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Synthesis, characterization, molecular docking studies and biological activity of coumarin linked 2-pyridone heterocycles

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In the present paper, the synthesis, characterization, antimicrobial activity and *in silico* molecular docking study of 6-((arylidene)amino)-4-(4-chlorophenyl)-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)ethylidene)amino)-1,2-dihydropyridine-3,5-dicarbonitriles **4a-o** have been reported. Compounds **4d**, **4g**, **4j**, **4k**, **4m** and **4o** show significant activity. Structure determination of the synthesized compounds has been done by the standard spectroscopic techniques. It is observed that biological activity is influenced by electronic environment of the molecules. Electron withdrawing group at *para* position plays a major role for enhancing the biological activity for antibacterial activity and the electron donating group at *para* position for antifungal activity. Compounds **4a-o** have been further evaluated for cytotoxicity on HeLa cells. From the cytotoxicity results, compounds have been found to possess low cytotoxicity with potent antimicrobial activity.

Keywords: Medicinal chemistry, antibacterial, antifungal, cytotoxicity, molecular docking

The synthesis and biological activities of coumarin based 2-pyridone derivatives **4a-o** occupied an important position in heterocyclic chemistry as well as in medicinal chemistry. Coumarin derivatives displayed a wide range of medicinal activity[1-6]. Moreover, these derivatives were also found to possess very good antimicrobial activity as shown in the literature[7-11]. They are responsible for positive activity of several types of cancer including malignant melanoma, renal cell carcinoma, leukemia, prostate and breast cancer cells progression with less cytotoxicity[12-15]. Many currently available antimicrobial agents possessed coumarin skeleton such as Chlorobiocin and Novobiocin[16] in their molecular frame work. 2-Pyridones denote versatile class of pharmacophores having very good therapeutic effect[17] on several diseases. Heterocyclic compounds having 2-pyridone moiety showed various biological activities, such as analgesic[18], anti-HIV[19] and antitumoral[20]. The most prominent examples of prescribed agents featuring the 2-pyridone nucleus include the Phosphodiester (PDE) III inhibitor Amrinone, Antifungal Ciclopirox, Cardiotonic agent Olprinone, Antitumor antibiotic Diazaquinomycin A, HIV-1 Inhibitors Pyridinone L as in shown Figure 1.

In this paper, we have adopted the concept of Michael addition having α-β unsaturated carbonyl attack followed by hydrolytic cyclization with subsequent oxidative aromatization is the most common method used to synthesize 2-pyridone heterocycles[21-23]. In continuation of our previous work for searching novel pharmacophores with potent antimicrobial activity[24-30], we have designed novel compounds having coumarin and 2-pyridone in a single molecular framework. Newly synthesized compounds were tested for antimicrobial potency and also evaluated cytotoxicity on HeLa cell line. Structural elucidation of newly synthesized compounds **4a-o** was carried out by different analytical techniques like IR, NMR and mass spectrometry.

Results and Discussion

Chemistry

The synthetic pathway of the titled compounds is given in Scheme I. 3-acetyl-2*H*-chromen-2-one **(1)** was synthesized with salicylaldehyde **(A)** and ethyl acetoacetate **(B).** Equimolar amount of the reaction

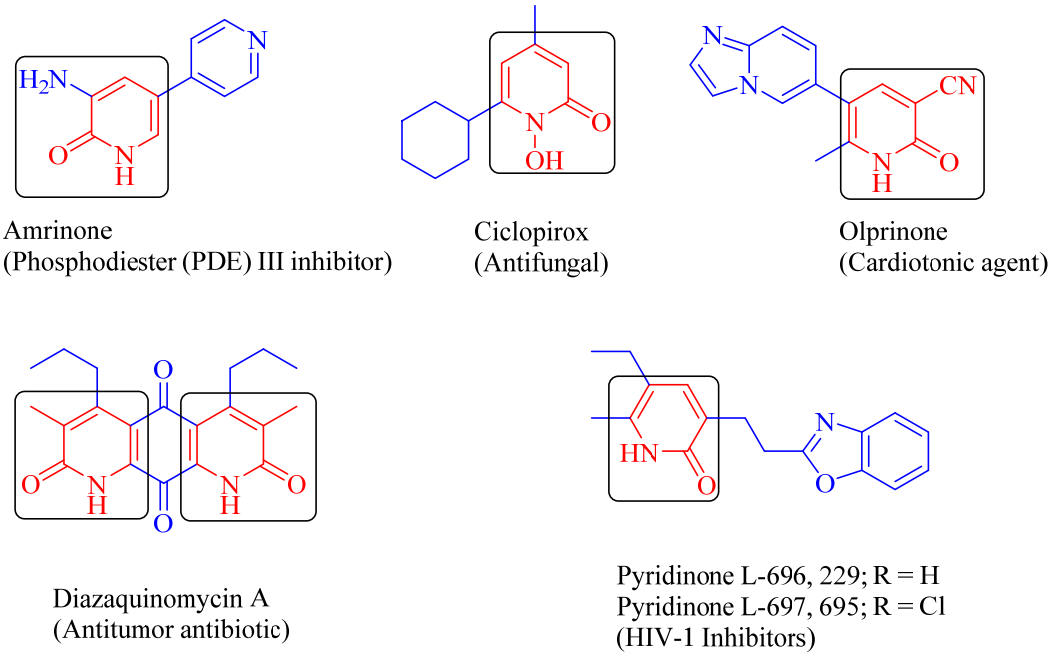


Figure 1 — Commercially available drugs containing 2-pyridone nucleus

mixture was stirred at RT in the presence of piperidine as a catalyst. Compound **(2)** was prepared by the reaction of 3-acetyl-2*H*-chromen-2-one **(1)** and 2-cyanoacetohydrazide which was mixed in an equimolar amount in methanol (30 mL), glacial acetic acid and refluxed at 50-60°C. In the consequent step, intermediate **(2)** (0.01 mol) was heated with 2-(4-chlorobenzylidene)malononitrile **(E)** (0.01 mol). In this reaction, piperidine was used as a catalyst in ethanol (99%, 30 mL). As a result of this, reaction mixture furnished compound **(3)**. Compound **(3)** (0.01 mol) was taken in ethanol and further reacted with aromatic aldehydes to produced novel compounds (**4a-o).**

Mechanism of the products **4a-o** is proposed in the Scheme II. In the first step, hydrazone **(A)** undertook Michael addition with **(B)** and produced the intermediate **(C)**. Intramolecular nucleophilic occurrence on the cyanide carbon was monitored to yield intermediate **(D)**. Compound **(E)** was produced by the intramolecular electron transfer to nitrogen atom. In the final step, intermediate **(E)** was altered to targeted molecules by intermolecular nucleophilic attack on carbonyl carbon of various aromatic aldehydes (Scheme II).

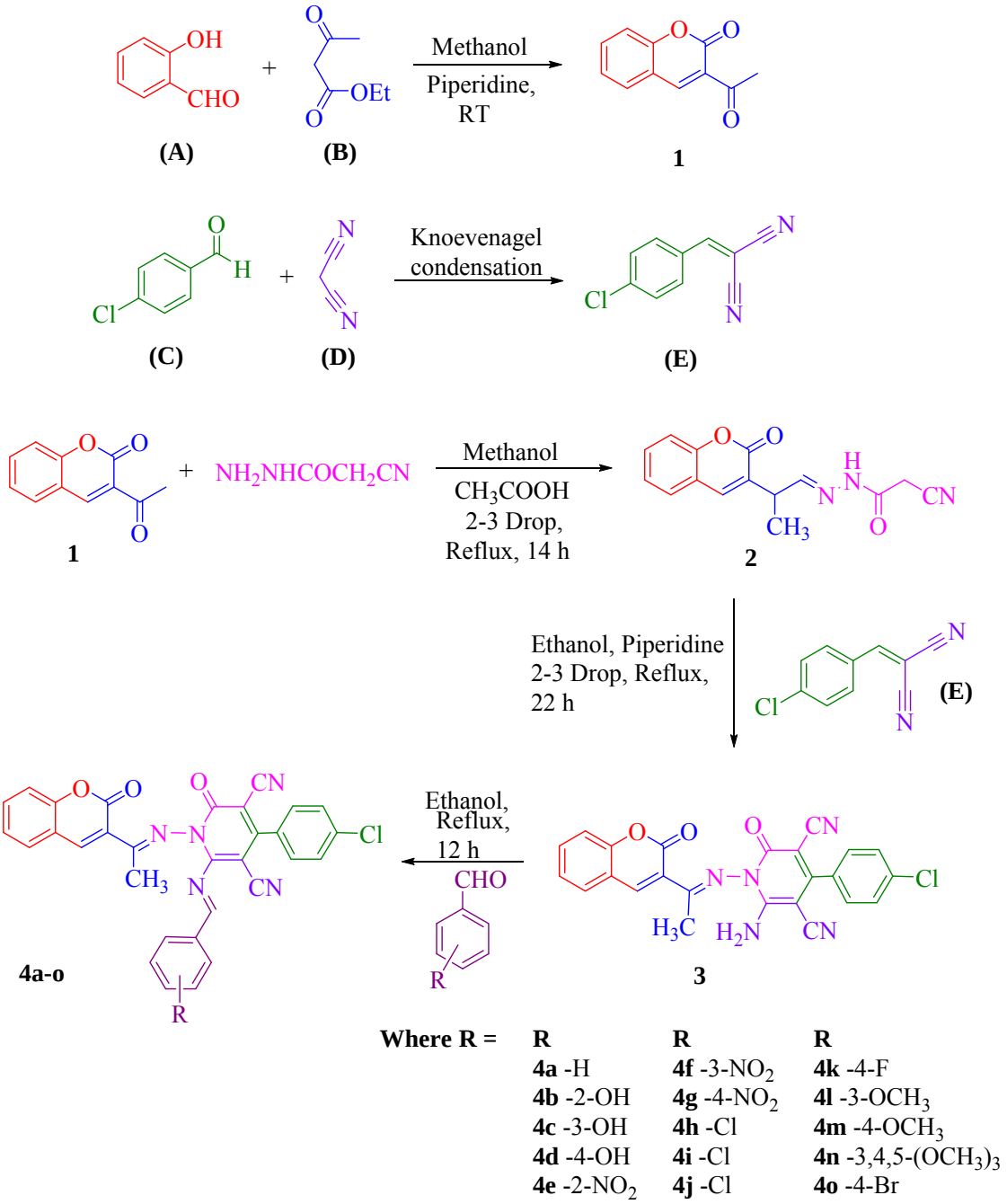
Discussion of Antimicrobial Activity

Antibacterial studies

All the synthesized compounds **4a-o** were screened for their *in vitro* activity against several panels of bacteria by the conventional broth micro-dilution method using Chloramphenicol as a standard drug and the results are presented in the Table I. It was observed that compounds **4g** (-4-NO_2), **4j** (-4-Cl), **4k** (-4-F) and **4o** (-4-Br) were the most effective antibacterial agents. Compounds **4g** (-4-NO_2) and **4o** (-4-Br) exhibited excellent activity against *P. aeruginosa* and *S. aureus* with 2 to 4 fold higher MIC (12.5-25 mg/mL) than chloramphenicol. Compounds **4j** (-4-Cl) and **4k** (-4-F) were the most effective against *S. Pyogenes* while compound **4j** (-4-Cl) showed excellent activity against *E. coli.* Compounds **4g** (-4-NO_2) and **4o** (-4-Br) have a very good activity against *S. Pyogenes* while compounds **4j** (-4-Cl) and **4k** (-4-F) were confirmed as very good agents against *S. aureus.* On the other hand, the presence of similar functional groups at the *ortho* position decrease the antibacterial activity as compared to **4e** (-4-NO_2) and **4h** (-4-Cl). Remarkable antibacterial activity is depicted by groups like -NO_2, -F, -Cl and -Br.

Antifungal studies

Preliminary screening results showed that compounds **4d** (-4-OH) and **4m** (-4-OCH_3) exhibited excellent activity against *A. clavatus.* While compound **4d** (-4-OH) showed excellent activity against *A. niger* having 2 to 4 fold higher MIC (12.5-25 mg/mL) in comparison with a standard drug Nystatin. The enhancement of the activity of compounds **4d** and **4m** (-4-OCH_3) may be attributed



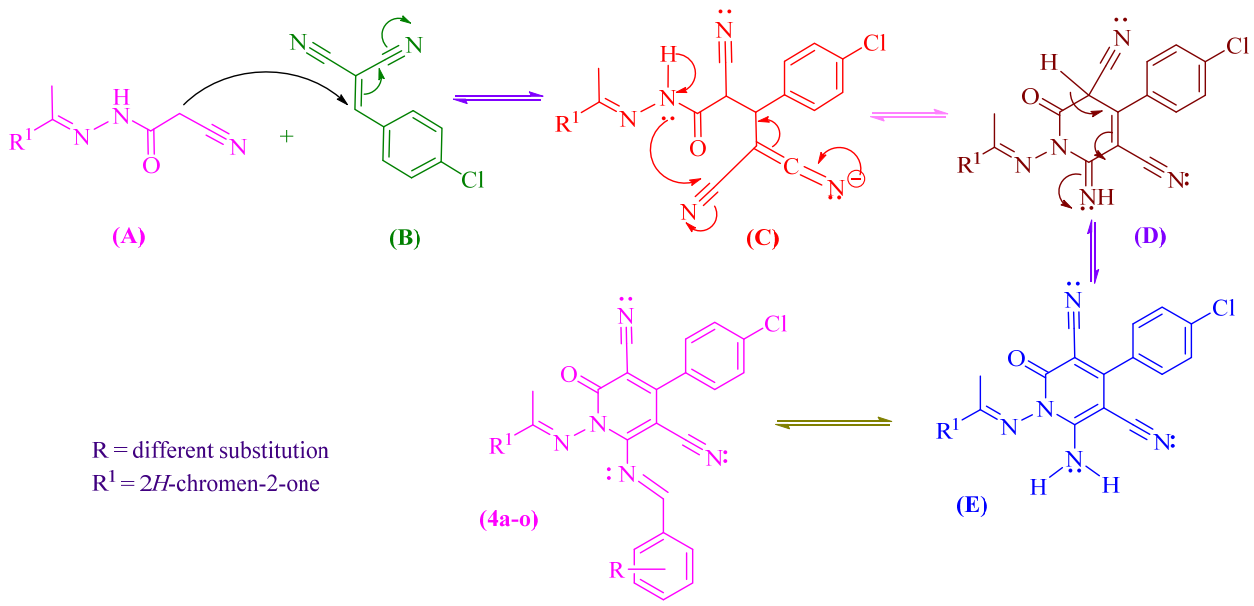
Scheme I — Synthetic route for the preparation of title compounds **4a-o**

to the presence of electron releasing group at *para* position. When we installed hydroxy group at *meta* position in **4c** (-3-OH), it possessed very good activity against *A. niger* and good activity against *A. clavatus*. Introduction of methoxy group in **4m** (-4-OCH_3) demonstrated very good potency against *A. niger*.

Cytotoxicity studies

In vitro cytotoxicity of **4a-o** was tested against human cervical cancer cell line (HeLa) by the MTT colorimetric assay[31,32] and Table II displayed IC_{50} values.

Cytotoxicity results exposed that the derivatives **4d, 4g, 4j, 4k, 4m** and **4o** having no toxicity at concentration level 100 µM (IC_{50} > 100 µM), while other compounds possessed moderate activity. It was confirmed that none of these tested compounds demonstrated any significant cytotoxic properties on HeLa cell lines. Above results indicated that none of



Scheme II — Plausible mechanistic pathway for the synthesis of compounds **4a-o**

Table I — Antimicrobial screening results of compounds **4a-o**

Compd	-R	Minimum inhibitory concentration (MIC) for bacteria (µg/mL)				Minimum inhibitory concentration (MIC) for fungi (µg/mL)		
		E.c.	*P.a.*	*S.a.*	*S.p.*	*C.a.*	*A.n.*	*A.c.*
4a	-H	250	250	150	150	200	500	500
4b	-2-OH	500	500	500	500	250	250	200
4c	-3-OH	500	500	250	500	500	50	100
4d	-4-OH	200	200	250	250	250	12.5	25
4e	-2-NO_2	500	250	500	250	500	>1000	>1000
4f	-3-NO_2	250	100	250	100	1000	1000	1000
4g	-4-NO_2	100	25	12.5	50	1000	500	500
4h	-2-Cl	500	500	200	250	500	1000	1000
4i	-3-Cl	200	250	250	200	250	250	250
4j	-4-Cl	12.5	100	50	25	500	500	1000
4k	-4-F	100	100	50	12.5	250	>1000	>1000
4l	-3-OCH_3	250	250	250	250	250	250	250
4m	-4-OCH_3	250	200	500	500	100	50	25
4n	-3,4,5-$(OCH_3)_3$	500	500	250	500	200	500	500
4o	-4-Br	100	25	25	50	1000	>1000	>1000
Chloramphenicol		50	50	50	50	–	–	–
Nystatin		–	–	–	–	100	100	100

Escherichia coli (*E.c.*) MTCC-442; *Pseudomonas aeruginosa* (*P.a.*) MTCC-441; *Staphylococcus aureus* (*S.a.*) MTCC-96; *Streptococcus pyogenes* (*S.p.*) MTCC-443; *Candida albicans* (*C.a.*) MTCC-227; *Aspergillus niger* (*A.n.*) MTCC-282; *Aspergillus clavatus* (*A.c.*) MTCC-1323.

the compounds displayed cytotoxicity against 3T3 cell lines (IC50 > 100 µM).

Molecular docking

Compounds **4b** and **4e** were used for docking poses representatives (Table III) as active molecules in transferase (1HNJ) of organism *E. coli* (strain K12). The beta-Ketoacyl-acyl carrier protein synthase III (FabH) is a condensing enzyme that plays central roles in fatty acid biosynthesis. This protein was downloaded from protein data bank (PDB). Binding of the ligands is shown in Figure 2, where, carbonyl

Table II — Levels of cytotoxicity prompted by selected compounds on HeLa cells

Compd	Cytotoxicity (IC_{50} μM) [a] HeLa [b]
4c	>100
4d	97.56
4g	>100
4j	>100
4k	98.16
4m	96.88
4o	>100
Doxorubicin	3.24

[a] IC_{50} is the concentration required to inhibit 50% of cell growth
[b] HeLa human cervical cancer cell line

Table III — Docking score

Compd	Transferase (g Score)
4b	−0.946
4e	−0.831
4d	−0.551
4g	−0.475
4c	−0.073
4l	0.509
4f	1.391
Chloroamphinicol	−2.538

Organism: *E. coli*

>C=O has hydrogen bonding with TYR 639 and ARG 630, and TYR 639 have pi-pi interaction with coumarin ring.

Experimental Section

Preparation of 3-acetyl-2*H*-chromen-2-one, 1 [33] and (*E*)2-(4-chlorobenzylidene)malononitrile [34] as per the literature method

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** (0.01mol) and 2-cyanoacetohydrazide (0.01 mol) in methanol (30 mL) was refluxed for 14 h and then cooled down to RT. The separated crystals were filtered, air dried and recrystallized from ethanol (95%). Yield: 72%, m.p. 159°C. Anal. Calcd for $C_{15}H_{13}N_3O_3$: C, 63.60; H, 4.63; N, 14.83. Found: C, 63.63; H, 4.56; N, 14.76%.

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

A mixture containing 2-cyano-*N*'-(2-(2-oxo-2*H*-chromen-yl)propylidene) acetohydrazide **2** (0.01 mol), (*E*)-2-(4-chlorobenzylidene)malononitrile (0.01

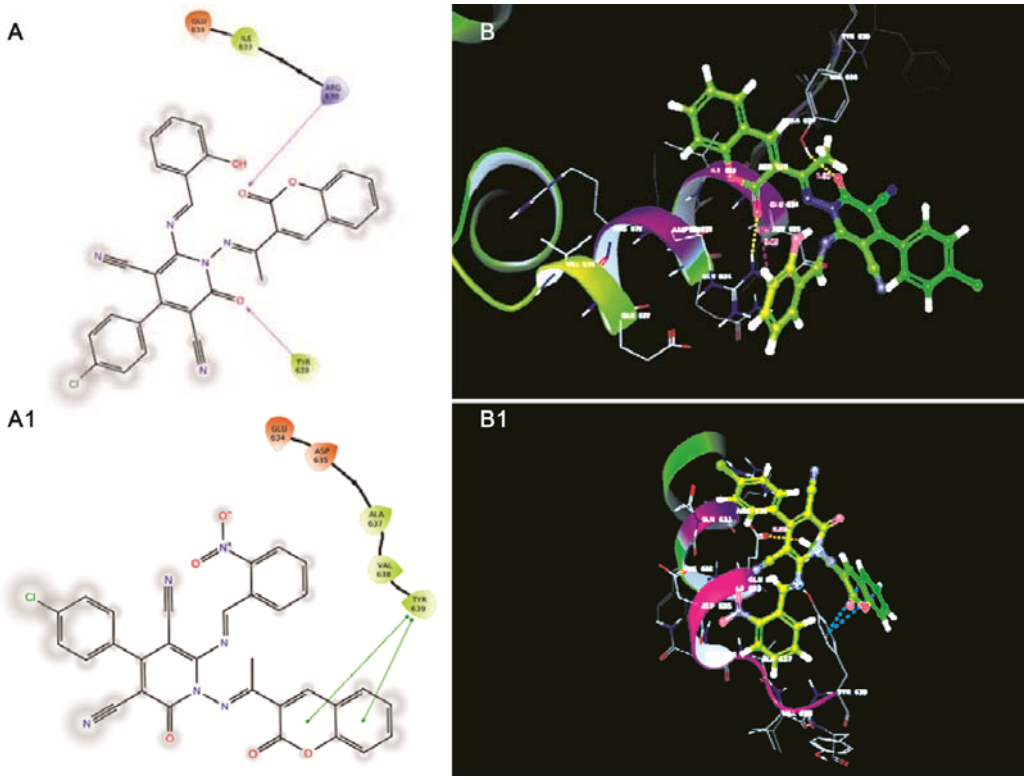


Figure 2 — Molecular docking poses of **4b** A (2D), B (3D). **4e** A1 (2D), B1 (3D).

mol) and 2 drops of piperidine in ethanol (95%) (30 mL) was refluxed for 22 h. The mixture was then cooled down to RT and diluted with a few drops of water. The crystals formed were filtered, air dried and recrystallized from ethanol. Yield: 68%, m.p. 190°C. Anal. Calcd for $C_{24}H_{14}ClN_5O_3$: C, 63.24; H, 3.10; N, 15.36. Found: C, 63.27; H, 3.04; N, 15.43%.

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

Compound **3** (0.01mol), 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 (95%). Yield: 73%. m.p. 157°C. Anal. Calcd for $C_{31}H_{18}ClN_5O_3$: C, 68.45; H, 3.34; N, 12.87. Found: C, 68.39; H, 3.39; N, 12.81%.

6-((Benzylidene)amino)-4-(4-chlorophenyl)-2-oxo-1-((1-(2-oxo-2*H*-chromen-3-yl)ethyli dene) amino)-1,2-dihydropyridine-3,5-dicarbonitrile, 4a: Yield: 73%. m.p. 157-161°C. IR (KBr): 2927 (C-H, CH_3), 2209 (-C≡N stretching, nitrile group), 1731 (>C=O stretching, coumarin ring), 1564, 1597 (>C=N-, >C=C< stretching, aromatic ring), 1588, 1407 (C-H bending, -CH=N linkage), 1254 (C-O-C coumarin ring), 729 (C-Cl stretching) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 9.18 (s, 1H, Ar-CH=N-), 8.91 (s, 1H, C_4 proton of coumarin), 7.25-7.89 (m, 13H, Ar-H of coumarin ring and phenyl ring), 1.38 (s, 3H, -C(CH_3)=N-); ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.4, 114.5, 115.5, 115.9(2), 116.3, 118.2, 123.1, 125.1, 127.7, 128.5, 128.8 (2), 128.9 (2), 129.4 (2), 130.3 (2), 130.8, 131.2, 133.2 (2), 133.5, 153.1, 153.4, 153.6, 155.4, 159.6, 160.0, 163.8, 169.6; LCMS: *m/z* 543.11 (M^+). Anal. Calcd for $C_{31}H_{18}ClN_5O_3$: C, 68.45; H, 3.34; N, 12.87. Found: C, 68.39; H, 3.39; N, 12.81%.

Conclusion

We have concluded on the basis of the biological activity results that the titled compounds **4a-o** depend on the different types of substituent pattern on phenyl ring. Many of the synthesized motifs (**4g**, **4j**, **4k** and **4o**) having electron withdrawing atoms /groups such as halogen and nitro at *para* and *meta* positions showed potent antibacterial results while compounds **4d** and **4m** having electron donating groups at *para* position showed potent antifungal results. The *para* position was more favorable as per the antimicrobial activity data of the compounds. Compounds (**4d**, **4g**, **4j**, **4k**, **4m** and **4o**) revealed notable cytotoxic action. This new class of novel coumarin linked 2-pyridone scaffolds can be optimized to get lead for further generation of antimicrobial agents. Therefore, the *in silico* studies and the biological activity can be specifically related with each other and optimization of potent molecules recognized in the present study can be taken out for further progress.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

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References

1 Kennedy R O & Thornes R D, *Coumarins*: *Biology*, *Applications*, *and Mode of Action* (Wiley and Sons, Chichester) (1997).
2 Phosrithong N, Samee W, Nunthanavanit P & Ungwitayatorn J, *Chem Biol Drug Des*, 79 (2012) 981.
3 Wu J, Wang X, Yi Y & Lee K, *Bioorg Med Chem Lett*, 13 (2003) 1813.
4 Seijas J, Tato M & Carballido-Reboredo R, *J Org Chem*, 70 (2005) 2855.
5 Shakil N A, Kumar J, Pandey R K & Saxena D B, *Phytochemistry*, 69 (2008) 759.
6 Lazarenkow A, Nawrot-Modranka J, Brzezinska E, Krajewska U & Rozalski M, *Med Chem Res*, 21 (2012) 1861.
7 Kawase M, Varu B, Shah A, Motohashi N, Tani S, Saito S, Debnath S, Mahapatra S, Dastidar S G & Chakrabarty A N, *Arzneim Forsch*, 51 (2001) 67.
8 Khan K M, Saify Z S, Khan M Z, Zia-Ullah, Choudhary M I, Atta-ur-Rahman, Perveen S, Chohan Z H & Supuran C T, *Enzyme Inhib Med Chem*, 19 (2004) 373.
9 Vyas K B, Nimavat K S, Jani G R & Hathi M V, *Orbital-The Electron J Chem*, 1 (2009) 183.
10 Bairagi S, Bhosale A & Deodhar M N, *E-J Chem*, 6 (2009) 759.
11 Lopez S E, Rosales M E, Canelon C E, Valverode E A, Narvaez R C, Charris J E, Giannini F A, Enriz R D, Carrasco M & Zacchino S, *Heterocycl Commun*, 7 (2000) 473.
12 Musa M A, Cooperwood J S, Khan M O F & Rahman T, *Arch Pharm* (*Weinheim*), 344 (2011) 102.
13 Musa M A, Badisa V L, Latinwo L M, Waryoba C & Ugochukwu N, *Anticancer Res*, 30 (2010) 4613.

14 Musa M A, Khan M O & Cooperwood J S, *Lett Drug Des Discov*, 6 (2009) 133.
15 Kokotos G, Theodorou V, Tzougraki C, Deforce D & Van den Eeckhout E, *Bioorg Med Chem Lett*, 7 (1997) 2165.
16 Dekic S V, Dekic V S, Dekic B R & Dekic M S, *Chemical Papers*, 61 (2007) 233.
17 Saiki A Y C, Shen L L, Chen C M, Baranowski J & Lerner C G, *Antimicrob Agents Chemother*, 43 (1999) 1574.
18 Ozturk G, Erol D D, Uzbay T & Aytemir M D, *Il Farmaco*, 56 (2001) 251.
19 Storck P, Aubertin A & Grierson D S, *Tetrahedron Lett*, 46 (2005) 2919.
20 Cocco M T, Congiu C & Onnis V, *Eur J Med Chem*, 38 (2003) 37.
21 Chen Y, Zhang H & Nan F, *J Comb Chem*, 6 (2004) 684.
22 Wang S, Tan T, Li J & Hu H, *Synlett*, 17 (2005) 2658.
23 Soto J L, Seoane C, Zamorano P, Rubio M J, Monforte A & Quinteiro M, *J Chem Soc Perkin Trans 1*, (1985) 1681.
24 Desai N C, Shihori N R, Kotadiya G M & Desai P, *Eur J Med Chem*, 82 (2014) 480.
25 Desai N C, Shihori N R & Kotadiya G M, *Chin Chem Lett*, 25 (2014) 305.
26 Desai N C, Dodiya A M & Shihori N R, *Med Chem Res*, 21 (2012) 2579.
27 Desai N C, Shihori N R, Bhatt M, Patel B & Karkar T, *Synth Commun*, 45 (2015) 2701.
28 Desai N C & Karkar T, *Int Lett Chem Phys Astron*, 66 (2016) 1.
29 Desai N C, Kotadiya G M & Trivedi A R, *Bioorg Med Chem Lett*, 24 (2014) 3126.
30 Hannan C T, *Vet Res*, 31 (2000) 373.
31 Teo W Z, Chua C K, Sofer Z & Pumera M, *Chem Eur J*, 21 (2015) 13020.
32 Upadhyay K, Manvar A, Rawal K, Joshi S, Trivedi J, Chaniyara R & Shah A, *Chem Biol Drug Des*, 80 (2012) 1003.
33 Abass S J, Muhammed J H & Salih L, *Journal of Kerbala University*, 13 (2015) 228.
34 Horner L & Kluepfel K, *Liebigs Ann Chem*, 591 (1955) 69.