Synthesis and characterization of some novel coumarin based 2-pyridone heterocycles with their broad spectrum antimicrobial potency

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Abstract: The synthesis of a novel series of 6-((arylidene)amino)-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)ethylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitriles **4a-o** were synthesized and structures of compounds have been elucidated by IR, ¹H NMR, ¹³C NMR and mass spectral data. Antimicrobial activity was measured against certain strains of bacteria and fungi by serial broth dilution. Evaluation of antimicrobial activity shown that the compounds (**4g**, **4j**, **4k** and **4o**) were found to be most active against selected bacterial strains and compounds (**4d**, **4m** and **4n**) were found to be most active against selected fungal strains.

1. Introduction

Coumarin and 2-pyridone derivatives play a vital role in the theoretical development of heterocyclic chemistry along with being used extensively in organic synthesis. The action of bacterial infections still remains a significant therapeutic problem. It led to develop infectious diseases and the increasing number of multidrug-resistant microbial pathogens. Therefore since the past few decades much attention has been given to the design and synthesis of new types of pharmacologically diverse structural hybrid molecules [1]. The present work is dealing with the synthesis of novel series of 6-((arylidene)amino)-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)ethylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitriles **4a-o**. It is well known that coumarin derivatives possess a wide range of medicinal indications, such as anthelmintic, anticoagulant, hypnotic and insecticidal properties [2]. The antimicrobial activity of coumarin derivatives is reported in the literature [3,4,5,6,7]. Potential of various natural and synthetic coumarin derivatives proved its importance as anti-inflammatory [8] antioxidant properties [9] anticancer [10,11] and monoamine oxidase inhibitors [12]. Novobiocin and chlorobiocin have reported as antimicrobials containing a coumarin skeleton [13].

Pyridone and their derivatives play a vital role in some biological processes and have significant chemical and pharmacological importance [14,15,16]. 2-Pyridones motifs found to possess useful pharmacological activities, such as analgesic [17], antimalarial [18], anti-HIV [19], phytotoxic [20], and antitumoral [21] properties. The versatility of 2-pyridone and its potential to yield derivatives with a wide range of biological activities has made it a useful structure for further molecular investigation. Moreover, 2-pyridones are a class of newly exposed potent antibacterial agents that are of specific concern due to their *in vitro* and *in vivo* antibacterial potencies against the bacterial type II DNA topoisomerases, which consist of two highly homologous enzymes-DNA gyrase and topoisomerase IV [22,23]. In accordance with our previous work [24,25,26] and medicinal importance of coumarin and 2-pyridone motifs we report herein the synthesis of a new class of 6-((arylidene)amino)-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)ethylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitriles **4a-o** as potential antimicrobial agents.

2. Results and discussion

2.1. Chemistry

The synthetic route to the proposed compounds is shown in Scheme 1. The synthesis of 6-((arylidene)amino)-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)ethylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitriles **4a-o** have carried out as follows: 3-acetyl-2*H*-chromen-2-one (**1**) was prepared by Salicylaldehyde (**A**) (0.01 mol) and equimolar amount of ethyl acetoacetate (**B**) in dry methanol using 1 mL piperidine as a catalyst. Reaction mixture was stirred at room temperature for 30 mins and after the stirring, crystals were observed at the bottom of the RBF. In the first step, compound (**2**) was prepared by mixture of 3-acetyl-2*H*-chromen-2-one (0.01 mol) (**1**) and 2-cyanoacetohydrazide (0.01 mol) in presence of methanol (30 mL) with catalytic amount of glacial acetic acid and refluxed at 50-60 °C. In the second step, intermediate (**2**) (0.01 mol) was refluxed with 2-benzylidenemalononitrile (**E**) (0.01 mol) and catalytic amount of piperidine in presence of ethanol (99%, 30 mL) as a solvent gave compound (**3**). Intermediate (**3**) (0.01 mol) was further treated with various aromatic aldehydes (0.01 mol) and ethanol (99%, 30 mL) refluxed and the solid separated to obtain novel compounds of the series **4a-o**. The structural assignments of the compounds **4a-o** were based on the characterization of their ¹H NMR, ¹³C NMR, and mass spectra. Satisfactory elemental analyses were obtained (Scheme 1).

Scheme 1. Synthetic route for preparation of title compounds (4a-o)

A plausible mechanistic pathway for the formation of compounds **4a-o** is suggested in Scheme 2. In first step, hydrazone (A) underwent Michael addition with Knoevenagel product (B) and produced the intermediate (C), which further undergoes intramolecular nucleophilic attack on cyanide carbon followed by annulation to yield intermediate (D). The intermediate (D) transformed to compound (E) by intramolecular electron transfer to nitrogen atom. In the last step, intermediate (E) was transformed to targeted compounds by intermolecular nucleophilic attack on carbonyl carbon of different aromatic aldehydes (Scheme 2).

Scheme 2. Plausible mechanistic pathway

2.2. Characterization of compounds 4a-o

The structure of compound **4a-o** was confirmed on the basis of spectral data. The IR spectrum of compound **4a-o** showed strong absorption band at 1700-1730 cm^{-1} due to $>\text{C}=\text{O}$ stretching, coumarin ring. Absorption band appeared at 2203-2221 cm^{-1} due to stretching vibrations corresponding to cyano group and absorption band at 1591-1599, 1551-1562 cm^{-1} corresponding to $\text{C}=\text{C}$, $\text{C}=\text{N}$ stretching, aromatic ring. Moreover absorption bands appeared in compounds **4a-o** at 1251-1268 cm^{-1} indicated the $\text{C}-\text{O}-\text{C}$ linkage present in coumarin ring. In ^1H NMR spectra, the appearance of singlet peaks in compounds **4a-o** at $\delta = 8.65$ -9.30 and 9.05-9.25 ppm was due to one proton of coumarin ring (C4-H) and $\text{Ar}-\text{CH}=\text{N}-$ linkage. Three proton of $\text{Ar}-\text{C}(\text{CH}_3)=\text{N}-$ displayed singlet at $\delta = 2.03$ -2.10 ppm. Remaining all aromatic protons appeared multiplet in the region $\delta = 6.90$ -8.53 ppm. The ^{13}C NMR spectrum of compound **4a-o** showed characteristic signal at $\delta = 159.2$ -159.9 ppm due to carbonyl carbon at coumarine motif as well as the appearance of signal around $\delta = 115.5$ (2) ppm was assignable to cyano group of the 2-pyridone ring. Carbon of $-\text{CH}=\text{N}-$ linkage showed signal around $\delta = 163.2$ ppm. The mass spectrum revealed a molecular ion peak in compound **4a-o** at $m/z = 509.15$ -599.18 in mass spectra, molecular ion peak was in agreement with proposed molecular weight and elemental analysis.

2.3. Antimicrobial Results

The activity of compounds was determined as per the National Committee for Clinical Laboratory Standards (NCCLS) protocol using Mueller-Hinton Broth (Becton–Dickinson, USA) [27,28,29,30]. All the newly synthesized compounds were evaluated against Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus pyogenes*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and fungi (*Candida albicans*, *Aspergillus niger* and *Aspergillus clavatus*) strains.

Table 1. Antibacterial and Antifungal activity of compounds 4a-o

Sr. No.	-R	Minimum inhibitory concentrations for bacteria (MIC _B) in µg/ml				Minimum inhibitory concentration for fungi (MIC _F) in µg/ml		
		<i>E. coli</i> MTCC 443	<i>P. aeruginosa</i> MTCC 1688	<i>S. aureus</i> MTCC 96	<i>S. pyogenes</i> MTCC 442	<i>C. albicans</i> MTCC 227	<i>A. niger</i> MTCC 282	<i>A. clavatus</i> MTCC 1323
4a	-H	>1000	500	1000	500	500	1000	250
4b	-2-OH	1000	1000	500	1000	500	100	100
4c	-3-OH	500	500	250	>1000	100	50	50
4d	-4-OH	250	1000	500	250	25	12.5	50
4e	-2-NO ₂	100	250	500	100	>1000	250	500
4f	-3-NO ₂	50	250	100	50	1000	>1000	>1000
4g	-4-NO ₂	12.5	25	25	100	1000	>1000	>1000
4h	-2-Cl	250	>1000	250	250	1000	500	500
4i	-3-Cl	100	500	50	1000	>1000	500	250
4j	-4-Cl	25	12.5	100	12.5	>1000	1000	250
4k	-4-F	12.5	12.5	100	12.5	500	500	>1000
4l	-3-OCH ₃	>1000	500	500	1000	100	50	250
4m	-4-OCH ₃	500	1000	500	250	50	12.5	12.5
4n	-3,4,5-(OCH ₃) ₃	1000	>1000	250	500	100	25	50
4o	-4-Br	50	12.5	12.5	50	>1000	500	500
	Ampicillin	100	100	250	100	-	-	-
	Griseofulvin	-	-	-	-	500	100	100

2.3.1. Antibacterial activity

In preliminary screening, compounds **4a-o** against the test microbes are listed in Table 1 along with MIC values of reference compounds ampicillin (for bacteria) and griseofulvin (for fungi). Although all coumarine base 2-pyridone derivatives **4a-o** were found to show antimicrobial activity against different strains in these two assays, compounds **4g**, **4j**, **4k** and **4o** are clearly outstanding in their antibacterial properties. For several pathogens, these compounds were more active than the reference drugs.

2.3.2. Antifungal activity

Compounds **4a-o** were tested for antifungal activity in six sets against *C. albicans*, *A. niger* and *A. clavatus* at various concentrations of 1000, 500, 200 and 100 µg/ml as shown in Table 1. Synthesized compounds are diluted at 1000 µg ml⁻¹ concentration, as a stock solution. The data of antimicrobial evaluation of compounds **4a-o** were collected in Table 1. Compound **4d** selectively inhibits the growth of *C. albicans* and *A. niger*. Compound **4m**, expressed excellent activity against *A. niger* and *A. clavatus* while compound **4n** exhibited excellent activity against *A. niger* with a two to four fold higher (12.5-25 µg/ml) MIC value than the reference drug griseofulvin. Detailed activity results are summarized in Table 1.

3. Experimental

3.1. Materials and methods

All reactions except those in aqueous media were carried out by standard techniques for the exclusion of moisture. Melting points were determined on an electro thermal melting point apparatus and were reported uncorrected. TLC on silica gel plates (Merck, 60 F₂₅₄) was used for purity checking and reaction monitoring. Elemental analysis (% C, H, N) was carried out by a Perkin-Elmer 2400 CHN analyzer. IR spectra of all compounds were recorded on a Perkin-Elmer FT-IR spectrophotometer in KBr. ¹H NMR spectra were recorded on Varian Gemini 300 MHz and ¹³C NMR spectra are recorded with a Varian Mercury-400 (100 MHz) NMR spectrometer, using tetramethylsilane as the internal reference, with dimethyl sulfoxide (DMSO-*d*₆) as solvent. Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer.

Preparation of 2-cyano-*N'*-(2-(2-oxo-2*H*-chromen-3-yl)propylidene) acetohydrazide (2)

A mixture of 3-acetyl-2*H*-chromen-2-one (**1**) (0.01 mol) and 2-cyanoacetohydrazide (0.01 mol) in methanol (30 mL) was refluxed for 14 h and then cooled down to room temperature. The separated crystals were filtered, air dried and recrystallized from ethanol (95%). Yield: 72%; m.p.: 159 °C; Anal. calcd. for C₁₅H₁₃N₃O₃: C-63.60, H-4.63, N-14.83; Found: C-63.63, H-4.56, N-14.76%.

Preparation of 6-amino-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)ethylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (3)

A mixture containing 2-cyano-*N'*-(2-(2-oxo-2*H*-chromen-3-yl)propylidene) acetohydrazide (**2**) (0.01 mol), 2-benzylidenemalononitrile (0.01 mol) and 2 drops of piperidine in ethanol (95%) (30 mL) was refluxed for 22 h. The mixture was then cooled down to room temperature and diluted with few drops of water. The crystals formed were filtered, air dried and recrystallized from ethanol. Yield: 68%; m.p.: 190 °C; Anal. Calcd. for C₂₄H₁₅N₅O₃: C-68.40, H-3.59, N-16.62; Found: C-68.27, H-3.64, N-16.69%.

General preparation of 6-((benzylidene)amino)-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)ethylidene) amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4a-o)

Compound (**3**) (0.01 mol), benzaldehyde (0.01 mol) and ethanol (95%) (30 mL) were taken in a round bottom flask and refluxed for 12 h. Separated solid was filtered, dried and recrystallized from ethanol.

6-((benzylidene)amino)-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)ethylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4a)

Yield: 69 %, m.p. 223-227 °C. IR (KBr, cm⁻¹): 3133, 3029 (C-H stretching, aromatic ring), 2917 (C-H, CH₃), 2205 (C≡N stretching, nitrile group), 1727 (>C=O stretching, coumarin ring), 1559, 1595 (>C=N-, >C=C< stretching, aromatic ring), 1586, 1402 (C-H bending, -CH=N linkage), 1251 (C-O-C coumarin ring). ¹H NMR (300 MHz, DMSO-*d*₆, δ ppm): 9.10 (s, 1H, Ar-CH=N-), 8.99 (s, 1H, C4 proton of coumarin), 7.13-7.83 (m, 14H, Ar-H of coumarin ring and phenyl ring), 2.03 (s, 3H, -C(CH₃)=N-). ¹³C NMR (100 MHz, DMSO-*d*₆, δ ppm): 169.1, 163.5, 160.2, 159.9, 155.3, 153.6, 153.2, 133.3, 133.1, 132.8, 131.1, 129.4, 129.4, 128.7, 128.7, 128.5, 128.5, 128.1, 128.1, 128.0, 127.6, 127.6, 125.7, 123.5, 118.3, 116.2, 115.6, 115.6, 115.2, 114.9, 4.6. MS: m/z 509.15 (M⁺). Anal. calcd. For C₃₁H₁₉N₅O₃, C, 73.08; H, 3.76; N, 13.75 %. Found: C, 73.02; H, 3.79; N, 13.71 %.

6-((2-hydroxybenzylidene)amino)-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)ethylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4b)

Yield: 77 %, m.p. 174-177 °C. IR (KBr, cm⁻¹): 3415 (O-H, Ar-OH), 3135, 3034 (C-H stretching, aromatic ring), 2914 (C-H, CH₃), 2209 (C≡N stretching, nitrile group), 1708 (>C=O stretching, coumarin ring), 1551, 1599 (>C=N-, >C=C< stretching, aromatic ring), 1404 (C-H bending, -CH=N linkage), 1252 (C-O-C coumarin ring). ¹H NMR (300 MHz, DMSO-*d*₆, δ ppm): 11.02 (s, 1H, Ar-OH), 9.18 (s, 1H, Ar-CH=N-), 9.25 (s, 1H, C4 proton of coumarin), 6.90-7.81 (m, 13H, Ar-H of coumarin ring and phenyl ring), 2.05 (s, 3H, Ar-C(CH₃)=N-). ¹³C NMR (100 MHz, DMSO-*d*₆, δ ppm): 169.6, 163.3, 161.5, 160.4, 159.7, 155.5, 153.3, 153.1, 133.7, 132.6, 132.4, 132.6, 128.7, 128.7, 128.4, 128.4, 128.1, 127.7, 127.7, 125.6, 123.6, 121.6, 118.6, 118.2, 117.5, 116.3, 115.6, 115.6, 115.5, 114.5, 4.6. MS: m/z 525.14 (M⁺). Anal. calcd. For C₃₁H₁₉N₅O₄, C, 70.85; H, 3.64; N, 13.33 %. Found: C, 70.82; H, 3.69; N, 13.36 %.

6-((3-hydroxybenzylidene)amino)-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)ethylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4c)

Yield: 71 %, m.p. 198-202 °C. IR (KBr, cm⁻¹): 3405 (O-H, Ar-OH), 3131, 3035 (C-H stretching, aromatic ring), 2914 (C-H, CH₃), 2212 (C≡N stretching, nitrile group), 1713 (>C=O stretching, coumarin ring), 1562, 1599 (>C=N-, >C=C< stretching, aromatic ring), 1406 (C-H bending, -CH=N linkage), 1266 (C-O-C coumarin ring). ¹H NMR (300 MHz, DMSO-*d*₆, δ ppm): 9.49 (s, 1H, Ar-

OH), 9.22 (s, 1H, Ar-CH=N-), 8.90 (s, 1H, C4 proton of coumarin), 6.94-7.84 (m, 13H, Ar-H of coumarin ring and phenyl ring), 2.05 (s, 3H, Ar-C(CH₃)=N-). ¹³C NMR (100 MHz, DMSO-*d*₆, δ ppm): 169.2, 163.9, 160.5, 159.7, 158.7, 155.7, 153.7, 153.3, 135.3, 133.6, 132.2, 130.6, 128.5, 128.5, 128.2, 128.2, 128.0, 127.8, 127.8, 125.1, 123.6, 121.5, 118.4, 118.2, 116.3, 115.7, 115.7, 115.3, 114.6, 114.5, 4.6. MS: *m/z* 525.14 (M⁺). Anal. calcd. For C₃₁H₁₉N₅O₄, C, 70.85; H, 3.64; N, 13.33 %. Found: C, 70.87; H, 3.61; N, 13.35 %.

6-((4-hydroxybenzylidene)amino)-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)ethylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4d)

Yield: 63 %, m.p. 162-166 °C. IR (KBr, cm⁻¹): 3419 (O-H, Ar-OH), 3127, 3039 (C-H stretching, aromatic ring), 2918 (C-H, CH₃), 2206 (-C≡N stretching, nitrile group), 1704 (>C=O stretching, coumarin ring), 1551, 1596 (>C=N-, >C=C< stretching, aromatic ring), 1409 (C-H bending, -CH=N linkage), 1261 (C-O-C coumarin ring). ¹H NMR (300 MHz, DMSO-*d*₆, δ ppm): 9.74 (s, 1H, Ar-OH), 9.20 (s, 1H, Ar-CH=N-), 8.65 (s, 1H, C4 proton of coumarin), 6.91-7.89 (m, 13H, Ar-H of coumarin ring and phenyl ring), 2.03 (s, 3H, Ar-C(CH₃)=N-). ¹³C NMR (100 MHz, DMSO-*d*₆, δ ppm): 169.5, 163.5, 160.9, 160.7, 159.7, 155.3, 153.5, 153.0, 133.7, 132.8, 130.4, 130.4, 128.8, 128.8, 128.5, 128.5, 128.1, 127.7, 127.7, 126.5, 125.4, 123.6, 118.4, 116.2, 116.0, 116.0, 115.6, 115.6, 115.1, 114.3, 4.4. MS: *m/z* 525.14 (M⁺). Anal. calcd. For C₃₁H₁₉N₅O₄, C, 70.85; H, 3.64; N, 13.33 %. Found: C, 70.78; H, 3.59; N, 13.26%.

6-((2-nitrobenzylidene)amino)-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)ethylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4e)

Yield: 68 %, m.p. 236-239 °C. IR (KBr, cm⁻¹): 3136, 3031 (C-H stretching, aromatic ring), 2920 (C-H, CH₃), 2205 (-C≡N stretching, nitrile group), 1711 (>C=O stretching, coumarin ring), 1553, 1593 (>C=N-, >C=C< stretching, aromatic ring), 1484, 1356 (-NO₂), 1401 (C-H bending, -CH=N linkage), 1262 (C-O-C coumarin ring). ¹H NMR (300 MHz, DMSO-*d*₆, δ ppm): 9.07 (s, 1H, Ar-CH=N-), 9.30 (s, 1H, C4 proton of coumarin), 7.15-8.09 (m, 13H, Ar-H of coumarin ring and phenyl ring), 2.05 (s, 3H, -C(CH₃)=N-). ¹³C NMR (100 MHz, DMSO-*d*₆, δ ppm): 169.5, 163.9, 160.3, 159.5, 155.4, 153.5, 153.1, 147.4, 134.7, 133.6, 132.3, 131.4, 130.3, 128.6, 128.6, 128.3, 128.3, 128.2, 128.0, 127.5, 127.5, 125.3, 124.1, 123.4, 118.5, 116.3, 115.5, 115.5, 115.1, 114.6, 4.7. MS: *m/z* 554.13 (M⁺). Anal. calcd. For C₃₁H₁₈N₆O₅, C, 67.15; H, 3.27; N, 15.16 %. Found: C, 67.21; H, 3.18; N, 15.23 %.

6-((3-nitrobenzylidene)amino)-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)ethylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4f)

Yield: 61 %, m.p. 244-247 °C. IR (KBr, cm⁻¹): 3130, 3034 (C-H stretching, aromatic ring), 2924 (C-H, CH₃), 2221 (-C≡N stretching, nitrile group), 1700 (>C=O stretching, coumarin ring), 1553, 1598 (>C=N-, >C=C< stretching, aromatic ring), 1480, 1352 (-NO₂), 1403 (C-H bending, -CH=N linkage), 1266 (C-O-C coumarin ring). ¹H NMR (300 MHz, DMSO-*d*₆, δ ppm): 9.15 (s, 1H, Ar-CH=N-), 8.87 (s, 1H, C4 proton of coumarin), 7.19-8.53 (m, 13H, Ar-H of coumarin ring and phenyl ring), 2.08 (s, 3H, -C(CH₃)=N-). ¹³C NMR (100 MHz, DMSO-*d*₆, δ ppm): 169.7, 163.3, 160.1, 159.2, 155.9, 153.4, 148.1, 135.4, 134.9, 133.7, 132.7, 129.4, 128.9, 128.9, 128.7, 128.7, 128.5, 128.0, 127.7, 127.7, 126.3, 125.4, 123.6, 121.5, 118.4, 116.3, 115.7, 115.7, 115.1, 114.9, 4.7. MS: *m/z* 554.13 (M⁺). Anal. calcd. For C₃₁H₁₈N₆O₅, C, 67.15; H, 3.27; N, 15.16 %. Found: C, 67.12; H, 3.21; N, 15.21 %.

6-((4-nitrobenzylidene)amino)-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)ethylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4g)

Yield: 72 %, m.p. 221-225 °C. IR (KBr, cm⁻¹): 3137, 3031 (C-H stretching, aromatic ring), 2926 (C-H, CH₃), 2215 (-C≡N stretching, nitrile group), 1730 (>C=O stretching, coumarin ring), 1557, 1594 (>C=N-, >C=C< stretching, aromatic ring), 1487, 1359 (-NO₂), 1268 (C-O-C coumarin ring), 1405 (C-H bending, -CH=N linkage). ¹H NMR (300 MHz, DMSO-*d*₆, δ ppm): 9.12 (s, 1H, Ar-CH=N-), 8.70 (s, 1H, C4 proton of coumarin), 7.22-8.37 (m, 13H, Ar-H of coumarin ring and

phenyl ring), 2.03 (s, 3H, $-\text{C}(\text{CH}_3)=\text{N}-$). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$, δ ppm): 169.1, 163.6, 160.3, 159.4, 155.7, 153.5, 153.1, 150.4, 139.9, 133.2, 132.4, 128.7, 128.7, 128.5, 128.5, 128.3, 127.5, 127.5, 127.3, 127.3, 125.8, 124.2, 124.2, 123.5, 118.3, 116.2, 115.9, 115.9, 115.5, 114.4, 4.6. MS: m/z 554.13 (M^+). Anal. calcd. For $\text{C}_{31}\text{H}_{18}\text{N}_6\text{O}_5$, C, 67.15; H, 3.27; N, 15.16 %. Found: C, 67.07; H, 3.34; N, 15.11 %.

6-((2-chlorobenzylidene)amino)-2-oxo-1-((1-(2-oxo-2H-chromen-3-yl)ethylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4h)

Yield: 73 %, m.p. 217-221 °C. IR (KBr, cm^{-1}): 3139, 3029 (C-H stretching, aromatic ring), 2928 (C-H, CH_3), 2208 ($-\text{C}\equiv\text{N}$ stretching, nitrile group), 1706 ($>\text{C}=\text{O}$ stretching, coumarin ring), 1560, 1594 ($>\text{C}=\text{N}-$, $>\text{C}=\text{C}<$ stretching, aromatic ring), 1267 (C-O-C coumarin ring), 1408 (C-H bending, $-\text{CH}=\text{N}$ linkage), 754 (C-Cl). ^1H NMR (300 MHz, $\text{DMSO}-d_6$, δ ppm): 9.18 (s, 1H, Ar- $\text{CH}=\text{N}-$), 8.94 (s, 1H, C4 proton of coumarin), 7.14-7.90 (m, 13H, Ar-H of coumarin ring and phenyl ring), 2.06 (s, 3H, $-\text{C}(\text{CH}_3)=\text{N}-$). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$, δ ppm): 169.9, 163.5, 160.3, 159.6, 155.3, 153.4, 153.2, 135.3, 134.5, 133.7, 132.3, 131.4, 130.6, 128.8, 128.8, 128.6, 128.6, 128.1, 127.7, 127.7, 127.5, 127.4, 125.8, 123.6, 118.3, 116.7, 115.9, 115.9, 115.5, 114.5, 4.6. MS: m/z 543.11 (M^+). Anal. calcd. For $\text{C}_{31}\text{H}_{18}\text{ClN}_5\text{O}_3$, C, 68.45; H, 3.34; N, 12.87 %. Found: C, 68.51; H, 3.29; N, 12.96 %.

6-((3-chlorobenzylidene)amino)-2-oxo-1-((1-(2-oxo-2H-chromen-3-yl)ethylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4i)

Yield: 65 %, m.p. 226-230 °C. IR (KBr, cm^{-1}): 3143, 3025 (C-H stretching, aromatic ring), 2923 (C-H, CH_3), 2203 ($-\text{C}\equiv\text{N}$ stretching, nitrile group), 1716 ($>\text{C}=\text{O}$ stretching, coumarin ring), 1557, 1597 ($>\text{C}=\text{N}-$, $>\text{C}=\text{C}<$ stretching, aromatic ring), 1410 (C-H bending, $-\text{CH}=\text{N}$ linkage), 1258 (C-O-C coumarin ring), 757 (C-Cl). ^1H NMR (300 MHz, $\text{DMSO}-d_6$, δ ppm): 9.10 (s, 1H, Ar- $\text{CH}=\text{N}-$), 9.11 (s, 1H, C4 proton of coumarin), 7.16-7.89 (m, 13H, Ar-H of coumarin ring and phenyl ring), 2.09 (s, 3H, $-\text{C}(\text{CH}_3)=\text{N}-$). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$, δ ppm): 169.4, 163.9, 160.5, 159.7, 155.3, 153.4, 153.2, 133.6, 133.5, 133.1, 132.7, 132.6, 130.3, 128.7, 128.7, 128.5, 128.5, 128.2, 127.8, 127.8, 127.3, 126.7, 125.6, 123.4, 118.2, 116.3, 115.7, 115.7, 115.3, 114.6, 4.7. MS: m/z 543.11 (M^+). Anal. calcd. For $\text{C}_{31}\text{H}_{18}\text{ClN}_5\text{O}_3$, C, 68.45; H, 3.34; N, 12.87 %. Found: C, 68.36; H, 3.26; N, 12.81 %.

6-((4-chlorobenzylidene)amino)-2-oxo-1-((1-(2-oxo-2H-chromen-3-yl)ethylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4j)

Yield: 62 %, m.p. 239-244 °C. IR (KBr, cm^{-1}): 3137, 3021 (C-H stretching, aromatic ring), 2930 (C-H, CH_3), 2217 ($-\text{C}\equiv\text{N}$ stretching, nitrile group), 1722 ($>\text{C}=\text{O}$ stretching, coumarin ring), 1559, 1591 ($>\text{C}=\text{N}-$, $>\text{C}=\text{C}<$ stretching, aromatic ring), 1416 (C-H bending, $-\text{CH}=\text{N}$ linkage), 1255 (C-O-C coumarin ring), 751 (C-Cl). ^1H NMR (300 MHz, $\text{DMSO}-d_6$, δ ppm): 9.20 (s, 1H, Ar- $\text{CH}=\text{N}-$), 9.15 (s, 1H, C4 proton of coumarin), 7.14-7.87 (m, 13H, Ar-H of coumarin ring and phenyl ring), 2.06 (s, 3H, $-\text{C}(\text{CH}_3)=\text{N}-$). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$, δ ppm): 169.7, 163.6, 160.2, 159.6, 155.5, 153.5, 153.4, 136.6, 133.3, 132.3, 131.7, 130.6, 130.6, 128.7, 128.7, 128.7, 128.7, 128.4, 128.4, 128.2, 127.7, 127.7, 125.7, 123.5, 118.3, 116.5, 115.9, 115.9, 115.6, 114.4, 4.5. MS: m/z 543.11 (M^+). Anal. calcd. For $\text{C}_{31}\text{H}_{18}\text{ClN}_5\text{O}_3$, C, 68.45; H, 3.34; N, 12.87 %. Found: C, 68.59; H, 3.42; N, 12.98 %.

6-((4-fluorobenzylidene)amino)-2-oxo-1-((1-(2-oxo-2H-chromen-3-yl)ethylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4k)

Yield: 69 %, m.p. 243-246 °C. IR (KBr, cm^{-1}): 3134, 3029 (C-H stretching, aromatic ring), 2934 (C-H, CH_3), 1135 (C-F), 2219 ($-\text{C}\equiv\text{N}$ stretching, nitrile group), 1719 ($>\text{C}=\text{O}$ stretching, coumarin ring), 1561, 1599 ($>\text{C}=\text{N}-$, $>\text{C}=\text{C}<$ stretching, aromatic ring), 1419 (C-H bending, $-\text{CH}=\text{N}$ linkage), 1260 (C-O-C coumarin ring). ^1H NMR (300 MHz, $\text{DMSO}-d_6$, δ ppm): 9.25 (s, 1H, Ar- $\text{CH}=\text{N}-$), 9.07 (s, 1H, C4 proton of coumarin), 7.11-7.92 (m, 13H, Ar-H of coumarin ring and phenyl ring), 2.10 (s, 3H, $-\text{C}(\text{CH}_3)=\text{N}-$). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$, δ ppm): 169.9, 165.6, 163.2, 160.6,

159.8, 155.9, 153.7, 153.4, 133.9, 132.1, 130.9, 130.9, 129.6, 128.7, 128.7, 128.5, 128.5, 128.1, 127.4, 127.4, 125.4, 123.2, 118.5, 116.4, 115.5, 115.5, 115.3, 115.3, 115.1, 114.9, 4.7. MS: m/z 527.14 (M^+). Anal. calcd. For $C_{31}H_{18}FN_5O_3$, C, 70.58; H, 3.44; N, 13.28 %. Found: C, 70.66; H, 3.52; N, 13.34 %.

6-((3-methoxybenzylidene)amino)-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)ethylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4l)

Yield: 74 %, m.p. 206-209 °C. IR (KBr, cm^{-1}): 3133, 3036 (C-H stretching, aromatic ring), 2939 (C-H, CH_3), 2214 ($-C\equiv N$ stretching, nitrile group), 1709 ($>C=O$ stretching, coumarin ring), 1558, 1595 ($>C=N-$, $>C=C<$ stretching, aromatic ring), 1426 (C-H bending, $-CH=N$ linkage), 1263 (C-O-C coumarin ring), 1214, 1152 (C-O-C). 1H NMR (300 MHz, $DMSO-d_6$, δ ppm): 9.05 (s, 1H, Ar-CH=N-), 8.77 (s, 1H, C4 proton of coumarin), 7.03-7.82 (m, 13H, Ar-H of coumarin ring and phenyl ring), 3.73 (s, 3H, $-OCH_3$), 2.05 (s, 3H, $-C(CH_3)=N-$). ^{13}C NMR (100 MHz, $DMSO-d_6$, δ ppm): 169.1, 163.9, 162.6, 160.4, 159.8, 155.9, 153.1, 153.0, 133.9, 132.6, 130.5, 130.5, 128.8, 128.8, 128.5, 128.5, 128.1, 127.6, 127.6, 126.6, 125.6, 123.9, 118.2, 116.3, 115.5, 115.5, 115.1, 114.8, 114.5, 114.5, 55.4, 4.6. MS: m/z 539.16 (M^+). Anal. calcd. For $C_{32}H_{21}N_5O_4$, C, 71.24; H, 3.92; N, 12.98 %. Found: C, 71.29; H, 3.83; N, 12.91 %.

6-((4-methoxybenzylidene)amino)-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)ethylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4m)

Yield: 70 %, m.p. 214-218 °C. IR (KBr, cm^{-1}): 3126, 3041 (C-H stretching, aromatic ring), 2943 (C-H, CH_3), 2218 ($-C\equiv N$ stretching, nitrile group), 1717 ($>C=O$ stretching, coumarin ring), 1555, 1597 ($>C=N-$, $>C=C<$ stretching, aromatic ring), 1429 (C-H bending, $-CH=N$ linkage), 1254 (C-O-C coumarin ring), 1210, 1155 (C-O-C). 1H NMR (300 MHz, $DMSO-d_6$, δ ppm): 9.20 (s, 1H, Ar-CH=N-), 9.10 (s, 1H, C4 proton of coumarin), 7.05-7.88 (m, 13H, Ar-H of coumarin ring and phenyl ring), 3.86 (s, 3H, $-OCH_3$), 2.03 (s, 3H, $-C(CH_3)=N-$). ^{13}C NMR (100 MHz, $DMSO-d_6$, δ ppm): 169.1, 163.9, 162.6, 160.4, 159.8, 155.9, 153.1, 153.0, 133.9, 132.6, 130.5, 130.5, 128.8, 128.8, 128.5, 128.5, 128.1, 127.6, 127.6, 126.6, 125.6, 123.9, 118.2, 116.3, 115.5, 115.5, 115.1, 114.8, 114.5, 114.5, 55.4, 4.6. MS: m/z 539.16 (M^+). Anal. calcd. For $C_{32}H_{21}N_5O_4$, C, 71.24; H, 3.92; N, 12.98 %. Found: C, 71.18; H, 3.89; N, 12.95 %.

2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)ethylidene)amino)-4-phenyl-6-((3,4,5-trimethoxybenzylidene)amino)-1,2-dihydropyridine-3,5-dicarbonitrile (4n)

Yield: 66 %, m.p. 229-233 °C. IR (KBr, cm^{-1}): 3129, 3044 (C-H stretching, aromatic ring), 2946 (C-H, CH_3), 2220 ($-C\equiv N$ stretching, nitrile group), 1702 ($>C=O$ stretching, coumarin ring), 1561, 1596 ($>C=N-$, $>C=C<$ stretching, aromatic ring), 1433 (C-H bending, $-CH=N$ linkage), 1251 (C-O-C coumarin ring), 1218, 1159 (C-O-C). 1H NMR (300 MHz, $DMSO-d_6$, δ ppm): 9.25 (s, 1H, Ar-CH=N-), 8.92 (s, 1H, C4 proton of coumarin), 7.12-7.78 (m, 11H, Ar-H of coumarin ring and phenyl ring), 3.83 (s, 9H, $-OCH_3$), 2.08 (s, 3H, $-C(CH_3)=N-$). ^{13}C NMR (100 MHz, $DMSO-d_6$, δ ppm): 169.7, 163.5, 160.2, 159.7, 155.3, 153.5, 153.5, 153.5, 153.2, 141.6, 133.5, 132.5, 128.8, 128.8, 128.5, 128.5, 128.2, 128.0, 127.7, 127.7, 125.6, 123.5, 118.4, 116.6, 115.9, 115.9, 115.5, 114.6, 104.3, 104.3, 60.4, 56.5, 56.5, 4.6. MS: m/z 599.18 (M^+). Anal. calcd. For $C_{34}H_{25}N_5O_6$, C, 68.11; H, 4.20; N, 11.68 %. Found: C, 68.02; H, 4.12; N, 11.72 %.

6-((4-bromobenzylidene)amino)-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)ethylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (4o)

Yield: 64 %, m.p. 208-212 °C. IR (KBr, cm^{-1}): 3140, 3035 (C-H stretching, aromatic ring), 2936 (C-H, CH_3), 2207 ($-C\equiv N$ stretching, nitrile group), 1719 ($>C=O$ stretching, coumarin ring), 1552, 1593 ($>C=N-$, $>C=C<$ stretching, aromatic ring), 1424 (C-H bending, $-CH=N$ linkage), 1267 (C-O-C coumarin ring), 1084, 1012 (C-Br). 1H NMR (300 MHz, $DMSO-d_6$, δ ppm): 9.11 (s, 1H, Ar-CH=N-), 9.27 (s, 1H, C4 proton of coumarin), 7.14-7.85 (m, 13H, Ar-H of coumarin ring and phenyl ring), 2.08 (s, 3H, $-C(CH_3)=N-$). ^{13}C NMR (100 MHz, $DMSO-d_6$, δ ppm): 169.2, 163.9, 160.3, 159.7, 155.8, 153.4, 153.2, 133.7, 132.3, 132.2, 131.6, 131.6, 128.9, 128.9, 128.7, 128.7,

128.4, 128.4, 128.2, 127.8, 127.8, 125.4, 125.4, 123.5, 118.3, 116.4, 115.7, 115.7, 115.5, 114.6, 4.5. MS: m/z 587.06 (M^+). Anal. calcd. For $C_{31}H_{18}BrN_5O_3$, C, 63.28; H, 3.08; N, 11.90 %. Found: C, 63.36; H, 3.02; N, 11.86 %.

SAR studies

The structure-activity relationships (SAR) of compounds **4a-o** were determined on the basis of results presented in Table 1. The substitution pattern on the coumarin based 2-pyridone ring system derivatives was carefully selected to confer different electronic environment of the molecules. From the activity data, compounds containing electron withdrawing group at *para* position lead to enhance bacterial activity. Compounds **4g** (-4-NO₂-C₆H₄), **4j** (-4-Cl-C₆H₄) and **4k** (-4-F-C₆H₄) containing 4-NO₂, 4-Cl and 4-F group showed highest inhibition at MIC = 12.5-25 $\mu\text{g ml}^{-1}$ against bacterial strain *E. coli* and *P. aeruginosa*. Same results observed in compounds **4j** (-4-Cl-C₆H₄) and **4k** (-4-F-C₆H₄) showed highest inhibition at MIC = 12.5-25 $\mu\text{g ml}^{-1}$ against *S. pyogenes* and compound **4o** -4-Br group showed highest inhibition at MIC = 12.5 $\mu\text{g ml}^{-1}$ against *P. aeruginosa* and *S. aureus*. On the basis of screening results, it has been observed that the compound containing electron releasing groups such as -OH, -OCH₃ lead to enhance fungal activity. Compounds **4d** (-4-OH-C₆H₄), **4m** (-4-OCH₃-C₆H₄) and **4n** (-3,4,5-(OCH₃)₃-C₆H₂) exhibited excellent inhibitory action against *A.niger*. While compounds **4d** (-4-OH-C₆H₄) and **4m** (-4-OCH₃-C₆H₄) also showed reasonably excellent inhibition against *C. albicans* and *A. clavatus* respectively. SAR studies revealed that the presence of electron withdrawing and electron releasing group in title compound increase the antibacterial and antifungal activity respectively.

Conclusion

New coumarin based 2-pyridone derivatives **4a-o** were synthesized and screened for their antimicrobial activity. Compounds **4g** (-4-NO₂-C₆H₄), **4j** (-4-Cl-C₆H₄), **4k** (-4-F-C₆H₄) and **4o** (-4-Br-C₆H₄) exhibit outstanding antibacterial and compounds **4d** (-4-OH-C₆H₄), **4m** (-4-OCH₃-C₆H₄) and **4n** (-3,4,5-(OCH₃)₃-C₆H₂) exhibit antifungal properties. On the basis biological activity results can be concluded that above scaffolds would play an important role in the development of antimicrobial agents in future.

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