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ORIGINAL RESEARCH

In vitro antimicrobial and antimycobacterial activity of some chalcones and their derivatives

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Abstract In this study, novel series of chalcone derivatives, namely, 4-[4-(3-phenyl-acryloyl)-phenylamino]-chromen-2-one (**5a–k**) have been synthesized from the intermediate 4-(4-acetyl-phenylamino)-chromen-2-one (**4**). Cyclization reaction of chalcone (**5a–k**) with hydrazine hydrate, guanidine nitrate, and malononitrile gives the corresponding 4-[4-(1-acetyl-5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)-phenylamino]-chromen-2-one (**6a–k**), 4-[4-(2-amino-6-phenyl-pyrimidin-4-yl)-phenylamino]-chromen-2-one (**7a–k**), and 2-amino-6-[4-(2-oxo-2*H*-chromen-4-ylamino)-phenyl]-4-phenyl-nicotinonitrile (**8a–k**) derivatives were synthesized. The newly synthesized compounds were evaluated for their antimycobacterial activity and antimicrobial activity against eight bacteria (*S. aureus*, *B. cereus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. typhi*, *P. vulgaris*, and *S. flexneri*) and four fungi (*A. niger*, *C. albicans*, *A. fumigatus*, and *A. clavatus*).

Keywords Coumarin · Chalcone and their derivatives · Antimicrobial activity · Antimycobacterial activity · Structure activity relationship

Introduction

Despite the rapid progress of science, the treatment of infectious diseases still remains a serious problem of concern to the scientific community, mainly because of the wide range of factors leading to the emergence of these diseases, as well as the increased number of pathogenic microorganisms with resistance to multiple drugs (Tenover *et al.*, 2005). To limit the emerging resistance of microorganisms, careful use of the existing antimicrobial drugs is required. However, there is a need for the design of novel antimicrobial agents, particularly for the treatment of the infections of the hospitalized patients and the protection of immunosuppressed or HIV-infected patients. Tuberculosis (TB) being one of the most common infectious diseases, mainly caused by *Mycobacterium tuberculosis*, is a public health threat in both the developing and the developed nations. Effective treatment of TB has been hampered by the emergence of drug-resistant strains of *M. tuberculosis*, calling for the search of new drugs (Laughon, 2007).

Chalcones and their derivatives are an attractive molecular scaffold for the search of new biologically active molecules. Chalcones or 1,3-diaryl-2-propen-1-ones are natural or synthetic compounds belonging to the flavonoids family (Nowakowska, 2007; Zhang *et al.*, 2006). Chalcone derivatives are very versatile as physiologically active compounds and substrates for the evaluation of various organic syntheses. Chalcones have been reported to possess many useful properties, including anti-inflammatory (Lee *et al.*, 2006), antimicrobial (Tomar *et al.*, 2007), antifungal (López *et al.*, 2001), antioxidant (Anto *et al.*, 1995), antileishmanial, antimalarial (Liu *et al.*, 2003), antitumor (Yi *et al.*, 2000), and anticancer (Hsu *et al.*, 2006) activities.

As part of our continuation study on coumarin-containing derivatives (Divyesh *et al.*, 2011), we have reported in this article coumarin-based chalcones and their derivatives. The coumarin moiety is distributed widely in nature. A number of natural products and synthetic analogues featuring coumarin structural motif display wide-ranging

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biological properties, and they are used as antibacterial (Khalid *et al.*, 2004), antifungal (Nida *et al.*, 2011), anticancer (Belluti *et al.*, 2010), antiHIV (Bedoya *et al.*, 2005), antioxidant (Kostova *et al.*, 2011), anti-inflammatory (Koneni *et al.*, 2011), antibiotics (Hussain *et al.*, 2003), anticoagulant (Manolov and Danchev, 1995), and antitumor (Al-Soud *et al.*, 2006) agents. Among the wide variety of heterocycles that have been explored for developing pharmaceutically important molecules, pyrazoles (Bhat *et al.*, 2005; Babasaheb *et al.*, 2009), pyrimidines (Kishor *et al.*, 2008; Amit *et al.*, 2008), and cyanopyridines (Anjani *et al.*, 2010; Nitin *et al.*, 2011) play an important role in the medicinal chemistry. Therefore, in light of above facts, we have reported the reaction of 4-(4-acetyl-phenylamino)-chromen-2-one with different aldehydes to form chalcones and their subsequent conversion to pyrazol, pyrimidine, and cyanopyridine components. All the final synthesized compounds were tested for their antimicrobial and antimycobacterial activities.

Results and discussion

Chemistry

The synthetic strategy adopted to obtain the target compounds is depicted in Scheme 1. 4-Hydroxy-chromen-2-one (**1**) on reaction with $POCl_3$ (**2**) yielded 4-chloro-chromen-2-one (**3**) and 4-chloro-3,4′,3′,4″-tercoumarin (**3a**). Further on reaction of compound **3** with 1-(4-amino-phenyl)-ethanone yielded 4-(4-acetyl-phenylamino)-chromen-2-one (**4**). Compound **4** when treated with aromatic aldehydes (**a**–**k**) yielded the corresponding chalcone derivatives **5a**–**k**. Cyclization of chalcone derivatives **5a**–**k** with hydrazine hydrate, guanidine nitrate, and malononitrile led to the formation of corresponding pyrazol (**6a**–**k**), pyrimidine (**7a**–**k**), and cyanopyridine (**8a**–**k**) derivatives.

The structures of the synthesized compounds were confirmed by spectral data and elemental analysis, and they were in full agreement with the proposed structures. In ^{1}H NMR spectra of compound **4**, singlet signal appeared at 8.82 for one proton of –NH and at 2.21 ppm because of –C*H*$_3$ of $COCH_3$. Protons corresponding to the coumarin moiety resonated at 6.11–7.75 ppm. In IR spectra, the bands at 1,671 cm^{-1} (C=O of coumarin) and 3,285 cm^{-1} (–NH) also confirmed the formation of compound **4**.

The formation of chalcone **5a** was supported by the appearance of doublet signals at δ 8.12 for Ar–CH and δ 7.05 for –CO–CH in ^{1}H NMR spectrum, and IR spectrum for CH=CH–, str. at 1,589 cm^{-1} also confirmed the formation of compound **5a**. The doublet of doublet signals observed at 3.58 and 3.30 ppm in ^{1}H NMR spectra of compound **6a** was attributed to –C*H*$_2$ of pyrazol ring. Furthermore, in the IR spectra, the band at 1,568 cm^{-1} (C=N) also confirmed the formation of compound **6a**. The presence of singlet signal at 5.31 ppm for two protons of –N*H*$_2$ in ^{1}H NMR spectra and the band at 3,385cm^{-1} for –NH_2 in IR spectrum confirms the formation of pyrimidine compound **7a**. Compound **8a** showed singlet peak at 8.05 ppm for one proton of pyridine ring, and the appearance of C≡N group at 2,217 cm^{-1} in IR spectra confirmed the formation of cyanopyridine ring.

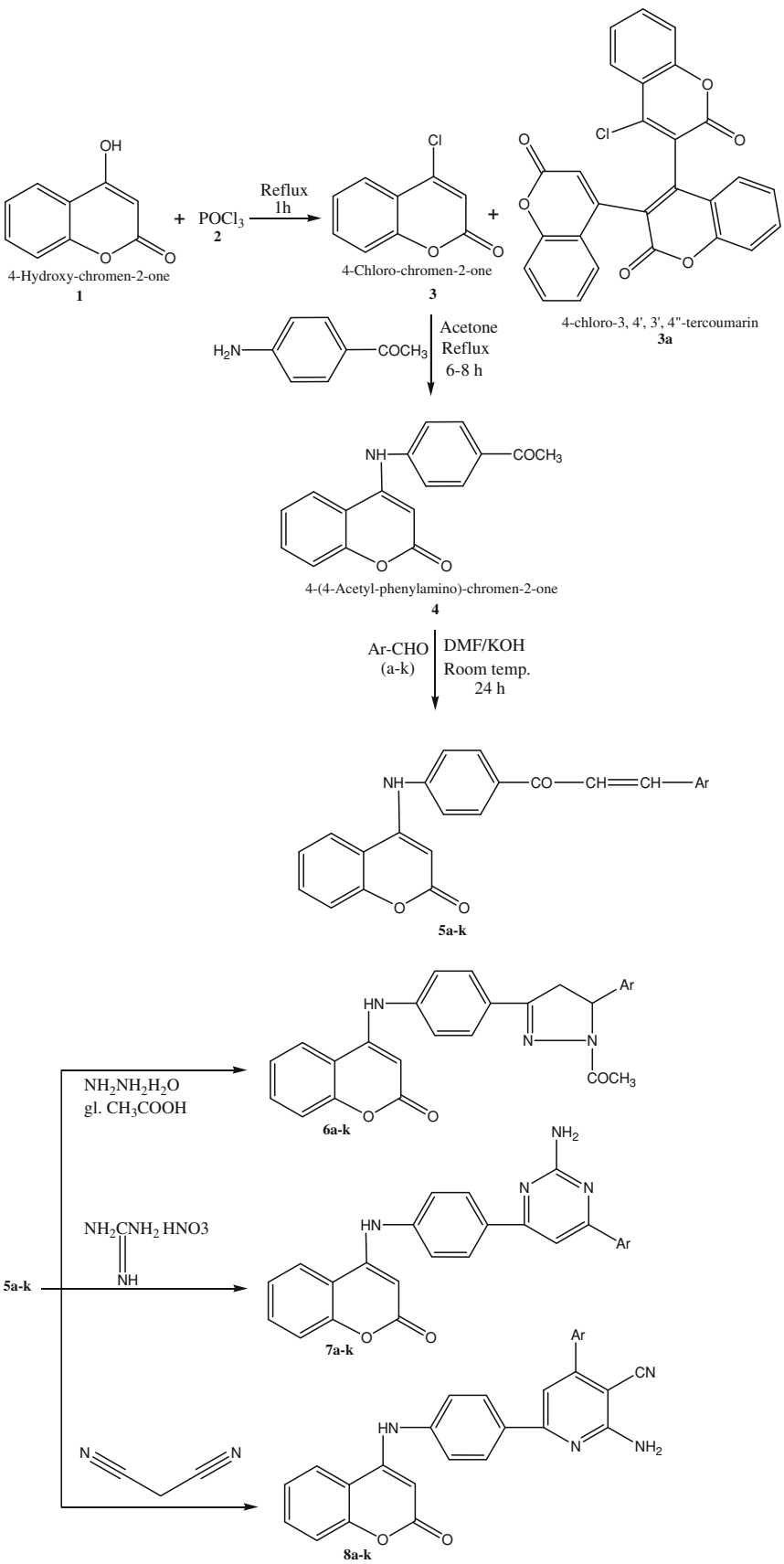
The synthesized compounds **5a**–**k**, **6a**–**k**, **7a**–**k**, and **8a**–**k** were evaluated for in vitro antibacterial and antifungal activities against various Gram-positive, Gram-negative bacteria, and fungal species. The results are shown in Tables 1, 2, and 3. Standard antibacterial ciprofloxacin and antifungal Ketoconazole were also tested for comparison with synthesized compounds **5a**–**k**, **6a**–**k**, **7a**–**k**, and **8a**–**k**.

A close survey of MIC values indicates that the chalcone compounds **5a**–**k** exhibited a varied range (25–100 μg/mL) of antibacterial activity against all the tested bacterial strains except **5a**, which were not having any substituents at *ortho*, *meta*, and *para* positions of the aryl ring and did not show any activity against *S. aureus*, *E. coli*, *P. aeruginosa*, *P. vulgaris*, and *S. flexneri* even at a maximum concentration of 100 μg/mL. Compound **5f** with nitro substituent at *para* position of aryl ring was also found to be inactive against *B. cereus*, *P. aeruginosa*, and *P. vulgaris* at 100 μg/mL. Compound **5b** which was having electron-withdrawing chloro substituents at the *ortho-para* position of aryl ring shows effective inhibitory effect in terms of MIC = 25 μg/mL against *S. aureus*. Compound **5j** which was having electron-donating methoxy substituent at *meta–para* position of aryl ring shows the best activity against *S. aureus*, *E. coli*, and *S. flexneri* among all the chalcone compounds, **5a**–**k**. Compound **5d** with fluoro substituent at the fourth position of aryl ring showed the highest activity (MIC = 25 μg/mL, 19-mm zone of inhibition) against *B. cereus* among compounds, **5a**–**k**. Gram-negative bacteria like *P. aeruginosa*, *K. pneumoniae*, *S. typhi*, and *P. vulgaris* were effectively inhibited by the compound **5k** which contains hydroxy substituent at *ortho–para* position of aryl ring.

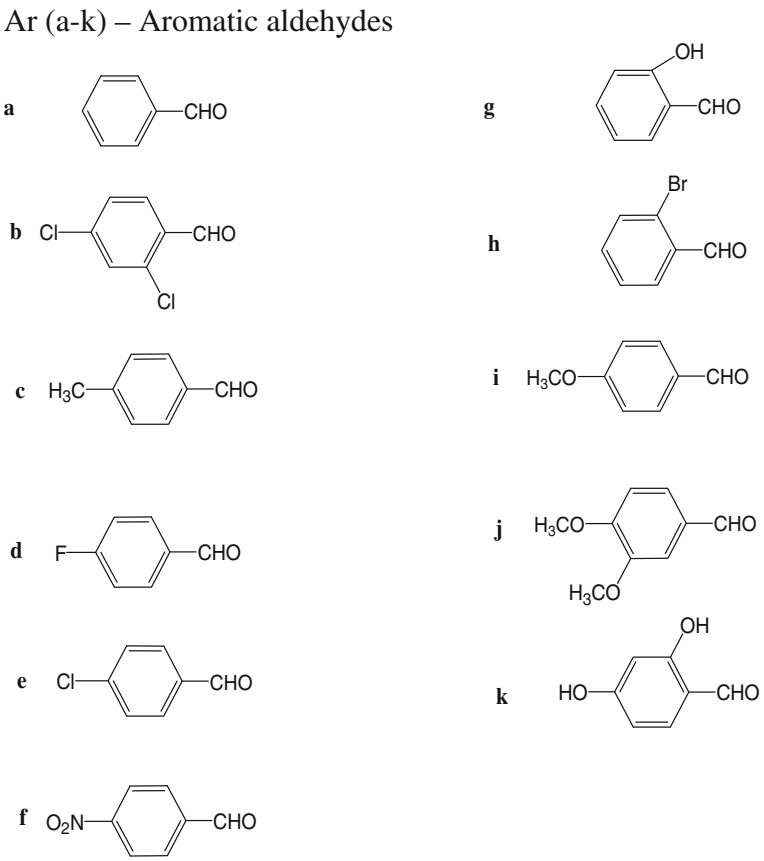
When chalcones **5a**–**k** were converted to pyrazol (**6a**–**k**), pyrimidine (**7a**–**k**), and cyanopyridine (**8a**–**k**) derivatives, then all these derivatives (**6a**–**8k**) showed increased antimicrobial activity.

Among all the synthesized derivatives, cyanopyridine derivatives (**8a**–**k**) showed very good antibacterial activity compared with chalcones, pyrazol, and pyrimidine derivatives. Some of the pyrazol compounds **6b**, **6d**, and **6j** displayed the MIC values in the range of 12.5–50 μg/mL against all the bacterial species. Pyrazol compounds **6b** and **6d** showed similar MIC = 12.5 μg/mL against *S. aureus* and *B. cereus*, respectively, which is the best MIC value

Scheme 1 Synthesis of chalcone and their derivatives



Scheme 1 continued



among compounds **6a**–**k**. Only one compound **6k** showed the significant MIC values in the range of 6.25–25 μg/mL against all Gram-negative bacterial strains. Compound **6k** was also found to be the most active against *K. pneumoniae* with excellent MIC = 6.25 μg/mL and maximum inhibitory zone 23 mm among **6a**–**k**.

Pyrimidine derivatives (**7a**–**k**) displayed better activity compared with chalcones and pyrazol derivatives. Pyrimidine compounds **7b**, **7d**, **7i**, and **7k** inhibit both the Gram-positive bacterial strain with similar MIC = 12.5 μg/mL. Besides compound **7j** showed similar activity in terms of MIC against *B. cereus*. Compound **7j** also found to be the most potent against *S. aureus* with the best activity (MIC = 6.25 μg/mL, 27 mm inhibitory zone) among all the pyrimidine derivatives (**7a**–**k**). In case of activity against Gram-negative bacterial strains, compounds **7d** and **7k** showed excellent MIC = 3.12 μg/mL against *K. pneumoniae* and *P. vulgaris*, respectively. Compound **7d** along with compound **7j** exerted similar activity (MIC = 6.25 μg/mL, 25 mm zone of inhibition) against *E. coli*.

A close investigation of the MIC values indicates that cyanopyridine compounds **8b**, **8d**, **8g**, **8i**, **8j**, and **8k** exhibited a varied range (3.12–12.5 μg/mL) of antibacterial activity against all the tested bacterial species, except compound **8j** which showed significant MIC values in the range of 3.12–6.25 μg/mL against all the bacterial species. Compound **8j** showed the highest zone of inhibition of 29 mm with excellent MIC = 3.12 μg/mL against *S. aureus* along with compound **8b** having slightly reduced inhibition zone (28 mm). Compound **8j** was also found to be the most active against *S. flexneri* among all the synthesized derivatives. Compound **8d** exhibited the greatest activity (MIC = 3.12 μg/mL, 28-mm inhibitory zone) against *B. cereus*. Same compound was found to be the most potent with similar MIC = 3.12 μg/mL and 29-mm inhibitory zone against *P. aeruginosa* and *P. vulgaris* among all the synthesized compounds. Compounds **8d** and **8i** found equipotent in terms of MIC = 3.12 μg/mL against *E. coli*, but **8i** showed maximum inhibition zone of 28 mm among all the compounds against *E. coli*. Strong inhibitory effect was shown by **8k** with MIC = 3.12 μg/mL (30-mm zone of inhibition) against *K. pneumoniae* along with similar efficacy of compounds **8d**, **8g**, and **8j** in terms of MIC = 3.12 μg/mL against the same bacterial specie. *S. typhi* was strongly inhibited by compound **8k** with excellent MIC = 3.12 μg/mL and 28-mm inhibition zone along with **8j** in terms of MIC = 3.12 μg/mL with a quite reduced inhibition zone of 27 mm.

Table 1 In vitro (Gram-positive) antibacterial activity of newly synthesized compounds

Compound (100 μg/disk)	Zone of inhibition in mm (MIC in μg/mL)	
	Gram-positive	
	S. aureus	*B. cereus*
5a	–	<10 (100)
5b	20 (25)	16 (50)
5c	14 (100)	15 (100)
5d	18 (50)	19 (25)
5e	18 (50)	14 (100)
5f	<10 (100)	–
5g	15 (100)	13 (100)
5h	13 (100)	11 (100)
5i	15(100)	13 (100)
5j	21 (25)	19 (50)
5k	20 (50)	16 (100)
6a	<10 (100)	13 (100)
6b	22 (12.5)	21 (12.5)
6c	16 (100)	16 (100)
6d	23 (12.5)	22 (12.5)
6e	19 (50)	17 (100)
6f	12 (100)	11 (100)
6g	17 (100)	16 (100)
6h	16 (100)	14 (100)
6i	16 (100)	15 (100)
6j	20 (25)	18 (50)
6k	19 (50)	20 (50)
7a	13 (100)	15 (100)
7b	24 (12.5)	22 (12.5)
7c	18 (50)	19 (50)
7d	24 (12.5)	23 (12.5)
7e	20 (50)	19 (50)
7f	15 (100)	16 (100)
7g	18 (50)	19 (50)
7h	17 (100)	16 (100)
7i	22 (12.5)	21 (12.5)
7j	27 (6.25)	20 (12.5)
7k	21 (12.5)	21 (12.5)
8a	17 (50)	19 (50)
8b	28 (3.12)	26 (6.25)
8c	21 (25)	23 (25)
8d	25 (6.25)	28 (3.12)
8e	24 (12.5)	21 (25)
8f	17 (50)	22 (25)
8g	21 (12.5)	22 (12.5)
8h	23 (25)	20 (25)
8i	27 (6.25)	27 (6.25)
8j	29 (3.12)	26 (6.25)
8k	22 (12.5)	26 (6.25)
Ciprofloxacin (100 μg/disk)	30 (1.0)	31 (1.0)
DMSO	–	–

Each value is the mean of three independent experiments

– Indicates compound is not active at higher concentration of 100 μg/mL

The in vitro antifungal activities of compounds **5a–k**, **6a–k**, **7a–k**, and **8a–k** were studied against the fungal strains viz., *A. niger*, *A. fumigatus*, *A. clavatus*, and *C. albicans*. Ketoconazole was used as a standard drug. The data of antifungal tests are depicted in Table 3. From the results, we found that chalcone compounds **5a–k** showed less antifungal activity compared with pyrazol (**6a–k**), pyrimidine (**7a–k**), and cyanopyridine (**8a–k**) derivatives. Most of the chalcone compounds **5a–k** displayed MIC in the range of 50–100 μg/mL, except compound **5j** which showed MIC at 25 μg/mL against *A. niger* and **5k** with same MIC against *A. fumigatus* and *A. clavatus*. In case of pyrazol derivatives, only one compound **6k** displayed good MIC at 12.5 μg/mL against *A. fumigatus*, while all other pyrazol compounds showed activity in terms of MIC in the range of 25–100 μg/mL. Pyrimidine ring-containing compound **7d** inhibits *A. clavatus* with MIC = 6.25 μg/mL, 26-mm inhibitory zone along with **7g** and **7k** in terms of MIC with quite reduced inhibition zone (25 mm). Compound **7j** found to be the most active against *A. niger* and *C. albicans* among other pyrimidine derivatives, while the best activity was observed against *A. fumigatus* by **7d** with MIC = 6.25 μg/mL and 27-mm inhibitory zone. Cyanopyridine ring-containing compound **8c** showed the excellent activity (MIC = 3.12 μg/mL, 28 mm inhibition zone) against *C. albicans*. Fungal specie *A. fumigatus* was strongly inhibited by compounds **8d** and **8j** with similar activity (MIC = 3.12 μg/mL, 28 mm-inhibitory zone). Compound **8d** was also found to be the most potent against *A. clavatus* with MIC = 6.25 μg/mL and 28-mm inhibitory zone. Besides, the two compounds **8g** and **8k** showed similar MIC with quite reduced inhibitory zone of 27 mm. Among all the cyanopyridine (**8a–k**) compounds, only compound **8j** inhibited *A. niger* at 6.25 μg/mL, while others showed MIC in the range of 12.5–25 μg/mL against same fungi.

In vitro antimycobacterial activity of all compounds was assessed against *M. tuberculosis* H37 RV. The results observed from Lowenstein Jensen MIC method along with the measurement of the potency of the standard drugs are

Table 2 In vitro (Gram-negative) antibacterial activity of newly synthesized compounds

Compound (100 μg/disk)	Zone of inhibition in mm (MIC in μg/mL)					
	Gram-negative					
	E. coli	*P. aeruginosa*	*K. pneumoniae*	*S. typhi*	*P. vulgaris*	*S. flexneri*
5a	–	–	<10 (100)	11 (100)	–	–
5b	14 (100)	<10 (100)	16 (100)	17 (50)	19 (50)	13 (100)
5c	<10 (100)	15 (100)	10 (100)	13 (100)	19 (50)	18 (50)
5d	18 (50)	16 (100)	17 (100)	17 (100)	18 (50)	14 (100)
5e	12 (100)	10 (100)	20 (50)	15 (100)	17 (100)	18 (50)
5f	<10 (100)	–	11 (100)	14 (100)	–	13 (100)
5g	14 (100)	15 (100)	19 (50)	13 (100)	15 (100)	13 (100)
5h	11 (100)	<10 (100)	14 (100)	11 (100)	<10 (100)	12 (100)
5i	18 (50)	16 (100)	16 (100)	14 (100)	18 (50)	15 (100)
5j	22 (25)	18 (50)	17 (100)	16 (100)	19 (50)	20 (25)
5k	19 (50)	19 (25)	21 (25)	21 (25)	20 (25)	17 (100)
6a	<10 (100)	10 (100)	11 (100)	13 (100)	13 (100)	<10 (100)
6b	18 (50)	20 (25)	19 (25)	17 (50)	22 (12.5)	17 (50)
6c	12 (100)	16 (100)	13 (100)	14 (100)	20 (50)	20 (50)
6d	20 (50)	17 (50)	23 (12.5)	18 (50)	21 (25)	20 (25)
6e	15 (100)	14 (100)	21 (50)	18 (50)	19 (50)	20 (50)
6f	14 (100)	11 (100)	13 (100)	17 (100)	<10 (100)	13 (100)
6g	16 (100)	16 (100)	20 (50)	16 (100)	17 (100)	18 (50)
6h	14 (100)	12 (100)	16 (100)	15 (100)	15 (100)	14 (100)
6i	19 (50)	18 (50)	17 (100)	17 (100)	20 (50)	16 (100)
6j	21 (25)	20 (50)	22 (25)	21 (25)	20 (50)	23 (12.5)
6k	20 (25)	22 (25)	23 (6.25)	21 (25)	22 (12.5)	20 (25)
7a	14 (100)	12 (100)	15 (100)	17 (50)	15 (100)	14 (100)
7b	19 (50)	22 (25)	21 (25)	20 (25)	25 (6.25)	21 (25)
7c	15 (100)	19 (50)	16 (100)	18 (50)	21 (25)	22 (25)
7d	25 (6.25)	24 (12.5)	28 (3.12)	23 (12.5)	25 (6.25)	21 (12.5)
7e	18 (50)	16 (100)	22 (12.5)	20 (25)	23 (12.5)	21 (25)
7f	17 (100)	16 (100)	18 (50)	21 (25)	14 (100)	16 (100)
7g	19 (50)	18 (50)	21 (12.5)	17 (100)	19 (50)	20 (25)
7h	16 (100)	15 (100)	18 (50)	20 (50)	17 (100)	19 (50)
7i	22 (25)	21 (12.5)	20 (25)	19 (50)	24 (12.5)	19 (50)
7j	25 (6.25)	23 (12.5)	23 (12.5)	22 (12.5)	21 (25)	24 (12.5)
7k	22 (12.5)	23 (12.5)	26 (6.25)	24 (12.5)	28 (3.12)	23 (12.5)
8a	19 (50)	18 (50)	21 (25)	23 (12.5)	19 (25)	17 (50)
8b	20 (12.5)	25 (6.25)	23 (12.5)	24 (12.5)	28 (3.12)	23 (12.5)
8c	20 (25)	22 (12.5)	18 (50)	21 (25)	24 (12.5)	25 (12.5)
8d	27 (3.12)	29 (3.12)	28 (3.12)	26 (6.25)	29 (3.12)	24 (12.5)
8e	21 (25)	19 (50)	25 (6.25)	24 (12.5)	26 (6.25)	23 (12.5)
8f	22 (25)	20 (50)	19 (25)	23 (12.5)	19 (50)	20 (50)
8g	24 (12.5)	24 (12.5)	28 (3.12)	25 (6.25)	22 (12.5)	21 (12.5)
8h	18 (50)	19 (50)	23 (12.5)	22 (25)	20 (25)	22 (12.5)
8i	28 (3.12)	25 (6.25)	22 (12.5)	22 (12.5)	26 (6.25)	24 (12.5)
8j	26 (6.25)	25 (6.25)	28 (3.12)	27 (3.12)	25 (6.25)	27 (6.25)
8k	24 (12.5)	27 (6.25)	30 (3.12)	28 (3.12)	23 (12.5)	26 (6.25)

Table 2 continued

Compound (100 μg/disk)	Zone of inhibition in mm (MIC in μg/mL)					
	Gram-negative					
	E. coli	*P. aeruginosa*	*K. pneumoniae*	*S. typhi*	*P. vulgaris*	*S. flexneri*
Ciprofloxacin (100 μg/disk)	32 (1.0)	33 (1.0)	33 (1.0)	30 (1.0)	31 (1.0)	32 (≤3)
DMSO	–	–	–	–	–	–

Each value is the mean of three independent experiments

– Indicates compound is not active at higher concentration of 100 μg/mL

shown in Table 4. The results revealed that compounds **7d**, **7i**, **7j**, **8b**, **8d**, **8g**, **8i**, and **8j** showed MIC <100 μg/mL. Compound **8j** which was having methoxy substituents on *meta–para* position of aryl ring exerted the best MIC = 12.5 μg/mL with 99 % inhibition among all the synthesized compounds. All the remaining derivatives were found to exert higher MIC ranging from 100 to >1,000 μg/mL.

Conclusion

In conclusion, the objectives of the present study was to synthesize and investigate the antimicrobial and antimycobacterial activities of some new chalcones and their derivatives (pyrazol, pyrimidine, and cyanopyridine) with the hope of discovering new structure leads serving as potent antimicrobial and antimycobacterial agents. From the results of antimicrobial and antimycobacterial activities, we found that cyanopyridine derivatives (**8a–k**) showed better activities compared with chalcone, pyrazol, and pyrimidine derivatives. We can also conclude that biological activities may be associated with the nature of the tested bacterial, fungal, *M. tuberculosis* strain, and it also depends on the substituent on aryl ring at chalcone, pyrazol, pyrimidine, and cyanopyridine moieties.

Experimental section

Chemicals and solvents were obtained from commercial sources and used as received throughout the investigation. Melting points were determined in open capillaries on a Veego electronic apparatus VMP-D (Veego Instrument Corporation, Mumbai, India) and are uncorrected. IR spectra (4,000–400 cm^{-1}) of the synthesized compounds were recorded on a Shimadzu 8400-S FT-IR spectrophotometer (Shimadzu India Pvt. Ltd., Mumbai, India) using KBr pellets. Thin layer chromatography was performed on object glass slides (2 × 7.5 cm) coated with silica gel-G, and spots were visualized under UV irradiation. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 400 MHz model spectrometer (Varian India Pvt. Ltd., Mumbai, India) using DMSO as a solvent and TMS as internal standard with ^{1}H and ^{13}C resonant frequencies of 400 MHz and 100 MHz, respectively. The ^{1}H NMR and ^{13}C NMR chemical shifts were reported as parts per million (ppm) downfield from TMS (Me_4Si) and were performed at the Centre for Excellence, Vapi, India. The splitting patterns are designated as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; and m, multiplet. The mass spectra were recorded on JEOL SX-102 (EI) model. All new compounds were subjected to elemental analysis using a Heraeus Carlo Erba 1180 CHN analyzer (Hanau, Germany).

Synthesis of 4-chloro-chromen-2-one (**3**)

4-Hydroxycoumarin 1 (30 g, 0.185 mol) and 60 mL $POCl_3$ were refluxed for 1 h, cooled, and slowly poured into crushed ice (700 g) with vigorous stirring. The solid was collected by filtration and washed successively with ice water. Azeotropic distillation with *n*-hexane, hot filtration of the by-product (15 g, 17 %), followed by evaporation of solvent and crystallization yielded 4-chloro coumarin (21.9 g, 65 %) with m.p. in the range of 87–89 °C (Kovác *et al.*, 2001).

*4-Chloro-3,4′,3′,4″-tercoumarin (by-product) (**3a**)*

Crystallization from acetic acid gave yellowish crystals, m.p. in the range of 322–327 °C (Kovác *et al.*, 2001).

Synthesis of 4-(4-acetyl-phenylamino)-chromen-2-one (**4**)

4-Chloro-chromen-2-one (10.0 g, 0.055 mol) and 1-(4-amino-phenyl)-ethanone (7.48 g, 0.055 mol) were dissolved in acetone (250 mL). The reaction mixture was refluxed for 6–8 h. Sodium carbonate was added to

Table 3 In vitro antifungal activity of newly synthesized compounds

Compound (100 μg/disk)	Zone of inhibition in mm (MIC in μg/mL)			
	A. niger	*A. fumigatus*	*A. clavatus*	*C. albicans*
5a	<10 (100)	12 (100)	15 (100)	<10 (100)
5b	15 (100)	17 (50)	14 (100)	12 (100)
5c	14 (100)	13 (100)	<10 (100)	11 (100)
5d	17 (50)	19 (50)	15 (100)	13 (100)
5e	12 (100)	14 (100)	14 (100)	15 (100)
5f	11 (100)	<10 (100)	12 (100)	10 (100)
5g	16 (100)	17 (50)	11 (100)	13 (100)
5h	12 (100)	15 (100)	12 (100)	18 (50)
5i	20 (50)	16 (100)	18 (50)	16 (100)
5j	21 (25)	17 (50)	16 (100)	17 (100)
5k	19 (50)	22 (25)	20 (25)	16 (100)
6a	<10 (100)	13 (100)	16 (100)	12 (100)
6b	17 (100)	19 (50)	16 (100)	15 (100)
6c	19 (50)	15 (100)	11 (100)	14 (100)
6d	20 (25)	22 (25)	20 (50)	16 (50)
6e	16 (100)	17 (50)	15 (100)	19 (50)
6f	14 (100)	13 (100)	17 (50)	15 (100)
6g	19 (50)	18 (25)	20 (50)	16 (100)
6h	15 (100)	17 (50)	14 (100)	21 (25)
6i	21 (25)	18 (50)	20 (25)	19 (50)
6j	23 (25)	19 (50)	20 (50)	22 (25)
6k	21 (25)	24 (12.5)	22 (25)	17 (50)
7a	17 (50)	19 (50)	21 (25)	17 (50)
7b	20 (25)	22 (12.5)	19 (50)	21 (25)
7c	21 (25)	18 (50)	16 (100)	16 (50)
7d	22 (12.5)	27 (6.25)	26 (6.25)	20 (25)
7e	17 (50)	19 (25)	17 (50)	21 (12.5)
7f	17 (50)	16 (100)	20 (25)	19 (50)
7g	21 (25)	23 (12.5)	25 (6.25)	18 (50)
7h	20 (50)	19 (50)	17 (50)	23 (25)
7i	23 (12.5)	20 (25)	23 (12.5)	24 (25)
7j	27 (6.25)	25 (12.5)	23 (12.5)	26 (6.25)
7k	22 (12.5)	25 (12.5)	25 (6.25)	21 (25)
8a	21 (25)	21 (25)	23 (12.5)	19 (25)
8b	22 (12.5)	23 (12.5)	22 (25)	23 (12.5)
8c	24 (12.5)	21 (25)	20 (25)	28 (3.12)
8d	23 (12.5)	28 (3.12)	28 (6.25)	22 (12.5)
8e	20 (25)	24 (12.5)	21 (25)	26 (6.25)
8f	23 (25)	20 (25)	22 (25)	23 (12.5)
8g	25 (12.5)	25 (6.25)	27 (6.25)	24 (25)
8h	22 (25)	22 (12.5)	19 (25)	24 (25)
8i	25 (12.5)	21 (12.5)	25 (6.25)	25 (12.5)
8j	27 (6.25)	28 (3.12)	25 (6.25)	27 (6.25)
8k	22 (12.5)	25 (6.25)	27 (6.25)	23 (12.5)
Ketoconazole (100 μg/disk)	30 (≤3)	29 (1.0)	31 (1.0)	33 (1.0)
DMSO	–	–	–	–

Each value is the mean of three independent experiments

– Indicates compound is not active at higher concentration of 100 μg/mL

Table 4 In vitro antimycobacterial activity of newly synthesized compounds

Compound	MIC in μg/mL	% Inhibition
5a	1,000	95
5b	1,000	95
5c	500	96
5d	250	97
5e	1,000	95
5f	1,000	95
5g	500	97
5h	500	97
5i	250	96
5j	200	97
5k	500	95
6a	1,000	95
6b	500	96
6c	200	98
6d	100	98
6e	250	97
6f	500	96
6g	250	98
6h	200	98
6i	100	99
6j	100	98
6k	250	97
7a	500	95
7b	200	98
7c	200	98
7d	62.5	99
7e	200	97
7f	500	96
7g	250	97
7h	100	98
7i	62.5	98
7j	50	98
7k	200	97
8a	500	96
8b	25	99
8c	100	98
8d	50	97
8e	100	98
8f	250	96
8g	25	97
8h	100	98
8i	62.5	96
8j	12.5	99
8k	100	97
Ethambutol	3.12	99
Pyrazinamide	6.25	99
Rifampicin	0.25	99
Isoniazid	0.20	99

neutralize HCl evolved during the reaction mixture. After completion of the reaction, the mixture was cooled and poured into crushed ice. The solid was separated, filtered, washed with water, dried, and recrystallized from ethanol to give compound **4**.

Yield: 82%. M.p. >300 °C; IR (KBr, cm^{-1}): 3,285 (–NH), 1,671 (C=O of coumarin), 1,633 (C=O of $COCH_3$); ^1H NMR (DMSO-d_6, δ, ppm): 8.82 (s, 1H, –NH), 7.75 (d, $J = 7.2$ Hz, 1H, coumarin), 7.42–7.10 (m, 4H, Ar–H), 6.68–6.49 (m, 3H, coumarin), 6.11 (s, 1H, coumarin), 2.21 (s, 1H, $–CH_3$); ^{13}C NMR (DMSO-d_6, δ, ppm): 193.21 (*C*=O of $COCH_3$), 162.37 (*C*=O of coumarin), 149.57 (*C*–NH at coumarin), 147.55, 143.27, 133.10, 132.77, 129.67, 129.25, 124.92, 124.89, 119.36, 118.76, 118.70, 117.15, 92.14 (13C, Ar–C), 27.35 (–*C*H_3 of $COCH_3$); EMI-MS (*m*/*z*): 280.19 (M^+); Anal. Calcd for $C_{17}H_{13}NO_3$: C, 73.11; H, 4.69; N, 5.02. Found: C, 73.16; H, 4.64; N, 5.08.

Synthesis of 4-[4-(3-phenyl-acryloyl)-phenylamino]-chromen-2-one (**5a**)

Compound **4** (4.0 g, 0.014 mol) was dissolved in DMF (50 mL), and benzaldehyde (1.54 g, 0.014 mol) was added with constant stirring at room temperature. Then KOH solution (40 wt%) was added to reaction mixture which was stirred for 24 h at room temperature. After completion of the reaction, the mixture was poured into crushed ice and neutralized with HCl. The product was separated out, filtered, washed with water, dried, and recrystallized from ethanol to give compound **5a**. Similarly, other compounds **5**(**b**–**k**) were synthesized.

Yield: 79 %. M.p. 145–147 °C; IR (KBr, cm^{-1}): 3,281 (–NH), 1,668 (C=O of coumarin), 1,641 (C=O), 1,589 (–CH=CH–, str.); ^1H NMR (DMSO-d_6, δ, ppm): 8.79 (s, 1H, –NH), 8.12 (d, $J = 7.5$ Hz, 1H, Ar–CH=), 7.78 (d, $J = 7.1$ Hz, 1H, coumarin), 7.75–7.15 (m, 9H, Ar–H), 7.05 (d, $J = 7.2$ Hz, 1H, –CO–CH=), 6.71–6.53 (m, 3H, coumarin), 6.15 (s, 1H, coumarin); ^{13}C NMR (DMSO-d_6, δ, ppm): 192.87 (*C*=O), 162.49 (*C*=O of coumarin), 149.69 (*C*–NH at coumarin), 141.56 (Ar–*C*H=), 122.29 (C=O–*C*H=), 147.52, 143.36, 133.17, 132.65, 131.25, 130.51, 130.34, 130.13, 129.73, 129.31, 128.29, 128.17, 124.85, 124.77, 119.42, 118.78, 118.63, 117.21, 92.38 (19C, Ar–C); EMI-MS (*m*/*z*): 368.33 (M^+); Anal. Calcd for $C_{24}H_{17}NO_3$: C, 78.46; H, 4.66; N, 3.81. Found: C, 78.38; H, 4.61; N, 3.88.

4-{4-[3-(2,4-Dichloro-phenyl)-acryloyl]-phenylamino}-chromen-2-one (**5b**)

Yield: 75 %. M.p. 156–158 °C; IR (KBr, cm^{-1}): 3,283 (–NH), 1,670 (C=O of coumarin), 1,645 (C=O), 1,592 (–CH=CH-, str.), 793 (–Cl); ^1H NMR (DMSO-d_6, δ, ppm):

8.85 (s, 1H, –NH), 8.15 (d, $J = 7.2$ Hz, 1H, Ar–CH=), 7.82 (d, $J = 7.2$ Hz, 1H, coumarin), 7.78–7.12 (m, 7H, Ar–H), 7.08 (d, $J = 7.4$ Hz, 1H, –CO–CH=), 6.68–6.50 (m, 3H, coumarin), 6.13 (s, 1H, coumarin); ^{13}C NMR (DMSO-d_6, δ, ppm): 192.95 (*C*=O), 162.57 (*C*=O of coumarin), 149.55 (*C*–NH at coumarin), 141.51 (Ar–*C*H=), 134.15 (*C*–Cl), 133.19 (*C*–Cl), 122.43 (C=O–*C*H=), 147.23, 142.98, 132.94, 132.74, 131.20, 130.43, 130.28, 129.40, 128.15, 128.11, 124.80, 124.69, 119.25, 118.65, 118.55, 117.29, 92.18 (17C, Ar–C); EMI-MS (*m*/*z*): 437.16 (M^+); Anal. Calcd for $C_{24}H_{15}Cl_2NO_3$: C, 66.07; H, 3.47; N, 3.21. Found: C, 66.13; H, 3.41; N, 3.29.

4-[4-(3-*p*-Tolyl-acryloyl)-phenylamino]-chromen-2-one (**5c**)

Yield: 68 %. M.p. 183–185 °C; IR (KBr, cm^{-1}): 3,278 (–NH), 1,671 (C=O of coumarin), 1,644 (C=O), 1,587 (–CH=CH–, str.); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.83 (s, 1H, –NH), 8.12 (d, $J = 7.5$ Hz, 1H, Ar–CH=), 7.85 (d, $J = 7.5$ Hz, 1H, coumarin), 7.81–7.10 (m, 8H, Ar–H), 7.04 (d, $J = 7.2$ Hz, 1H, –CO–CH=), 6.65–6.47 (m, 3H, coumarin), 6.16 (s, 1H, coumarin), 2.15 (s, 3H, Ph–CH_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 193.10 (*C*=O), 162.55 (*C*=O of coumarin), 149.43 (*C*–NH at coumarin), 141.24 (Ar–*C*H=), 138.54 (*C*–CH_3 of phenyl), 122.47 (C=O–*C*H=), 147.14, 142.96, 133.29, 132.75, 131.21, 130.59, 129.96, 129.70, 129.26, 128.45, 128.13, 124.81, 124.70, 119.35, 118.63, 118.60, 117.14, 92.58 (18C, Ar–C), 21.29 (C–CH_3 of phenyl); EMI-MS (*m*/*z*): 382.57 (M^+); Anal. Calcd for $C_{25}H_{19}NO_3$: C, 78.72; H, 5.02; N, 3.67. Found: C, 78.75; H, 5.07; N, 3.62.

4-{4-[3-(4-Fluoro-phenyl)-acryloyl]-phenylamino}-chromen-2-one (**5d**)

Yield: 73 %. M.p. 171–173 °C; IR (KBr, cm^{-1}): 3,287 (–NH), 1,674 (C=O of coumarin), 1,593 (–CH=CH–, str.), 1,642 (C=O), 1,155 (C–F); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.77 (s, 1H, –NH), 8.16 (d, $J = 7.3$ Hz, 1H, Ar–CH=), 7.84 (d, $J = 7.2$ Hz, 1H, coumarin), 7.82–7.14 (m, 8H, Ar–H), 7.08 (d, $J = 7.1$ Hz, 1H, –CO–CH=), 6.70–6.52 (m, 3H, coumarin), 6.13 (s, 1H, coumarin); ^{13}C NMR (DMSO-d_6, δ, ppm): 192.95 (*C*=O), 168.22 (*C*–F), 162.42 (*C*=O of coumarin), 149.66 (*C*–NH at coumarin), 141.33 (Ar–*C*H=), 122.25 (C=O–*C*H=), 147.67, 143.15, 133.56, 132.87, 131.13, 130.63, 129.85, 129.68, 129.20, 128.39, 128.10, 124.77, 124.63, 119.26, 118.73, 118.69, 117.35, 92.16 (18C, Ar–C); EMI-MS (*m*/*z*): 386.62 (M^+); Anal. Calcd for $C_{24}H_{16}FNO_3$: C, 74.80; H, 4.18; N, 3.63. Found: C, 74.84; H, 4.13; N, 3.67.

4-{4-[3-(4-Chloro-phenyl)-acryloyl]-phenylamino}-chromen-2-one (**5e**)

Yield: 76 %. M.p. 163–165 °C; IR (KBr, cm^{-1}): 3,291 (–NH), 1,670 (C=O of coumarin), 1,639 (C=O), 1,590 (–CH=CH–, str.), 810 (–Cl); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.80 (s, 1H, –NH), 8.11 (d, $J = 7.6$ Hz, 1H, Ar–CH=), 7.81 (d, $J = 7.1$ Hz, 1H, coumarin), 7.77–7.12 (m, 8H, Ar–H), 7.10 (d, $J = 7.4$ Hz, 1H, –CO–CH=), 6.67–6.48 (m, 3H, coumarin), 6.11 (s, 1H, coumarin); ^{13}C NMR (DMSO-d_6, δ, ppm): 192.91 (*C*=O), 162.70 (*C*=O of coumarin), 149.61 (*C*–NH at coumarin), 141.52 (Ar–*C*H=), 133.45 (*C*–Cl), 122.58 (C=O–*C*H=), 147.88, 143.22, 133.24, 132.61, 131.29, 130.69, 130.22, 129.78, 129.25, 128.50, 128.23, 124.80, 124.67, 119.21, 118.86, 118.53, 117.41, 92.45 (18C, Ar–C); EMI-MS (*m*/*z*): 402.72 (M^+); Anal. Calcd for $C_{24}H_{16}ClNO_3$: C, 71.73; H, 4.01; N, 3.49. Found: C, 71.66; H, 4.07; N, 3.44.

4-{4-[3-(4-Nitro-phenyl)-acryloyl]-phenylamino}-chromen-2-one (**5f**)

Yield: 70 %. M.p. 172–174 °C; IR (KBr, cm^{-1}): 3,287 (–NH), 1,666 (C=O of coumarin), 1,585 (–CH=CH–, str.), 1,637 (C=O), 1,545 (N=O str.); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.86 (s, 1H, –NH), 8.17 (d, $J = 7.2$ Hz, 1H, Ar–CH=), 7.79 (d, $J = 7.3$ Hz, 1H, coumarin), 7.77–7.16 (m, 8H, Ar–H), 7.09 (d, $J = 7.2$ Hz, 1H, –CO–CH=), 6.70–6.54 (m, 3H, coumarin), 6.13 (s, 1H, coumarin); ^{13}C NMR (DMSO-d_6, δ, ppm): 193.17 (*C*=O), 161.97 (*C*=O of coumarin), 149.80 (*C*–NH at coumarin), 148.19 (*C*–NO_2), 141.15 (Ar–*C*H=), 122.39 (C=O–*C*H=), 148.12, 142.96, 132.93, 132.56, 131.20, 130.36, 130.15, 129.62, 129.21, 128.12, 128.10, 124.65, 124.52, 119.18, 118.77, 118.50, 117.10, 91.89 (18C, Ar–C); EMI-MS (*m*/*z*): 412.52 (M^+); Anal. Calcd for $C_{24}H_{16}N_2O_5$: C, 69.90; H, 3.91; N, 6.79. Found: C, 69.94; H, 3.87; N, 6.71.

4-{4-[3-(2-Hydroxy-phenyl)-acryloyl]-phenylamino}-chromen-2-one (**5g**)

Yield: 77 %. M.p. 187–189 °C; IR (KBr, cm^{-1}): 3,284 (–NH), 3,241 (–OH), 1,672 (C=O of coumarin), 1,647 (C=O), 1,593 (–CH=CH–, str.); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.83 (s, 1H, –NH), 8.15 (d, $J = 7.3$ Hz, 1H, Ar–CH=), 7.75 (d, $J = 7.3$ Hz, 1H, coumarin), 7.73–7.10 (m, 8H, Ar–H), 7.03 (d, $J = 7.5$ Hz, 1H, –CO–CH=), 6.67–6.49 (m, 3H, coumarin), 6.13 (s, 1H, coumarin), 4.51 (s, 1H, –O*H*); ^{13}C NMR (DMSO-d_6, δ, ppm): 193.25 (*C*=O), 162.43 (*C*=O of coumarin), 158.45 (*C*–OH), 149.54 (*C*–NH at coumarin), 141.38 (Ar–*C*H=), 122.20 (C=O–*C*H=), 147.45, 143.49, 133.23, 132.70, 131.45, 130.61, 130.54, 129.86, 129.57, 128.56, 128.13, 124.79, 124.66,

119.32, 118.80, 118.68, 117.33, 92.24 (18C, Ar–C); EMI-MS (*m*/*z*): 384.39 (M^+); Anal. Calcd for $C_{24}H_{17}NO_4$: C, 75.19; H, 4.47; N, 3.65. Found: C, 75.26; H, 4.40; N, 3.69.

4-{4-[3-(2-Bromo-phenyl)-acryloyl]-phenylamino}-chromen-2-one (**5h**)

Yield: 66 %. M.p. 210–212 °C; IR (KBr, cm^{-1}): 3,279 (–NH), 1,669 (C=O of coumarin), 1,645 (C=O), 1,588 (–CH=CH–, str.), 731 (–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.81 (s, 1H, –NH), 8.18 (d, $J = 7.1$ Hz, 1H, Ar–CH=), 7.79 (d, $J = 7.5$ Hz, 1H, coumarin), 7.76–7.14 (m, 8H, Ar–H), 7.08 (d, $J = 7.1$ Hz, 1H, –CO–CH=), 6.70–6.51 (m, 3H, coumarin), 6.15 (s, 1H, coumarin); ^{13}C NMR (DMSO-d_6, δ, ppm): 192.95 (*C*=O), 161.89 (*C*=O of coumarin), 149.43 (*C*–NH at coumarin), 141.20 (Ar–*C*H=), 122.36 (C=O–*C*H=), 121.73 (*C*–Br), 147.57, 143.33, 133.09, 132.85, 131.21, 130.47, 130.32, 129.53, 129.24, 128.21, 127.97, 124.67, 124.35, 119.45, 118.89, 118.61, 117.45, 92.11 (18C, Ar–C); EMI-MS (*m*/*z*): 447.23 (M^+); Anal. Calcd for $C_{24}H_{16}BrNO_3$: C, 64.59; H, 3.61; N, 3.14. Found: C, 64.54; H, 3.67; N, 3.19.

4-{4-[3-(4-Methoxy-phenyl)-acryloyl]-phenylamino}-chromen-2-one (**5i**)

Yield: 83 %. M.p. 195–197 °C; IR (KBr, cm^{-1}): 3,290 (–NH), 1,666 (C=O of coumarin), 1,647 (C=O), 1,595 (–CH=CH–, str.), 1,275 (–OCH_3); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.86 (s, 1H, –NH), 8.15 (d, $J = 7.5$ Hz, 1H, Ar–CH=), 7.85 (d, $J = 7.5$ Hz, 1H, coumarin), 7.80–7.16 (m, 8H, Ar–H), 7.11 (d, $J = 7.4$ Hz, 1H, –CO–CH=), 6.69–6.50 (m, 3H, coumarin), 6.16 (s, 1H, coumarin), 3.81 (s, 3H, –O*C*H_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 193.33 (*C*=O), 162.41 (*C*=O of coumarin), 160.55 (*C*–OCH_3), 149.53 (*C*–NH at coumarin), 141.79 (Ar–*C*H=), 122.65 (C=O–*C*H=), 147.88, 143.93, 132.87, 132.52, 131.46, 130.64, 130.21, 129.79, 129.38, 128.18, 128.11, 124.72, 124.56, 119.15, 118.82, 118.70, 117.54, 91.98 (18C, Ar–C), 56.13 (–O*C*H3); EMI-MS (*m*/*z*): 398.25 (M^+); Anal. Calcd for $C_{25}H_{19}NO_4$: C, 75.55; H, 4.82; N, 3.52. Found: C, 75.59; H, 4.80; N, 3.47.

4-{4-[3-(3,4-Dimethoxy-phenyl)-acryloyl]-phenylamino}-chromen-2-one (**5j**)

Yield: 75 %. M.p. 225–227 °C; IR (KBr, cm^{-1}): 3,285 (–NH), 1,671 (C=O of coumarin), 1,642 (C=O), 1,591 (–CH=CH–, str.), 1,278 (–OCH_3); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.83 (s, 1H, –NH), 8.11 (d, $J = 7.2$ Hz, 1H, Ar–CH=), 7.83 (d, $J = 7.3$ Hz, 1H, coumarin), 7.79–7.18 (m, 7H, Ar–H), 7.09 (d, $J = 7.5$ Hz, 1H, –CO–CH=), 6.66–6.45 (m, 3H, coumarin), 6.19 (s, 1H, coumarin), 3.83 (s, 3H, –O*C*H_3), 3.81 (s, 3H, –O*C*H_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 193.17 (*C*=O), 162.29 (*C*=O of coumarin), 160.67 (*C*–OCH_3), 159.27 (*C*–OCH_3), 149.72 (*C*–NH at coumarin), 141.27 (Ar–*C*H=), 122.68 (C=O–*C*H=), 147.52, 143.11, 133.39, 132.75, 131.33, 130.87, 129.89, 129.24, 128.56, 128.25, 124.74, 124.62, 119.22, 118.65, 118.54, 117.45, 92.11 (17C, Ar–C), 56.43 (–O*C*H3); EMI-MS (*m*/*z*): 428.45 (M^+); Anal. Calcd for $C_{26}H_{21}NO_5$: C, 73.06; H, 4.95; N, 3.28. Found: C, 73.13; H, 4.91; N, 3.34.

4-{4-[3-(2,4-Dihydroxy-phenyl)-acryloyl]-phenylamino}-chromen-2-one (**5k**)

Yield: 80 %. M.p. 213–216 °C; IR (KBr, cm^{-1}): 3,288 (–NH), 3,271 (–OH), 1,674 (C=O of coumarin), 1,648 (C=O), 1,595 (–CH=CH–, str.); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.77 (s, 1H, –NH), 8.45 (s, 1H, –O*H*), 8.15 (d, $J = 7.1$ Hz, 1H, Ar–CH=), 7.76 (d, $J = 7.3$ Hz, 1H, coumarin), 7.72–7.13 (m, 7H, Ar–H), 7.10 (d, $J = 7.5$ Hz, 1H, –CO–CH=), 6.67–6.48 (m, 3H, coumarin), 6.13 (s, 1H, coumarin), 4.58 (s, 1H, –O*H*); ^{13}C NMR (DMSO-d_6, δ, ppm): 192.97 (*C*=O), 162.54 (*C*=O of coumarin), 157.63 (*C*–OH), 156.35 (*C*–OH), 149.77 (*C*–NH at coumarin), 141.43 (Ar–*C*H=), 122.18 (C=O–*C*H=), 148.13, 143.55, 133.29, 132.39, 131.45, 130.63, 129.70, 129.25, 128.10, 127.87, 124.82, 124.70, 119.31, 118.56, 118.24, 117.56, 92.17 (17C, Ar–C); EMI-MS (*m*/*z*): 400.15 (M^+); Anal. Calcd for $C_{24}H_{17}NO_5$: C, 72.17; H, 4.29; N, 3.51. Found: C, 72.11; H, 4.20; N, 3.54.

Synthesis of 4-[4-(1-acetyl-5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)-phenylamino]-chromen-2-one (**6a**)

A mixture of compound **5a** (2.0 g, 0.005 mol) and hydrazine hydrate (0.27 g, 0.005 mol) in 1,4-dioxane (30 mL) in the presence of glacial acetic acid (10 mL) was refluxed for 6–8 h. After completion of the reaction, mixture was cooled and poured into crushed ice, and the product separated out was filtered, washed with water, dried, and recrystallized from DMF to give compound **6a**. Similarly, other compounds **6**(**b**–**k**) were synthesized.

Yield: 74 %. M.p. 223–224 °C; IR (KBr, cm^{-1}): 3,284 (–NH), 1,670 (C=O of coumarin), 1,645 (–$COCH_3$), 1,568 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.83 (s, 1H, –NH), 7.81 (d, $J = 7.2$ Hz, 1H, coumarin), 7.77–7.13 (m, 9H, Ar–H), 6.69–6.51 (m, 3H, coumarin), 6.16 (s, 1H, coumarin), 5.48 (dd, $J = 7.3$, 1.4 Hz, 1H, –C*H*–CH_2), 3.58 (dd, $J = 12.2$, 6.1 Hz, 1H, –CH_2), 3.30 (dd, $J = 12.5$, 6.3 Hz, 1H, –CH_2), 2.33 (s, 3H, –$COCH_3$); ^{13}C NMR (DMSO-d_6, δ, ppm): 171.23 (*C*=O of $COCH_3$), 162.77 (*C*=O of coumarin), 157.21 (*C*=N), 149.67 (*C*–NH at coumarin), 148.12, 143.32, 133.39, 132.77, 131.34, 131.13, 130.84, 130.45, 129.85, 129.56, 128.38, 128.11, 125.15, 124.89, 119.53, 118.74, 118.61, 117.29, 92.58 (19C,

Ar–C), 63.42 (–*C*H–Ar), 38.25 (–*C*H_2–CH–Ar), 24.45 (–CO*C*H_3); EMI-MS (*m*/*z*): 424.38 (M^+); Anal. Calcd for $C_{26}H_{21}N_3O_3$: C, 73.74; H, 5.00; N, 9.92. Found: C, 73.71; H, 5.06; N, 9.86.

4-{4-[1-Acetyl-5-(2,4-dichloro-phenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-phenylamino}-chromen-2-one (**6b**)

Yield: 71 %. M.p. 211–212 °C; IR (KBr, cm^{-1}): 3,286 (–NH), 1,671 (C=O of coumarin), 1,647 (–$COCH_3$), 1,565 (C=N), 812 (–Cl); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.88 (s, 1H, –NH), 7.85 (d, $J = 7.4$ Hz, 1H, coumarin), 7.80–7.17 (m, 7H, Ar–H), 6.70–6.54 (m, 3H, coumarin), 6.15 (s, 1H, coumarin), 5.42 (dd, $J = 7.1$, 1.5 Hz, 1H, –C*H*–CH_2), 3.52 (dd, $J = 12.4$, 6.4 Hz, 1H, –CH_2), 3.34 (dd, $J = 12.1$, 6.2 Hz, 1H, –CH_2), 2.37 (s, 3H, –$COCH_3$); ^{13}C NMR (DMSO-d_6, δ, ppm): 171.45 (*C*=O of $COCH_3$), 162.68 (*C*=O of coumarin), 157.34 (*C*=N), 149.62 (*C*–NH at coumarin), 134.37 (*C*–Cl), 133.42 (*C*–Cl), 147.92, 143.12, 133.13, 132.85, 131.24, 130.49, 129.55, 129.46, 128.30, 128.16, 124.90, 124.56, 119.47, 118.70, 118.59, 117.33, 92.29 (17C, Ar–C), 63.25 (–*C*H–Ar), 38.17 (–*C*H_2–CH–Ar), 24.67 (–CO*C*H_3); EMI-MS (*m*/*z*): 493.19 (M^+); Anal. Calcd for $C_{26}H_{19}Cl_2N_3O_3$: C, 63.43; H, 3.89; N, 8.53. Found: C, 63.49; H, 3.96; N, 8.45.

4-[4-(1-Acetyl-5-*p*-tolyl-4,5-dihydro-1*H*-pyrazol-3-yl)-phenylamino]-chromen-2-one (**6c**)

Yield: 65 %. M.p. 251–253 °C; IR (KBr, cm^{-1}): 3,281 (–NH), 1,673 (C=O of coumarin), 1,645 (–$COCH_3$), 1,572 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.84 (s, 1H, –NH), 7.88 (d, $J = 7.1$ Hz, 1H, coumarin), 7.83–7.12 (m, 8H, Ar–H), 6.68–6.49 (m, 3H, coumarin), 6.14 (s, 1H, coumarin), 5.50 (dd, $J = 7.6$, 1.4 Hz, 1H, –C*H*–CH_2), 3.53 (dd, $J = 12.1$, 6.5 Hz, 1H, –CH_2), 3.32 (dd, $J = 12.4$, 6.1 Hz, 1H, –CH_2), 2.31 (s, 3H, –$COCH_3$), 2.19 (s, 3H, Ph–CH_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 170.95 (*C*=O of $COCH_3$), 162.65 (*C*=O of coumarin), 157.78 (*C*=N), 149.63 (*C*–NH at coumarin), 138.74 (*C*–CH_3 of phenyl), 147.52, 143.11, 133.43, 132.55, 131.39, 130.47, 129.95, 129.89, 129.76, 128.58, 128.41, 124.78, 124.89, 119.40, 118.33, 118.11, 116.90, 92.38 (18C, Ar–C), 62.91 (–*C*H–Ar), 38.67 (–*C*H_2–CH–Ar), 24.59 (–CO*C*H_3), 21.57 (C–*C*H_3 of phenyl); EMI-MS (*m*/*z*): 438.62 (M^+); Anal. Calcd for $C_{27}H_{23}N_3O_3$: C, 74.12; H, 5.30; N, 9.60. Found: C, 74.18; H, 5.35; N, 9.53.

4-{4-[1-Acetyl-5-(4-fluoro-phenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-phenylamino}-chromen-2-one (**6d**)

Yield: 76 %. M.p. 239–241 °C; IR (KBr, cm^{-1}): 3,289 (–NH), 1,677 (C=O of coumarin), 1,643 (–$COCH_3$), 1,569 (C=N), 1,162 (C–F); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.80 (s, 1H, –NH), 7.79 (d, $J = 7.2$ Hz, 1H, coumarin), 7.76–7.11 (m, 8H, Ar–H), 6.71–6.48 (m, 3H, coumarin), 6.11 (s, 1H, coumarin), 5.44 (dd, $J = 7.5$, 1.1 Hz, 1H, –C*H*–CH_2), 3.52 (dd, $J = 12.4$, 6.4 Hz, 1H, –CH_2), 3.29 (dd, $J = 12.6$, 6.2 Hz, 1H, –CH_2), 2.30 (s, 3H, –$COCH_3$); ^{13}C NMR (DMSO-d_6, δ, ppm): 170.83 (*C*=O of $COCH_3$), 168.76 (*C*–F), 162.70 (*C*=O of coumarin), 157.46 (*C*=N), 149.42 (*C*–NH at coumarin), 148.15, 143.39, 133.77, 133.07, 132.94, 131.25, 130.24, 129.97, 129.66, 128.68, 128.51, 124.75, 124.69, 119.58, 118.65, 118.42, 117.52, 92.31 (18C, Ar–C), 62.97 (–*C*H–Ar), 37.75 (–*C*H_2–CH–Ar), 24.52 (–CO*C*H_3); EMI-MS (*m*/*z*): 442.56 (M^+); Anal. Calcd for $C_{26}H_{20}FN_3O_3$: C, 70.74; H, 4.57; N, 9.52. Found: C, 70.71; H, 4.52; N, 9.59.

4-{4-[1-Acetyl-5-(4-chloro-phenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-phenylamino}-chromen-2-one (**6e**)

Yield: 73 %. M.p. 262–264 °C; IR (KBr, cm^{-1}): 3,294 (–NH), 1,672 (C=O of coumarin), 1,642 (–$COCH_3$), 1,563 (C=N), 817 (–Cl); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.87 (s, 1H, –NH), 7.85 (d, $J = 7.4$ Hz, 1H, coumarin), 7.81–7.16 (m, 8H, Ar–H), 6.70–6.54 (m, 3H, coumarin), 6.16 (s, 1H, coumarin), 5.52 (dd, $J = 7.5$, 1.2 Hz, 1H, –C*H*–CH_2), 3.51 (dd, $J = 12.1$, 6.6 Hz, 1H, –CH_2), 3.34 (dd, $J = 12.3$, 6.5 Hz, 1H, –CH_2), 2.39 (s, 3H, –$COCH_3$); ^{13}C NMR (DMSO-d_6, δ, ppm): 171.67 (*C*=O of $COCH_3$), 162.55 (*C*=O of coumarin), 157.36 (*C*=N), 149.55 (*C*–NH at coumarin), 133.80 (*C*–Cl), 148.22, 143.53, 133.59, 132.75, 131.49, 130.78, 130.15, 129.89, 129.42, 128.48, 128.27, 125.11, 124.84, 119.35, 118.80, 118.49, 117.52, 91.88 (18C, Ar–C), 63.85 (–*C*H–Ar), 38.41 (–*C*H_2–CH–Ar), 25.09 (–CO*C*H_3); EMI-MS (*m*/*z*): 458.11 (M^+); Anal. Calcd for $C_{26}H_{20}ClN_3O_3$: C, 68.20; H, 4.40; N, 9.18. Found: C, 68.26; H, 4.32; N, 9.14.

4-{4-[1-Acetyl-5-(4-nitro-phenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-phenylamino}-chromen-2-one (**6f**)

Yield: 68 %. M.p. 243–244 °C; IR (KBr, cm^{-1}): 3,282 (–NH), 1,669 (C=O of coumarin), 1,640 (–$COCH_3$), 1,552 (N=O str.), 1,565 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.83 (s, 1H, –NH), 7.81 (d, $J = 7.2$ Hz, 1H, coumarin), 7.78–7.14 (m, 8H, Ar–H), 6.72–6.57 (m, 3H, coumarin), 6.19 (s, 1H, coumarin), 5.43 (dd, $J = 7.3$, 1.1 Hz, 1H, –C*H*–CH_2), 3.54 (dd, $J = 12.3$, 6.2 Hz, 1H, –CH_2), 3.26 (dd, $J = 12.4$, 6.1 Hz, 1H, –CH_2), 2.31 (s, 3H, –$COCH_3$); ^{13}C NMR (DMSO-d_6, δ, ppm): 171.65 (*C*=O of $COCH_3$), 162.19 (*C*=O of coumarin), 157.78 (*C*=N), 149.87 (*C*–NH at coumarin), 148.85 (*C*–NO_2), 148.25, 143.16, 133.13, 132.78, 131.45, 130.89, 130.55, 129.74, 129.43, 129.02, 128.34, 123.95, 124.88, 119.56, 119.17, 118.48, 116.70,

92.19 (18C, Ar–C), 62.79 (–*C*H–Ar), 38.13 (–*C*H_2–CH–Ar), 23.91 (–CO*C*H_3); EMI-MS (*m*/*z*): 469.25 (M^+); Anal. Calcd for $C_{26}H_{20}N_4O_5$: C, 66.66; H, 4.30; N, 11.96. Found: C, 66.73; H, 4.38; N, 11.91.

4-{4-[1-Acetyl-5-(2-hydroxy-phenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-phenylamino}-chromen-2-one (**6g**)

Yield: 79 %. M.p. 256–258 °C; IR (KBr, cm^{-1}): 3,286 (–NH), 3,252 (–OH), 1,666 (C=O of coumarin), 1,643 (–$COCH_3$), 1,574 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.85 (s, 1H, –NH), 7.78 (d, $J = 7.2$ Hz, 1H, coumarin), 7.76–7.13 (m, 8H, Ar–H), 6.70–6.54 (m, 3H, coumarin), 6.15 (s, 1H, coumarin), 5.53 (dd, $J = 7.3$, 1.5 Hz, 1H, –C*H*–CH_2), 3.57 (dd, $J = 12.3$, 6.4 Hz, 1H, –CH_2), 3.35 (dd, $J = 12.2$, 6.1 Hz, 1H, –CH_2), 4.58 (s, 1H, –O*H*) 2.40 (s, 3H, –$COCH_3$); ^{13}C NMR (DMSO-d_6, δ, ppm): 171.75 (*C*=O of $COCH_3$), 162.71 (*C*=O of coumarin), 158.64 (*C*–OH), 157.40 (*C*=N), 149.66 (*C*–NH at coumarin), 147.62, 143.53, 133.50, 133.15, 131.40, 130.88, 129.94, 129.82, 129.51, 128.66, 127.83, 125.99, 124.85, 118.92, 118.86, 118.35, 117.55, 92.66 (18C, Ar–C), 63.49 (–*C*H–Ar), 38.44 (–*C*H_2–CH–Ar), 24.13 (–CO*C*H_3); EMI-MS (*m*/*z*): 440.31 (M^+); Anal. Calcd for $C_{26}H_{21}N_3O_4$: C, 71.06; H, 4.82; N, 9.56. Found: C, 71.02; H, 4.88; N, 9.50.

4-{4-[1-Acetyl-5-(2-bromo-phenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-phenylamino}-chromen-2-one (**6h**)

Yield: 63 %. M.p. 220–221 °C; IR (KBr, cm^{-1}): 3,283 (–NH), 1,675 (C=O of coumarin), 1,641 (–$COCH_3$), 1,569 (C=N), 745 (–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.83 (s, 1H, –NH), 7.82 (d, $J = 7.5$ Hz, 1H, coumarin), 7.80–7.17 (m, 8H, Ar–H), 6.67–6.50 (m, 3H, coumarin), 6.21 (s, 1H, coumarin), 5.41 (dd, $J = 7.3$, 1.3 Hz, 1H, –C*H*–CH_2), 3.55 (dd, $J = 12.3$, 6.5 Hz, 1H, –CH_2), 3.32 (dd, $J = 12.1$, 6.3 Hz, 1H, –CH_2), 2.29 (s, 3H, –$COCH_3$); ^{13}C NMR (DMSO-d_6, δ, ppm): 171.86 (*C*=O of $COCH_3$), 162.15 (*C*=O of coumarin), 156.81 (*C*=N), 149.88 (*C*–NH at coumarin), 121.35 (*C*–Br), 148.22, 142.93, 133.27, 133.15, 131.47, 130.68, 130.14, 129.66, 129.45, 128.40, 128.17, 125.10, 124.63, 119.66, 118.75, 118.52, 118.10, 92.54 (18C, Ar–C), 62.72 (–*C*H–Ar), 38.79 (–*C*H_2–CH–Ar), 23.80 (–CO*C*H_3); EMI-MS (*m*/*z*): 503.24 (M^+); Anal. Calcd for $C_{26}H_{20}BrN_3O_3$: C, 62.16; H, 4.01; N, 8.36. Found: C, 62.23; H, 4.07; N, 8.31.

4-{4-[1-Acetyl-5-(4-methoxy-phenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-phenylamino}-chromen-2-one (**6i**)

Yield: 81 %. M.p. 247–249 °C; IR (KBr, cm^{-1}): 3,294 (–NH), 1,670 (C=O of coumarin), 1,649 (–$COCH_3$), 1,561 (C=N), 1,282 (–OCH_3); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.84 (s, 1H, –NH), 7.88 (d, $J = 7.4$ Hz, 1H, coumarin), 7.83–7.19 (m, 8H, Ar–H), 6.73–6.55 (m, 3H, coumarin), 6.13 (s, 1H, coumarin), 5.46 (dd, $J = 7.4$, 1.2 Hz, 1H, –C*H*–CH_2), 3.85 (s, 3H, –O*C*H_3), 3.54 (dd, $J = 12.1$, 6.3 Hz, 1H, –CH_2), 3.33 (dd, $J = 12.4$, 6.2 Hz, 1H, –CH_2), 2.37 (s, 3H, –$COCH_3$); ^{13}C NMR (DMSO-d_6, δ, ppm): 170.44 (*C*=O of $COCH_3$), 162.69 (*C*=O of coumarin), 159.87 (*C*–OCH_3), 157.82 (*C*=N), 149.51 (*C*–NH at coumarin), 148.18, 144.12, 132.98, 132.66, 132.12, 131.24, 130.46, 129.80, 129.22, 128.37, 128.31, 124.70, 124.42, 119.51, 118.63, 118.60, 117.51, 92.25 (18C, Ar–C), 63.76 (–*C*H–Ar), 55.80 (–O*C*H3), 37.79 (–*C*H_2–CH–Ar), 24.13 (–CO*C*H_3); EMI-MS (*m*/*z*): 454.23 (M^+); Anal. Calcd for $C_{27}H_{23}N_3O_4$: C, 71.51; H, 5.11; N, 9.27. Found: C, 71.57; H, 5.07; N, 9.34.

4-{4-[1-Acetyl-5-(3,4-dimethoxy-phenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-phenylamino}-chromen-2-one (**6j**)

Yield: 72 %. M.p. 281–283 °C; IR (KBr, cm^{-1}): 3,280 (–NH), 1,675 (C=O of coumarin), 1,645 (–$COCH_3$), 1,565 (C=N), 1,285 (–OCH_3); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.85 (s, 1H, –NH), 7.87 (d, $J = 7.6$ Hz, 1H, coumarin), 7.84–7.21 (m, 7H, Ar–H), 6.70–6.49 (m, 3H, coumarin), 6.17 (s, 1H, coumarin), 5.42 (dd, $J = 7.4$, 1.3 Hz, 1H, –C*H*–CH_2), 3.88 (s, 3H, –O*C*H_3), 3.85 (s, 3H, –O*C*H_3) 3.51 (dd, $J = 12.3$, 6.2 Hz, 1H, –CH_2), 3.36 (dd, $J = 12.5$, 6.2 Hz, 1H, –CH_2), 2.28 (s, 3H, –$COCH_3$); ^{13}C NMR (DMSO-d_6, δ, ppm): 171.54 (*C*=O of $COCH_3$), 162.45 (*C*=O of coumarin), 160.75 (*C*–OCH_3), 159.49 (*C*–OCH_3), 156.90 (*C*=N), 149.35 (*C*–NH at coumarin), 148.10, 142.85, 133.31, 132.89, 131.20, 131.16, 130.91, 129.84, 128.73, 128.42, 124.77, 124.55, 119.47, 119.15, 118.69, 117.87, 92.06 (17C, Ar–C), 63.11 (–*C*H–Ar), 55.84 (–O*C*H3), 38.59 (–*C*H_2–CH–Ar), 24.87 (–CO*C*H_3); EMI-MS (*m*/*z*): 484.49 (M^+); Anal. Calcd for $C_{28}H_{25}N_3O_5$: C, 69.55; H, 5.21; N, 8.69. Found: C, 69.59; H, 5.14; N, 8.73.

4-{4-[1-Acetyl-5-(2,4-dihydroxy-phenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-phenylamino}-chromen-2-one (**6k**)

Yield: 77 %. M.p. 266–267 °C; IR (KBr, cm^{-1}): 3,291 (–NH), 3,277 (–OH), 1,669 (C=O of coumarin), 1,650 (–$COCH_3$), 1,559 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.82 (s, 1H, –NH), 8.49 (s, 1H, –O*H*), 7.80 (d, $J = 7.2$ Hz, 1H, coumarin), 7.75–7.17 (m, 7H, Ar–H), 6.70–6.50 (m, 3H, coumarin), 6.18 (s, 1H, coumarin), 5.41 (dd, $J = 7.2$, 1.1 Hz, 1H, –C*H*–CH_2), 4.53 (s, 1H, –O*H*), 3.55 (dd, $J = 12.5$, 6.3 Hz, 1H, –CH_2), 3.38 (dd, $J = 12.3$, 6.2 Hz, 1H, –CH_2), 2.29 (s, 3H, –$COCH_3$); ^{13}C NMR (DMSO-d_6, δ, ppm): 171.60 (*C*=O of $COCH_3$), 162.88 (*C*=O of coumarin), 157.82 (*C*–OH), 157.29 (*C*–OH), 157.11 (*C*=N),

149.33 (*C*–NH at coumarin), 148.29, 143.67, 133.45, 132.65, 131.49, 131.12, 130.74, 129.56, 128.75, 128.17, 125.12, 124.83, 119.47, 118.61, 118.30, 117.89, 91.87 (17C, Ar–C), 62.82 (–*C*H–Ar), 37.96 (–*C*H_2–CH–Ar), 24.07 (–CO*C*H_3); EMI-MS (*m*/*z*): 456.35 (M^+); Anal. Calcd for $C_{26}H_{21}N_3O_5$: C, 68.56; H, 4.65; N, 9.23. Found: C, 68.51; H, 4.60; N, 9.28.

Synthesis of 4-[4-(2-amino-6-phenyl-pyrimidin-4-yl)-phenylamino]-chromen-2-one (**7a**)

A mixture of compound **5a** (2.0 g, 0.005 mol), guanidine nitrate (0.66 g, 0.005 mol), and 1–2 mL of 40 % KOH solution in ethanol (30 mL) was refluxed for 10–12 h. The reaction mixture was cooled, then poured into crushed ice, and the product separated out was filtered, washed with water, dried, and recrystallized from DMF to give compound **7a**. Similarly, other compounds **7**(**b**–**k**) were synthesized.

Yield: 68 %. M.p. 229–231 °C; IR (KBr, cm^{-1}): 3,385 (–NH_2), 3,289 (–NH), 1,673 (C=O of coumarin), 1,561 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.79 (s, 1H, –NH), 7.86 (d, $J = 7.1$ Hz, 1H, coumarin), 7.83–7.11 (m, 10H, Ar–H), 6.65–6.46 (m, 3H, coumarin), 6.13 (s, 1H, coumarin), 5.31 (s, 2H, –NH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 162.55 (*C*=O of coumarin), 159.88, 159.56 (2C, pyrimidine), 155.13 (C–NH_2), 148.86 (C–NH at coumarin), 113.27 (1C, pyrimidine), 146.99, 142.92, 133.77, 133.16, 131.56, 131.33, 130.24, 130.49, 129.65, 129.22, 128.32, 128.20, 124.93, 124.59, 119.44, 118.69, 118.54, 117.13, 93.24 (19C, Ar–C); EMI-MS (*m*/*z*): 407.57 (M^+); Anal. Calcd for $C_{25}H_{18}N_4O_2$: C, 73.88; H, 4.46; N, 13.78. Found: C, 73.81; H, 4.52; N, 13.70.

4-{4-[2-Amino-6-(2,4-dichloro-phenyl)-pyrimidin-4-yl]-phenylamino}-chromen-2-one (**7b**)

Yield: 72 %. M.p. 257–259 °C; IR (KBr, cm^{-1}): 3,381 (–NH_2), 3,284 (–NH), 1,669 (C=O of coumarin), 1,568 (C=N), 819 (–Cl); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.86 (s, 1H, –NH), 7.87 (d, $J = 7.2$ Hz, 1H, coumarin), 7.79–7.10 (m, 8H, Ar–H), 6.69–6.49 (m, 3H, coumarin), 6.18 (s, 1H, coumarin), 5.27 (s, 2H, –NH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 162.75 (*C*=O of coumarin), 159.67, 159.43 (2C, pyrimidine), 154.83 (C–NH_2), 149.27 (C–NH at coumarin), 112.96 (1C, pyrimidine), 135.17 (*C*–Cl), 134.82 (*C*–Cl), 147.73, 142.92, 133.46, 132.15, 131.39, 130.55, 129.95, 129.76, 128.66, 127.70, 125.10, 124.96, 119.33, 118.68, 118.38, 117.20, 92.75 (17C, Ar–C); EMI-MS (*m*/*z*): 476.22 (M^+); Anal. Calcd for $C_{25}H_{16}Cl_2N_4O_2$: C, 63.17; H, 3.39; N, 11.79. Found: C, 63.21; H, 3.34; N, 11.73.

4-[4-(2-Amino-6-p-tolyl-pyrimidin-4-yl)-phenylamino]-chromen-2-one (**7c**)

Yield: 65 %. M.p. 245–246 °C; IR (KBr, cm^{-1}): 3,391 (–NH_2), 3,284 (–NH), 1,671 (C=O of coumarin), 1,568 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.81 (s, 1H, –NH), 7.91 (d, $J = 7.4$ Hz, 1H, coumarin), 7.86–7.16 (m, 9H, Ar–H), 6.73–6.56 (m, 3H, coumarin), 6.17 (s, 1H, coumarin), 5.33 (s, 2H, –NH_2), 2.25 (s, 3H, Ph–CH_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 162.61 (*C*=O of coumarin), 159.54, 159.32 (2C, pyrimidine), 155.29 (C–NH_2), 149.66 (C–NH at coumarin), 138.15 (*C*–CH_3 of phenyl), 113.55 (1C, pyrimidine), 148.43, 142.92, 133.56, 132.41, 131.35, 130.69, 130.05, 129.81, 129.54, 128.44, 128.12, 125.18, 124.70, 119.59, 118.84, 118.62, 117.46, 93.08 (18C, Ar–C), 22.07 (C–*C*H_3 of phenyl); EMI-MS (*m*/*z*): 421.50 (M^+); Anal. Calcd for $C_{26}H_{20}N_4O_2$: C, 74.27; H, 4.79; N, 13.33. Found: C, 74.22; H, 4.85; N, 13.38.

4-{4-[2-Amino-6-(4-fluoro-phenyl)-pyrimidin-4-yl]-phenylamino}-chromen-2-one (**7d**)

Yield: 75 %. M.p. 279–281 °C; IR (KBr, cm^{-1}): 3,388 (–NH_2), 3,276 (–NH), 1,672 (C=O of coumarin), 1,564 (C=N), 1,169 (C–F); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.78 (s, 1H, –NH), 7.82 (d, $J = 7.1$ Hz, 1H, coumarin), 7.80–7.09 (m, 9H, Ar–H), 6.68–6.52 (m, 3H, coumarin), 6.14 (s, 1H, coumarin), 5.39 (s, 2H, –NH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 168.53 (*C*–F), 161.88 (*C*=O of coumarin), 160.12, 159.86 (2C, pyrimidine), 154.92 (C–NH_2), 148.54 (C–NH at coumarin), 113.59 (1C, pyrimidine), 147.70, 143.40, 133.65, 132.17, 132.14, 131.18, 130.57, 129.84, 129.55, 128.86, 128.43, 124.63, 124.54, 119.63, 118.40, 118.32, 117.56, 92.15 (18C, Ar–C); EMI-MS (*m*/*z*): 425.34 (M^+); Anal. Calcd for $C_{25}H_{17}FN_4O_2$: C, 70.75; H, 4.04; N, 13.20. Found: C, 70.79; H, 4.10; N, 13.15.

4-{4-[2-Amino-6-(4-chloro-phenyl)-pyrimidin-4-yl]-phenylamino}-chromen-2-one (**7e**)

Yield: 68 %. M.p. >300 °C; IR (KBr, cm^{-1}): 3,380 (–NH_2), 3,291 (–NH), 1,675 (C=O of coumarin), 1,562 (C=N), 832 (–Cl); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.89 (s, 1H, –NH), 7.83 (d, $J = 7.5$ Hz, 1H, coumarin), 7.81–7.19 (m, 9H, Ar–H), 6.67–6.53 (m, 3H, coumarin), 6.15 (s, 1H, coumarin), 5.23 (s, 2H, –NH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 162.70 (*C*=O of coumarin), 159.51, 159.23 (2C, pyrimidine), 155.39 (C–NH_2), 148.35 (C–NH at coumarin), 113.66 (1C, pyrimidine), 134.10 (*C*–Cl), 147.80, 142.83, 133.66, 132.45, 131.40, 130.56, 130.11, 129.67, 129.32, 128.80, 128.55, 124.16, 123.84, 119.49, 118.87, 118.43, 117.13, 92.19 (18C, Ar–C); EMI-MS (*m*/*z*): 441.72 (M^+);

Anal. Calcd for $C_{25}H_{17}ClN_4O_2$: C, 68.11; H, 3.89; N, 12.71. Found: C, 68.16; H, 3.82; N, 12.75.

4-{4-[2-Amino-6-(4-nitro-phenyl)-pyrimidin-4-yl]-phenylamino}-chromen-2-one (**7f**)

Yield: 69 %. M.p. 266–267 °C; IR (KBr, cm^{-1}): 3,388 ($–NH_2$), 3,292 (–NH), 1,674 (C=O of coumarin), 1,555 (N=O str.), 1,559 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.89 (s, 1H, –NH), 7.85 (d, $J = 7.3$ Hz, 1H, coumarin), 7.80–7.12 (m, 9H, Ar–H), 6.75–6.56 (m, 3H, coumarin), 6.17 (s, 1H, coumarin), 5.36 (s, 2H, $–NH_2$); ^{13}C NMR (DMSO-d_6, δ, ppm): 161.10 (*C*=O of coumarin), 159.47, 159.13 (2C, pyrimidine), 154.44 (C–NH_2), 149.15 (C–NH at coumarin), 148.24 (*C*–NO_2), 113.60 (1C, pyrimidine), 147.36, 143.52, 133.83, 132.19, 131.56, 130.28, 129.85, 129.44, 129.03, 128.55, 128.11, 124.15, 123.88, 119.38, 118.57, 118.15, 116.84, 92.47 (18C, Ar–C); EMI-MS (*m*/*z*): 452.31 (M^+); Anal. Calcd for $C_{25}H_{17}N_5O_4$: C, 66.51; H, 3.80; N, 15.51. Found: C, 66.44; H, 3.71; N, 15.56.

4-{4-[2-Amino-6-(2-hydroxy-phenyl)-pyrimidin-4-yl]-phenylamino}-chromen-2-one (**7g**)

Yield: 75 %. M.p. 293–295 °C; IR (KBr, cm^{-1}): 3,383 ($–NH_2$), 3,278 (–NH), 3,248 (–OH), 1,668 (C=O of coumarin), 1,572 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.83 (s, 1H, –NH), 7.79 (d, $J = 7.3$ Hz, 1H, coumarin), 7.76–7.10 (m, 9H, Ar–H), 6.72–6.50 (m, 3H, coumarin), 6.13 (s, 1H, coumarin), 5.26 (s, 2H, $–NH_2$), 4.63 (s, 1H, –O*H*); ^{13}C NMR (DMSO-d_6, δ, ppm): 162.23 (*C*=O of coumarin), 159.75, 159.21 (2C, pyrimidine), 158.35 (*C*–OH), 155.48 (C–NH_2), 149.77 (C–NH at coumarin), 113.20 (1C, pyrimidine), 147.27, 143.58, 133.67, 132.15, 131.39, 130.65, 129.87, 129.13, 128.81, 128.51, 127.97, 124.82, 124.33, 119.17, 118.13, 118.05, 117.80, 93.16 (18C, Ar–C); EMI-MS (*m*/*z*): 423.26 (M^+); Anal. Calcd for $C_{25}H_{18}N_4O_3$: C, 71.08; H, 4.29; N, 13.26. Found: C, 71.14; H, 4.33; N, 13.21.

4-{4-[2-Amino-6-(2-bromo-phenyl)-pyrimidin-4-yl]-phenylamino}-chromen-2-one (**7h**)

Yield: 69 %. M.p. 250–252 °C; IR (KBr, cm^{-1}): 3,395 ($–NH_2$), 3,281 (–NH), 1,670 (C=O of coumarin), 1,562 (C=N), 757 (-Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.87 (s, 1H, –NH), 7.81 (d, $J = 7.4$ Hz, 1H, coumarin), 7.75–7.11 (m, 9H, Ar–H), 6.74–6.58 (m, 3H, coumarin), 6.19 (s, 1H, coumarin), 5.22 (s, 2H, $–NH_2$); ^{13}C NMR (DMSO-d_6, δ, ppm): 161.84 (*C*=O of coumarin), 160.17, 159.87 (2C, pyrimidine), 155.49 (C–NH_2), 149.33 (C–NH at coumarin), 113.49 (1C, pyrimidine), 121.88 (*C*–Br), 147.82, 143.18, 133.87, 132.15, 131.56, 130.87, 130.22, 129.43, 129.32, 128.56, 128.11, 124.80, 124.26, 119.69, 118.73, 118.26, 117.90, 93.03 (18C, Ar–C); EMI-MS (*m*/*z*): 486.53 (M^+); Anal. Calcd for $C_{25}H_{17}BrN_4O_2$: C, 61.87; H, 3.53; N, 11.54. Found: C, 61.93; H, 3.45; N, 11.59.

4-{4-[2-Amino-6-(4-methoxy-phenyl)-pyrimidin-4-yl]-phenylamino}-chromen-2-one (**7i**)

Yield: 78 %. M.p. >300 °C; IR (KBr, cm^{-1}): 3,387 ($–NH_2$), 3,285 (–NH), 1,674 (C=O of coumarin), 1,566 (C=N), 1,280 ($–OCH_3$); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.88 (s, 1H, –NH), 7.83 (d, $J = 7.4$ Hz, 1H, coumarin), 7.81–7.17 (m, 9H, Ar–H), 6.69–6.51 (m, 3H, coumarin), 6.10 (s, 1H, coumarin), 5.28 (s, 2H, $–NH_2$), 3.79 (s, 3H, –O*C*H_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 162.75 (*C*=O of coumarin), 160.81 (*C*–OCH_3), 159.53, 159.37 (2C, pyrimidine), 155.31 (C–NH_2), 149.17 (C–NH at coumarin), 113.60 (1C, pyrimidine), 147.82, 143.22, 132.91, 132.86, 131.92, 131.04, 130.57, 129.88, 129.15, 128.83, 128.50, 124.66, 124.43, 119.55, 118.21, 118.19, 117.60, 92.64 (18C, Ar–C), 55.93 (–O*C*H3); EMI-MS (*m*/*z*): 437.55 (M^+); Anal. Calcd for $C_{26}H_{20}N_4O_3$: C, 71.55; H, 4.62; N, 12.84. Found: C, 71.59; H, 4.55; N, 12.81.

4-{4-[2-Amino-6-(3,4-dimethoxy-phenyl)-pyrimidin-4-yl]-phenylamino}-chromen-2-one (**7j**)

Yield: 71 %. M.p. 288–289 °C; IR (KBr, cm^{-1}): 3,379 ($–NH_2$), 3,292 (–NH), 1,671 (C=O of coumarin), 1,568 (C=N), 1,280 ($–OCH_3$); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.87 (s, 1H, –NH), 7.85 (d, $J = 7.1$ Hz, 1H, coumarin), 7.80–7.18 (m, 8H, Ar–H), 6.72–6.55 (m, 3H, coumarin), 6.19 (s, 1H, coumarin), 3.87 (s, 3H, –O*C*H_3), 3.82 (s, 3H, –O*C*H_3) 5.30 (s, 2H, $–NH_2$); ^{13}C NMR (DMSO-d_6, δ, ppm): 162.89 (*C*=O of coumarin), 160.75 (*C*–OCH_3), 160.14 (*C*–OCH_3), 159.44, 159.16 (2C, pyrimidine), 155.65 (C–NH_2), 148.90 (C–NH at coumarin), 112.79 (1C, pyrimidine), 147.82, 142.93, 133.20, 132.53, 131.47, 130.16, 129.67, 129.13, 128.70, 128.38, 124.51, 124.36, 119.53, 119.67, 118.54, 117.79, 93.17 (17C, Ar–C), 56.11 (–O*C*H3); EMI-MS (*m*/*z*): 467.49 (M^+); Anal. Calcd for $C_{27}H_{22}N_4O_4$: C, 69.52; H, 4.75; N, 12.01. Found: C, 69.56; H, 4.70; N, 12.09.

4-{4-[2-Amino-6-(2,4-dihydroxy-phenyl)-pyrimidin-4-yl]-phenylamino}-chromen-2-one (**7k**)

Yield: 82 %. M.p. >300 °C; IR (KBr, cm^{-1}): 3,390 ($–NH_2$), 3,295 (–NH), 3,271 (–OH), 1,667 (C=O of coumarin), 1,562 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.79 (s, 1H, –NH), 8.54 (s, 1H, –O*H*), 7.83 (d, $J = 7.2$ Hz, 1H, coumarin), 7.76–7.10 (m, 8H, Ar–H), 6.68–6.51 (m, 3H, coumarin), 6.16 (s, 1H, coumarin), 5.26 (s, 2H, $–NH_2$),

4.49 (s, 1H, –O*H*); ^{13}C NMR (DMSO-d_6, δ, ppm): 162.71 (*C*=O of coumarin), 160.17, 159.88 (2C, pyrimidine), 158.02 (*C*–OH), 157.85 (*C*–OH), 154.89 (C–NH_2), 149.72 (*C*–NH at coumarin), 113.40 (1C, pyrimidine), 148.16, 142.87, 133.41, 132.49, 131.53, 130.18, 130.04, 129.86, 128.90, 128.32, 124.56, 124.12, 119.33, 118.51, 118.32, 117.82, 92.18 (17C, Ar–C); EMI-MS (*m/z*): 439.13 (M^+); Anal. Calcd for $C_{25}H_{18}N_4O_4$: C, 68.49; H, 4.14; N, 12.78. Found: C, 68.54; H, 4.19; N, 12.72.

Synthesis of 2-amino-6-[4-(2-oxo-2*H*-chromen-4-ylamino)-phenyl]-4-phenyl-nicotinonitrile (**8a**)

A mixture of **5a** (2.0 g, 0.005 mol), malononitrile (0.33 g, 0.005 mol), and ammonium acetate (0.77 g, 0.01) in ethanol (30 mL) was refluxed for 8–10 h. After cooling, the reaction mixture was poured into crushed ice. The product separated out was filtered, washed with water, dried, and recrystallized from DMF to give compound **8a**. Similarly, other compounds **8**(**b**–**k**) were synthesized.

Yield: 73 %. M.p. 280–281 °C; IR (KBr, cm^{-1}): 3,391 (–NH_2), 3,287 (–NH), 2,217 (C≡N), 1,670 (C=O of coumarin); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.82 (s, 1H, –NH), 8.05 (s, 1H, pyridine), 7.82 (d, $J = 7.4$ Hz, 1H, coumarin), 7.89–7.17 (m, 9H, Ar–H), 6.74–6.59 (m, 3H, coumarin), 6.20 (s, 1H, coumarin), 5.38 (s, 2H, –NH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 163.25 (1C, pyridine), 162.63 (*C*=O of coumarin), 157.11 (1C, pyridine), 156.13 (*C*–NH_2), 149.28 (*C*–NH at coumarin), 116.29 (*C*≡N), 115.69 (1C, pyridine), 148.56, 143.50, 133.10, 131.95, 131.55, 130.21, 130.17, 130.11, 129.94, 129.53, 128.80, 128.57, 125.15, 124.89, 119.31, 118.70, 118.57, 117.28, 93.15 (19C, Ar–C), 86.13 (*C*–C≡N); EMI-MS (*m/z*): 431.52 (M^+); Anal. Calcd for $C_{27}H_{18}N_4O_2$: C, 75.34; H, 4.21; N, 13.02. Found: C, 75.30; H, 4.26; N, 13.08.

2-Amino-4-(2,4-dichloro-phenyl)-6-[4-(2-oxo-2*H*-chromen-4-ylamino)-phenyl]-nicotinonitrile (**8b**)

Yield: 71 %. M.p. 246–248 °C; IR (KBr, cm^{-1}): 3,386 (–NH_2), 3,283 (–NH), 2,211 (C≡N), 1,674 (C=O of coumarin), 804 (–Cl); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.89 (s, 1H, –NH), 8.09 (s, 1H, pyridine), 7.84 (d, $J = 7.1$ Hz, 1H, coumarin), 7.80–7.20 (m, 7H, Ar–H), 6.71–6.53 (m, 3H, coumarin), 6.11 (s, 1H, coumarin), 5.32 (s, 2H, –NH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 163.57 (1C, pyridine), 162.34 (*C*=O of coumarin), 157.47 (1C, pyridine), 155.83 (*C*–NH_2), 149.67 (*C*–NH at coumarin), 134.42 (*C*–Cl), 133.87 (*C*–Cl), 116.61 (*C*≡N), 115.52 (1C, pyridine), 147.19, 143.18, 133.27, 132.52, 131.35, 130.88, 130.21, 129.54, 128.86, 128.53, 124.72, 124.66, 119.43, 118.30, 118.15, 117.44, 92.56 (17C, Ar–C), 85.78 (*C*–C≡N); EMI-MS (*m/z*): 500.14 (M^+); Anal. Calcd for $C_{27}H_{16}Cl_2N_4O_2$: C, 64.94; H, 3.23; N, 11.22. Found: C, 64.89; H, 3.27; N, 11.17.

2-Amino-6-[4-(2-oxo-2*H*-chromen-4-ylamino)-phenyl]-4-*p*-tolyl-nicotinonitrile (**8c**)

Yield: 67 %. M.p. 284–285 °C; IR (KBr, cm^{-1}): 3,394 (–NH_2), 3,290 (–NH), 2,215 (C≡N), 1,668 (C=O of coumarin); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.87 (s, 1H, –NH), 8.11 (s, 1H, pyridine), 7.83 (d, $J = 7.2$ Hz, 1H, coumarin), 7.78–7.12 (m, 8H, Ar–H), 6.67–6.50 (m, 3H, coumarin), 6.14 (s, 1H, coumarin), 5.37 (s, 2H, –NH_2), 2.11 (s, 3H, Ph–CH_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 162.89 (1C, pyridine), 162.70 (*C*=O of coumarin), 156.81 (1C, pyridine), 156.43 (*C*–NH_2), 149.17 (*C*–NH at coumarin), 138.87 (*C*–CH_3 of phenyl), 116.38 (*C*≡N), 115.41 (1C, pyridine), 148.12, 143.16, 133.49, 132.40, 131.90, 130.08, 129.85, 129.77, 129.15, 128.67, 128.11, 125.11, 124.79, 119.69, 118.57, 118.44, 117.10, 93.07 (18C, Ar–C), 22.13 (C–*C*H_3 of phenyl), 85.91 (*C*–C≡N); EMI-MS (*m/z*): 445.20 (M^+); Anal. Calcd for $C_{28}H_{20}N_4O_2$: C, 75.66; H, 4.54; N, 12.60. Found: C, 75.74; H, 4.61; N, 12.52.

2-Amino-4-(4-fluoro-phenyl)-6-[4-(2-oxo-2*H*-chromen-4-ylamino)-phenyl]-nicotinonitrile (**8d**)

Yield: 77 %. M.p. 297–299 °C; IR (KBr, cm^{-1}): 3,383 (–NH_2), 3,281 (–NH), 2,206 (C≡N), 1,669 (C=O of coumarin), 1,167 (C–F); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.80 (s, 1H, –NH), 8.03 (s, 1H, pyridine), 7.85 (d, $J = 7.3$ Hz, 1H, coumarin), 7.83–7.15 (m, 8H, Ar–H), 6.67–6.49 (m, 3H, coumarin), 6.15 (s, 1H, coumarin), 5.27 (s, 2H, –NH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 168.79 (*C*–F), 163.10 (1C, pyridine), 162.50 (*C*=O of coumarin), 157.23 (1C, pyridine), 155.70 (*C*–NH_2), 149.72 (*C*–NH at coumarin), 116.47 (*C*≡N), 115.55 (1C, pyridine), 148.15, 142.95, 133.91, 133.06, 131.45, 130.13, 129.48, 129.10, 128.90, 128.56, 127.10, 124.80, 124.51, 119.52, 118.71, 118.66, 117.07, 92.22 (18C, Ar–C), 86.28 (*C*–C≡N); EMI-MS (*m/z*): 449.28 (M^+); Anal. Calcd for $C_{27}H_{17}FN_4O_2$: C, 72.31; H, 3.82; N, 12.49. Found: C, 72.24; H, 3.73; N, 12.57.

2-Amino-4-(4-chloro-phenyl)-6-[4-(2-oxo-2*H*-chromen-4-ylamino)-phenyl]-nicotinonitrile (**8e**)

Yield: 75 %. M.p. >300 °C; IR (KBr, cm^{-1}): 3,393 (–NH_2), 3,285 (–NH), 2,215 (C≡N), 1,663 (C=O of coumarin), 819 (–Cl); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.82 (s, 1H, –NH), 8.13 (s, 1H, pyridine), 7.84 (d, $J = 7.5$ Hz, 1H, coumarin), 7.83–7.16 (m, 8H, Ar–H), 6.69–6.51 (m, 3H, coumarin), 6.17 (s, 1H, coumarin), 5.40 (s, 2H, –NH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 163.89 (1C, pyridine), 162.43 (*C*=O of coumarin), 156.63 (1C, pyridine), 155.78

(*C*–NH_2), 149.44 (*C*–NH at coumarin), 115.92 (*C*≡N), 115.17 (1C, pyridine), 134.05 (*C*–Cl), 147.68, 143.56, 133.22, 132.59, 130.97, 130.62, 130.11, 129.90, 129.75, 128.89, 128.05, 123.87, 124.17, 119.86, 118.42, 118.15, 117.66, 93.16 (18C, Ar–C), 86.25 (*C*–C≡N); EMI-MS (*m/z*): 465.76 (M^+); Anal. Calcd for $C_{27}H_{17}ClN_4O_2$: C, 69.75; H, 3.69; N, 12.05. Found: C, 69.83; H, 3.63; N, 12.10.

2-Amino-4-(4-nitro-phenyl)-6-[4-(2-oxo-2*H*-chromen-4-ylamino)-phenyl]-nicotinonitrile (**8f**)

Yield: 70 %. M.p. 236–237 °C; IR (KBr, cm^{-1}): 3,390 (–NH_2), 3,288 (–NH), 2,212 (C≡N), 1,549 (N=O str.), 1,672 (C=O of coumarin); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.84 (s, 1H, –NH), 8.10 (s, 1H, pyridine), 7.88 (d, $J = 7.4$ Hz, 1H, coumarin), 7.85–7.24 (m, 8H, Ar–H), 6.72–6.51 (m, 3H, coumarin), 6.16 (s, 1H, coumarin), 5.31 (s, 2H, –NH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 162.85 (1C, pyridine), 162.60 (*C*=O of coumarin), 157.09 (1C, pyridine), 155.43 (*C*–NH_2), 149.76 (*C*–NH at coumarin), 116.55 (*C*≡N), 115.83 (1C, pyridine), 148.87 (*C*–NO_2), 147.16, 143.22, 133.76, 132.87, 131.53, 130.85, 130.11, 129.79, 129.45, 128.23, 128.15, 124.69, 124.44, 119.54, 118.33, 117.83, 117.29, 92.20 (18C, Ar–C), 86.10 (*C*–C≡N); EMI-MS (*m/z*): 476.61 (M^+); Anal. Calcd for $C_{27}H_{17}N_5O_4$: C, 68.21; H, 3.60; N, 14.73. Found: C, 68.16; H, 3.69; N, 14.68.

2-Amino-4-(2-hydroxy-phenyl)-6-[4-(2-oxo-2*H*-chromen-4-ylamino)-phenyl]-nicotinonitrile (**8g**)

Yield: 82 %. M.p. >300 °C; IR (KBr, cm^{-1}): 3,395 (–NH_2), 3,280 (–NH), 3,252 (–OH), 2,221 (C≡N), 1,667 (C=O of coumarin); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.81 (s, 1H, –NH), 8.15 (s, 1H, pyridine), 7.80 (d, $J = 7.3$ Hz, 1H, coumarin), 7.77–7.12 (m, 8H, Ar–H), 6.68–6.52 (m, 3H, coumarin), 6.21 (s, 1H, coumarin), 5.28 (s, 2H, –NH_2), 4.61 (s, 1H, –O*H*); ^{13}C NMR (DMSO-d_6, δ, ppm): 163.91 (1C, pyridine), 161.92 (*C*=O of coumarin), 158.78 (*C*–OH), 157.18 (1C, pyridine), 156.06 (*C*–NH_2), 149.44 (*C*–NH at coumarin), 116.78 (*C*≡N), 115.31 (1C, pyridine), 148.33, 143.51, 133.69, 132.54, 131.16, 130.83, 130.14, 130.06, 129.97, 128.85, 128.67, 125.59, 124.87, 119.13, 118.53, 118.37, 117.46, 93.11 (18C, Ar–C), 85.92 (*C*–C≡N); EMI-MS (*m/z*): 447.19 (M^+); Anal. Calcd for $C_{27}H_{18}N_4O_3$: C, 72.64; H, 4.06; N, 12.55. Found: C, 72.72; H, 4.02; N, 12.47.

2-Amino-4-(2-bromo-phenyl)-6-[4-(2-oxo-2*H*-chromen-4-ylamino)-phenyl]-nicotinonitrile (**8h**)

Yield: 74 %. M.p. 260–270 °C; IR (KBr, cm^{-1}): 3,389 (–NH_2), 3,284 (–NH), 2,218 (C≡N), 1,662 (C=O of coumarin), 740 (–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.87 (s, 1H, –NH), 8.11 (s, 1H, pyridine), 7.84 (d, $J = 7.2$ Hz, 1H, coumarin), 7.80–7.15 (m, 8H, Ar–H), 6.71–6.49 (m, 3H, coumarin), 6.17 (s, 1H, coumarin), 5.35 (s, 2H, –NH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 163.34 (1C, pyridine), 162.15 (*C*=O of coumarin), 156.91 (1C, pyridine), 156.47 (*C*–NH_2), 149.37 (*C*–NH at coumarin), 116.88 (*C*≡N), 115.20 (1C, pyridine), 122.16 (*C*–Br), 147.13, 142.70, 133.18, 133.05, 131.80, 130.56, 129.32, 129.13, 129.04, 128.42, 127.77, 124.62, 124.53, 119.30, 118.87, 118.32, 117.22, 92.67 (18C, Ar–C), 85.63 (*C*–C≡N); EMI-MS (*m/z*): 510.35 (M^+); Anal. Calcd for $C_{27}H_{17}BrN_4O_2$: C, 63.67; H, 3.36; N, 11.00. Found: C, 63.74; H, 3.30; N, 11.09.

2-Amino-4-(4-methoxy-phenyl)-6-[4-(2-oxo-2*H*-chromen-4-ylamino)-phenyl]-nicotinonitrile (**8i**)

Yield: 84 %. M.p. >300 °C; IR (KBr, cm^{-1}): 3,391 (–NH_2), 3,285 (–NH), 2,204 (C≡N), 1,676 (C=O of coumarin), 1,279 (–OCH_3); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.83 (s, 1H, –NH), 8.07 (s, 1H, pyridine), 7.90 (d, $J = 7.5$ Hz, 1H, coumarin), 7.86–7.23 (m, 8H, Ar–H), 6.73–6.55 (m, 3H, coumarin), 6.20 (s, 1H, coumarin), 5.25 (s, 2H, –NH_2), 3.76 (s, 3H, –O*C*H_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 163.11 (1C, pyridine), 162.58 (*C*=O of coumarin), 160.87 (*C*–OCH_3), 157.18 (1C, pyridine), 156.25 (*C*–NH_2), 149.57 (*C*–NH at coumarin), 116.33 (*C*≡N), 115.47 (1C, pyridine), 148.27, 143.83, 132.62, 131.92, 131.13, 130.33, 130.01, 129.80, 129.45, 128.78, 128.27, 125.12, 124.67, 119.53, 118.73, 118.61, 117.22, 92.17 (18C, Ar–C), 55.72 (–O*C*H3), 86.05 (*C*–C≡N); EMI-MS (*m/z*): 461.44 (M^+); Anal. Calcd for $C_{28}H_{20}N_4O_3$: C, 73.03; H, 4.38; N, 12.17. Found: C, 73.10; H, 4.28; N, 12.27.

2-Amino-4-(3,4-dimethoxy-phenyl)-6-[4-(2-oxo-2*H*-chromen-4-ylamino)-phenyl]-nicotinonitrile (**8j**)

Yield: 78 %. M.p. 285–287 °C; IR (KBr, cm^{-1}): 3,396 (–NH_2), 3,290 (–NH), 2,213 (C≡N), 1,668 (C=O of coumarin), 1,284 (–OCH_3); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.79 (s, 1H, –NH), 8.05 (s, 1H, pyridine), 7.86 (d, $J = 7.4$ Hz, 1H, coumarin), 7.77–7.08 (m, 7H, Ar–H), 6.68–6.47 (m, 3H, coumarin), 6.17 (s, 1H, coumarin), 5.33 (s, 2H, –NH_2), 3.89 (s, 3H, –O*C*H_3), 3.86 (s, 3H, –O*C*H_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 163.76 (1C, pyridine), 162.55 (*C*=O of coumarin), 160.43 (*C*–OCH_3), 159.87 (*C*–OCH_3), 157.28 (1C, pyridine), 156.04 (*C*–NH_2), 149.47 (*C*–NH at coumarin), 116.13 (*C*≡N), 115.58 (1C, pyridine), 147.64, 143.28, 133.56, 132.30, 131.67, 130.35, 130.19, 129.85, 128.86, 128.39, 124.33, 124.15, 119.49, 118.53, 118.24, 117.22, 92.54 (17C, Ar–C), 85.87 (*C*–C≡N), 56.15 (–O*C*H3); EMI-MS (*m/z*): 491.63 (M^+);

Anal. Calcd for $C_{29}H_{22}N_4O_4$: C, 71.01; H, 4.52; N, 11.42. Found: C, 71.05; H, 4.56; N, 11.34.

2-Amino-4-(2,4-dihydroxy-phenyl)-6-[4-(2-oxo-2*H*-chromen-4-ylamino)-phenyl]-nicotinonitrile (**8k**)

Yield: 73 %. M.p. >300 °C; IR (KBr, cm^{-1}): 3,386 ($-NH_2$), 3,282 (–NH), 3,266 (–OH), 2,216 (C≡N), 1,671 (C=O of coumarin); ^1H NMR (DMSO-d_6, δ, ppm): 8.84 (s, 1H, –NH), 8.51 (s, 1H, –O*H*), 8.12 (s, 1H, pyridine), 7.79 (d, $J = 7.6$ Hz, 1H, coumarin), 7.76–7.19 (m, 7H, Ar–H), 6.64–6.42 (m, 3H, coumarin), 6.15 (s, 1H, coumarin), 5.36 (s, 2H, $-NH_2$), 4.53 (s, 1H, –O*H*); ^{13}C NMR (DMSO-d_6, δ, ppm): 163.20 (1C, pyridine), 162.17 (*C*=O of coumarin), 157.83 (*C*–OH), 157.21 (1C, pyridine), 156.24 (*C*–OH), 155.85 (*C*–NH_2), 149.69 (*C*–NH at coumarin), 116.13 (*C*≡N), 115.70 (1C, pyridine), 147.26, 143.32, 133.58, 132.87, 131.40, 130.21, 129.54, 129.05, 128.82, 127.46, 125.02, 124.86, 119.44, 118.32, 118.12, 117.61, 92.44 (17C, Ar–C), 86.08 (*C*–C≡N); EMI-MS (*m*/*z*): 463.29 (M^+); Anal. Calcd for $C_{27}H_{18}N_4O_4$: C, 70.12; H, 3.92; N, 12.12. Found: C, 70.20; H, 3.83; N, 12.05.

Antimicrobial assay

The synthesized compounds **8a**–**n**, **10a**–**n**, and **12a**–**n** were examined for antimicrobial activity against several bacteria (*Staphylococcus aureus* MTCC 96, *Bacillus cereus* MTCC 430, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 741, *Klebsiella pneumoniae* MTCC 109, *Salmonella typhi* MTCC 733, *Proteus vulgaris* MTCC 1771, *and Shigella flexneri* MTCC 1457); and fungi (*Aspergillus niger* MTCC 282, *Aspergillus fumigatus* MTCC 343, *Aspergillus clavatus* MTCC 1323, and *Candida albicans* MTCC 183) species using paper disk diffusion technique (Gillespie, 1994). The sterilized (autoclaved at 120 °C for 30 min) medium (40–50 °C) was inoculated (1 mL/100 mL of medium) with the suspension (10^5 cfu mL^{-1}) of the microorganism (matched to McFarland barium sulfate standard) and poured into a petri dish to give a depth of 3–4 mm. The paper impregnated with the test compounds (μg mL^{-1}) in dimethyl sulfoxide) was placed on the solidified medium. The plates were pre-incubated for 1 h at room temperature and incubated at 37 °C for 24 and 48 h for antibacterial and antifungal activities, respectively. Ciprofloxacin (100 μg/disk) and Ketoconazole (100 μg/disk) were used as standards for antibacterial and antifungal activities, respectively. The observed zone of inhibition is presented in Tables 1, 2, and 3. MIC of the compound was determined by agar streak dilution method (Hawkey and Lewis, 1994). A stock solution of the synthesized compound (100 μg mL^{-1}) in dimethyl sulfoxide was prepared, and graded quantities of the test compounds were incorporated in specified quantity of molten sterile agar (nutrient agar for antibacterial activity and sabouraud dextrose agar medium for antifungal activity). A specified quantity of the medium (40–50 °C) containing the compound was poured into a petri dish to give a depth of 3–4 mm and allowed to solidify. A suspension of the microorganism was prepared to contain approximately 10^5 cfu mL^{-1} and applied to plates with serially diluted compounds in dimethyl sulfoxide to be tested and incubated at 37 °C for 24 and 48 h for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate. The observed MIC is presented in Tables 1, 2, and 3.

Antimycobacterial assay

The antimycobacterial screening for the test compounds was performed against *M. tuberculosis* H37Rv using L. J. (Lowenstein and Jensen) MIC method (Isenberg, 1992) for the measurement of MIC. Stock solutions of primary: 1000, 500, and 250 μg/mL, and secondary: 200, 100, 62.5, 50, 25, 12.5, 6.25, and 3.25 μg/mL dilutions of each test compound in dimethylsulfoxide (DMSO) were added in the liquid L. J. Medium, and then media were sterilized by inspissation method. A culture of *M. tuberculosis* H37Rv growing on L. J. medium was harvested in 0.85 % saline in bijou bottles. These tubes were then incubated at (37 ± 1) °C for 24 h followed by streaking of *M. tuberculosis* H37Rv (5×10^4 bacilli per tube). These tubes were then incubated at (37 ± 1) °C. Growth of bacilli was seen after 12 days, 22 days, and finally 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* H37Rv. The concentration, at which no development of colonies occurred or <20 colonies, was taken as MIC concentration of test compound. The standard strain *M. tuberculosis* H37Rv was tested with known drugs rifampicin, isoniazid, ethambutol, and pyrazinamide.

References

Al-Soud YA, Al-Masoudi IA, Saeed B, Beifuß U, Al-Masoudi NA (2006) Synthesis of new 1H-1,2,4-triazolylcoumarins and their antitumor and anti-HIV activities. Chem Heterocycl Compd 42:583–590

Amit RT, Dipti KD, Naresh RR, Viresh HS (2008) Synthesis and biological evaluation of new pyrimidines via a novel chalcone series. Arkivoc 11:131–141

Anjani S, Kishor K, Ana C, Marina S, Irini D, Athina G (2010) Synthesis of some new s-triazine based chalcones and their derivatives as potent antimicrobial agents. Eur J Med Chem 45:510–518

Anto RJ, Sukumaran K, Kuttana G, Rao MNA, Subbaraju V, Kuttana R (1995) Anticancer and antioxidant activity of synthetic chalcones and related compounds. Cancer Lett 97:33–37

Babasaheb PB, Shrikant SG, Ragini GB, Nalini MG, Chandrahasya NK (2009) Synthesis and biological evaluation of a novel series of pyrazole chalcones as anti-inflammatory, antioxidant and antimicrobial agents. Bioorg Med Chem 17:8168–8173

Bedoya LM, Beltrán M, Sancho R, Olmedo DA, Sánchez-Palomino S, del Olmo E, López-Pérez JL, Muñoz E, San Feliciano A, Alcamí J (2005) 4-Phenylcoumarins as HIV transcription inhibitors. Bioorg Med Chem Lett 15:4447–4450

Belluti F, Fontana G, Dal Bo L, Carenini N, Giommarelli C, Zunino F (2010) Design, Synthesis and anticancer activities of stilbene-coumarin hybrid compounds: Identification of novel proapoptotic agents. Bioorg Med Chem 18:3543–3550

Bhat BA, Dhar KL, Puri SC, Saxena AK, Shanmugavel M, Qazi GN (2005) Synthesis and biological evaluations of chalcones and their derived pyrazoles as potential cytotoxic agents. Bioorg Med Chem Lett 15:3177–3180

Divyesh P, Rahul P, Premlata K, Navin P (2011) In vitro antimicrobial assessment of coumarin-based s-triazinyl piperazines. Med Chem Res. doi:10.1007/s00044-011-9676-3

Gillespie SH (1994) Medical microbiology-illustrated. Butterworth Heinemann Ltd., United Kingdom, pp 234–247

Hawkey PM, Lewis DA (1994) Medical bacteriology—a practical approach. Oxford University Press, United Kingdom, pp 181–194

Hsu YL, Kuo PL, Tzeng WS, Lin CC (2006) Chalcone inhibits the proliferation of human breast cancer cell by blocking cell cycle progression and inducing apoptosis. Food Chem Toxicol 44:704–713

Hussain M, Hussain MT, Rama NH, Hameed S, Malik A, Khan KM (2003) Synthesis and antimicrobial activities of some isocoumarin and dihydroisocoumarin derivatives. Nat Prod Res 17: 207–214

Isenberg HD (1992) Clinical microbiology procedures handbook, vol 1. American Society for Microbiology, Washington, DC

Khalid MK, Zafar SS, Muhammad ZK, Zia-Ullah, Iqbal Choudhary M, Atta-ur-Rahman, Shahnaz P, Zahid HC, Claudiu TS (2004) Synthesis of coumarin derivatives with cytoxic, antibacterial and antifungal activity. J Enzyme Inhib Med Chem 19:373–379

Kishor HC, Mayank JP, DHaval BV (2008) Design, synthesis and evaluation of novel quinolyl chalcones as antibacterial agents. Arkivoc 13:189–197

Koneni VS, Manoj K, Ram KM, Ravi S, Gitika B, Khanna Ak, Shivika R, Rakesh S (2011) Synthesis and anti-inflammatory activity of novel biscoumarin–chalcone hybrids. Bioorg Med Chem Lett 21:4480–4484

Kostova I, Bhatia S, Grigorov P, Balkansky S, Parmar VS, Prasad AK, Saso L (2011) Coumarins as antioxidants. Curr Med Chem 18:3929–3951

Kováč M, Sabatié A, Floch Ľ (2001) Synthesis of coumarin sulfonamides and sulfonylurea. Arkivoc 6:100–108

Laughon BE (2007) New tuberculosis drugs in development. Curr Top Med Chem 7:463–467

Lee SH, Seo GS, Kim JY, Jin XY, Kim HD, Sohn DH (2006) Heme oxygenase 1 mediates anti-inflammatory effects of 2′,4′,6′-tris(methoxymethoxy) chalcone. Eur J Pharmacol 532:178–186

Liu M, Wilairat P, Croft SL, Tan AL, GO M (2003) Structure-activity relationship of anti-leishmanial and antimalarial chalcones. Bioorg Med Chem 11:2729–2738

López SN, Castelli MV, Zacchino SA, Domínguez JN, Lobo G, Charris-Charris J, Cortés JC, Ribas, Devia C, Rodríguez AM, Enriz RD (2001) In vitro antifungal evaluation and structure–activity relationships of a new series of chalcone derivatives and synthetic analogues, with inhibitory properties against polymers of the fungal cell wall. Bioorg Med Chem 9:1999–2013

Manolov I, Danchev ND (1995) Synthesis, toxicological and pharmacological assessment of some 4-hydroxycoumarin. Eur J Med Chem 30:531–536

Nida NF, Mudasir RB, Anis A, Asad UK, Abdul R (2011) 7-Hydroxycoumarin derivatives: synthesis, characterization and preliminary antimicrobial activities. Med Chem Res 20:535–541

Nitin K, Alka C, Sushma D (2011) Synthesis of cyanopyridine and pyrimidine analogues as new anti-inflammatory and antimicrobial agents. Biomed Pharmacother 65:375–380

Nowakowska Z (2007) A review of antiinfective and anti-inflammatory chalcones. Eur J Med Chem 42:125–137

Tenover FC, McDonald LC (2005) Vancomycin-resistant staphylococci and enterococci: epidemiology and control. Curr Opin Infect Dis 18:300–305

Tomar V, Bhattacharjee G, Kamaluddin, Kumar A (2007) Synthesis and antimicrobial evaluation of new chalcones containing piperazine or 2,5-dichlorothiophene moiety. Bioorg Med Chem Lett 17:5321–5324

Yi X, Zheng-Yu Y, Peng X, Kenneth FB, Yuka N, Kuo-Hsiung L (2000) Antitumor agents. Part 202: Novel 2′-amino chalcones: design, synthesis and biological evaluation. Bioorg Med Chem Lett 10:699–701

Zhang Y, Shi S, Zhao M, Jiang Y, Tu P (2006) A novel chalcone from *Coreopsis tinctoria* nutt. Biochem Syst Ecol 34:766–769