.0 (CH), 143.2 (CH), 143.6

(C), 144.4 (C), 145.5 (C), 145.9 (C), 147.8 (C), 148.4 (CH), 155.4 (C), 158.5 (C), 161.2 (C). Anal. Calcd. for $C_{35}H_{23}NO_5$: C, 78.20; H, 4.31; N, 2.61%. Found: C, 78.14; H, 4.22; N, 2.54%.

4-(4-Methoxyphenyl)-2-(benzo[*f*]coumarin-3-yl)-6-(8-methoxycoumarin-3-yl)pyridine (3r). Yield 66%; mp >300 ºC; yellow solid; IR(KBr): 3060 (m), 1715 (vs), 1610 (s), 1550 (s), 825 (s) cm^{-1}; ^1H NMR: δ 3.95 (3H, s, OCH_3), 4.07 (3H, s, OCH_3), 7.15-8.59 (14H, m, Ar-H + C_5-H of pyridine ring), 8.73 (1H, s, 3-H of pyridine ring), 9.08 (1H, s, 4"-H of coumarin ring), 9.79 (1H, s, 4'-H of coumarin ring); ^{13}C NMR: δ 55.7 (OCH_3), 56.6 (OCH_3), 113.7 (C), 114.7 (C), 116.1 (CH), 118.0 (CH), 119.0 (C), 119.8 (CH), 119.9 (CH), 121.6 (CH), 121.7 (CH), 125.9 (C), 126.8 (CH), 127.9 (CH), 129.1 (C), 129.7 (CH), 129.9 (CH), 130.3 (CH), 130.8 (C), 139.0 (CH), 143.3 (CH), 143.6 (C), 145.3 (C), 145.7 (C), 147.2 (C), 148.4 (CH), 155.4 (C), 161.5 (C), 163.8 (C). Anal. Calcd. for $C_{35}H_{23}NO_6$: C, 75.94; H, 4.19; N, 2.53%. Found: C, C, 75.85; H, 4.10; N, 2.68%.

Antimicrobial activity

All the synthesized compounds **3a-r** were screened for their antibacterial activities against *Escherichia coli* (Gram-negative bacteria), *Bacillus subtilis* (Gram-positive bacteria) and antifungal activity against *Aspergillus niger* (Fungi) by the agar cup diffusion method.[16] The zone of inhibition was measured in mm and was compared with standard drug. DMF was used as blank, Ciprofloxacin and Ampicillin were used as antibacterial standards and Griseofulvin was used as antifungal standard. All the compounds were tested at 1000 μg/ml concentration.

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