Full Paper

Synthesis, *in-vitro* Antimicrobial and Cytotoxic Studies of Novel Azetidinone Derivatives

Rangappa S. Keri, Kallappa M. Hosamani, Harisha S. Reddy, and Ramya V. Shingalapur

P. G. Department of Studies in Chemistry, Karnatak University, Pavate Nagar, Dharwad, India

Developing novel antimicrobial drugs is increasingly important in the modern pharmaceutical industry. A series of novel 3-chloro-4-[4-(2-oxo-2*H*-chromen-4-ylmethoxy)phenyl]-1-phenylazetidin-2-ones **5a–o** have been synthesized from 4-bromomethylcoumarins **1a–e** and 4-arylimino-methyl-phenols **3a–c**. These compounds were screened for their *in-vitro* antibacterial activity against two Gram-positive (*Staphylococcus aureus* and *Vancomycin resistant enteroccoccus*) and two Gram-negative (*Escherichia coli* and *Shigella dysentery*) bacterial strains and antifungal activity against *Aspergillus fumigatus*, *Candida albicans*, and *Penicillium.* Results revealed that compounds **5c**, **5f**, **5h**, **5j**, and **5m** showed excellent activity against a panel of microorganisms. The brine-shrimp bioassay was also carried out to study their *in-vitro* cytotoxic properties and two compounds, **5h** and **5m**, possessing $LD_{50} = 7.154 \times 10^{-4}$ M and 5.782×10^{-4} M, respectively, displayed potent cytotoxic activity against *Artemia salina*. The presence of a chlorine group in the coumarin moiety, its effect on their antibacterial, antifungal, and cytotoxic activities is discussed. All newly synthesized compounds were characterized by elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR, and MS.

Keywords: Antibacterial / Antifungal / Azetidinone / Coumarin / Cytotoxicity

Received: August 7, 2009; accepted: November 25, 2009

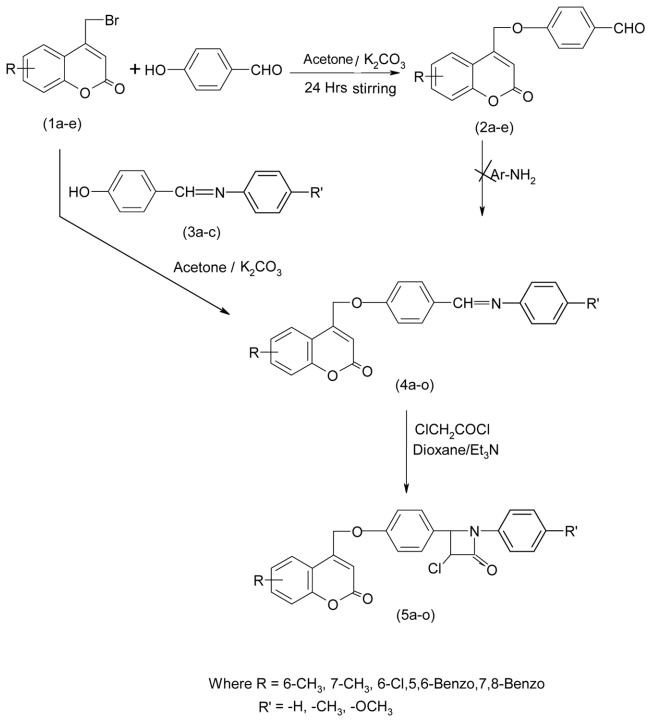
DOI 10.1002/ardp.200900188

Introduction

The treatment of infectious diseases still remains an important and challenging problem caused by a combination of factors; among them emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens. In spite of a large number of antibiotics and chemotherapeutics available for medical use, the emergence of old and new antibiotic resistance created in the last decades revealed a substantial medical need for new classes of antimicrobial agents. There is a real perceived need for the discovery of new compounds endowed with antimicrobial activity, possibly acting through mechanisms, which are distinct from those of well-known classes of antibacterial agents, to which many clinically relevant pathogens are now resistant. Through various molecules designed and synthesized for this aim, it was demonstrated that azetidinone and its derivatives could be considered as possible antimicrobial agents [1, 2].

2-Azetidinones, commonly known as β-lactams, are heterocyclic compounds well-known among organic and medicinal chemists [3]. The activity of the famous antibiotics such as penicillins, cephalosporins, thienamycine, nocardicins, aztreonam, and carbapenems are attributed to the presence of the 2-azetidinone ring in them. Azetidinones are a very important class of compounds possessing a wide range of biological activities such as antimicrobial [4], anti-inflammatory [5], anticonvulsant [6], antibiotic [7], anticancer [8], anti-elastase [9], antiviral [10], antitumor [4], and anti-HCMV [11] activities. Recently, it has been reported that β-lactams have novel biological properties such as acting as cytomegalovirus protease inhibitors [12], thrombin and tryptase inhibitors [13], cholesterol absorption inhibitors [14], human leukocyte

Correspondence: Kallappa M. Hosamani, P .G. Department of Studies in Chemistry, Karnatak University, Pavate Nagar, Dharwad-580 003, India.
E-mail: dr_hosamani@yahoo.com
Fax: +91 836 274-7884 and +91 836 277-1275



Scheme 1. Schematic representation for the synthesis of azetidinone derivatives from 4-bromo-methyl coumarin.

elastease (HLE) inhibitors [15], porcine pancreatic elastase (PPE) inhibitors [16], HIV-1 protease [17], and peroxisome proliferator-activated receptors (PPARs) [18]. Besides their biological activities, the importance of β-lactams as synthetic intermediates has been widely recognized in organic synthesis [19] for example in the semi-synthesis of Taxol [20]. Like azetidinone, coumarin also exhibits diverse biological properties [21].

In the design of new drugs, the development of hybrid molecules through the combination of different pharmacophores in one frame may lead to compounds with interesting biological profiles. Owing to the importance and in continuation of our work of the synthesis of antimicrobially active compounds [22–24], we now wish to describe the synthesis of new azetidinone derivatives from 4-aryloxy-methyl coumarins (Scheme 1) and the *in-vitro* screening results of their antibacterial, antifungal, and cytotoxic activities.

Results and discussion

Chemistry

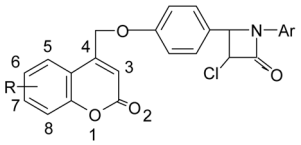
The present synthetic strategy begins with the generation of the required 4-bromo-methyl coumarins **1a–e** by Pechmann cyclization of phenols with 4-bromo-ethyl acetoacetate. Condensation of **1a** with 4-hydroxy benzaldehyde in presence of anhydrous K_2CO_3 yielded 2-oxo-2*H*-chromen-4-ylmethoxy)benzaldehydes **2a–e**. In the IR spectrum of 4-(2-oxo-2*H*-chromen-4-yl-methoxy)-benzaldehyde **2a**, the lactone-carbonyl stretching frequency was observed at 1718 cm^{-1}, whereas the aldehydic-carbonyl stretching appeared at 1690 cm^{-1}. In the ^{1}H-NMR spectrum of compound **2a**, a singlet was observed at δ = 2.38 ppm due to C_6-CH_3 protons. The C_4-CH_2 protons were observed downfield as a singlet at δ = 5.31 ppm. The C_3-H of coumarin appeared at δ = 6.64 ppm. The aldehydic proton appeared as a singlet in the downfield at δ = 9.90 ppm. The ^{13}C-NMR spectral data of compound **2a** is given in the experimental section. In view of the poor reactivity of the *p*-carbonyl group observed in 4-aryloxy-methyl coumarins, pre-functionalized phenols **3a–c** (obtained by the reaction of *p*-formyl phenol and aromatic amines) were used for room-temperature allylic S_N reaction under standard acetone potassium carbonate conditions generating the required precursors **4a–o** in high yields. The IR spectrum of 6-methyl-4-(4-phenyliminomethyl-phenoxymethyl)-chromen-2-one **4a** (R = 6-CH_3, R_1 = H) showed a lactone-carbonyl stretching frequency at 1718 cm^{-1} and the stretching frequency for the C=N group at 1605 cm^{-1}. The PMR spectrum of **4a** exhibited a singlet due to the CH_3 protons at *d* = 2.32 ppm, C_4-CH_2 at δ = 5.31 ppm, and C_3-H at δ = 6.43 ppm. The aromatic protons resonated in the range of δ = 7.10–8.32 ppm. The azomethine proton appeared downfield as a singlet at δ = 9.83 ppm. The azomethine group in **4a–o** undergoing cycloaddition with chloroacetylchloride in the presence of triethylamine [Et_3N] as a catalyst in DMF/benzene afforded 3-chloro-4-[4-(2-oxo-2*H*-chromen-4-ylmethoxy)-phenyl]-1-phenyl-azetidin-2-ones **5a–o** (Scheme 1). The IR spectrum of 3-chloro-4-[4-(6-methyl-2-oxo-2*H*-chromen-4-ylmethoxy)-phenyl]-1-phenyl-azetidin-2-one **5a** showed bands at 1768 cm^{-1} (>C=O of β-lactam) and the coumarin-carbonyl stretching at 1722 cm^{-1}. In the PMR spectra of compound **5a**, the peak was observed at δ = 5.32 ppm due to the >CH–Cl in the β-lactam ring; in the ^{13}C-NMR spectrum of compound **5a**, a peak at δ = 53.3 ppm was observed due to >CH–Cl, at 184.4 ppm (cyclic, >C=O), and at 158.2 ppm (hetero-aromatics) in the β-lactam moiety. The mass spectrum of compound **5a** showed the molecular ion peak at 446 [M^+ + 1].

Biological evaluation

Antibacterial bioassay

The synthesized compounds **5a–o** were screened *in vitro* for their antibacterial activity against two Gram-positive (*Staphylococcus aureus*, *Vancomycin*-resistant *enteroccoccus*) and two Gram-negative (*Escherichia coli*, *Shigella dysentery*) bacterial strains by agar well diffusion method [25]. The

Table 1. Physical and analytical data of the 2-azetidinone derivatives.



Entry	Product[a)]	R	Ar	Yield (%)	M. p.[b)] (°C)	Mol. Formula / Mol. Wt.	Elemental Analysis (Calcd./Found)		
							C	H	N
1	**5a**	6-CH_3	Ph	88.00	138 – 140	$C_{26}H_{20}ClNO_4$	70.03	4.52	3.14
						446	70.08	4.56	3.17
2	**5b**	7-CH_3	Ph	71.00	114 – 116	$C_{26}H_{20}ClNO_4$	70.03	4.52	3.14
						446	70.06	4.55	3.18
3	**5c**	6-Cl	Ph	62.80	118 – 120	$C_{25}H_{17}Cl_2NO_4$	64.39	3.67	3.00
						466	64.43	3.69	2.98
4	**5d**	5,6-Benzo	Ph	74.10	198 – 200	$C_{29}H_{20}ClNO_4$	72.27	4.18	2.92
						482	72.29	4.23	2.96
5	**5e**	7,8-Benzo	Ph	65.12	232 – 234	$C_{29}H_{20}ClNO_4$	72.27	4.18	2.92
						482	72.26	4.20	2.90
6	**5f**	6-CH_3	4-MeC_6H_4	64.20	132 – 134	$C_{27}H_{22}ClNO_4$	70.51	4.82	3.05
						460	70.50	4.84	3.04
7	**5g**	7-CH_3	4-MeC_6H_4	71.80	208 – 210	$C_{27}H_{22}ClNO_4$	70.51	4.82	3.05
						460	70.55	4.82	3.01
8	**5h**	6-Cl	4-MeC_6H_4	82.4	183 – 185	$C_{26}H_{19}Cl_2NO_4$	65.01	3.99	2.92
						481	65.05	4.00	2.90
9	**5i**	5,6-Benzo	4-MeC_6H_4	71.12	220 – 222	$C_{30}H_{22}ClNO_4$	72.65	4.47	2.82
						496	72.61	4.50	2.84
10	**5j**	7,8-Benzo	4-MeC_6H_4	65.20	110 – 112	$C_{30}H_{22}ClNO_4$	72.65	4.47	2.82
						496	72.67	4.45	2.80
11	**5k**	6-CH_3	4-$MeOC_6H_4$	63.8	170 – 172	$C_{27}H_{22}ClNO_5$	68.14	4.66	2.94
						477	68.17	4.63	2.97
12	**5l**	7-CH_3	4-$MeOC_6H_4$	70.8	98 – 100	$C_{27}H_{22}ClNO_5$	68.14	4.66	2.94
						477	68.12	4.69	2.93
13	**5m**	6-Cl	4-$MeOC_6H_4$	63.4	130 – 132	$C_{26}H_{19}Cl_2NO_5$	62.92	3.86	2.82
						497	62.95	3.83	2.80
14	**5n**	5,6-Benzo	4-$MeOC_6H_4$	61.02	282 – 284	$C_{30}H_{22}ClNO_5$	70.38	4.33	2.74
						512	70.40	4.35	2.70
15	**5o**	7,8-Benzo	4-$MeOC_6H_4$	70.13	195 – 197	$C_{30}H_{22}ClNO_5$	70.38	4.33	2.74
						512	70.36	4.32	2.77

[a)] Products were characterized by IR, NMR, MS, and elemental analysis.
[b)] Yields of isolated compounds.
[c)] Melting points are uncorrected.

results were compared with those of the standard drug Ciprofloxacin. The zone of inhibition and percentage inhibition of the compounds are given in (Table 2). The results indicate that all the synthesized compounds are good to moderately active against a panel of microorganisms. Compounds **5c**, **5f**, **5h**, **5j**, and **5m** exhibited excellent activity against *S. aureus* and *V.*-r. *enteroccoccus.* Out of these, **5m** was found to be the most active. However, compound **5a**, **5b**, **5d**, and **5e** showed moderate activity against *S. aureus* and *V.*-r. *enteroccoccus.* Compound **5n** and **5o** were found to be inactive against *S. aureus* and *S. dysentery.* The compounds **5c**, **5h**, and **5m** with 6-Cl substitution in the coumarin ring have shown good activity for both the bacterial and fungal strains at 25 μg/mL concentration.

Antifungal bioassay

The antifungal screening of all compounds was carried out against *Aspergillus fumigatus*, *Candida albicans*, and *Penicillium* fungal strains. The results were compared with those of standard drug Gentamycin. These results (see Table 3) indicate that the compounds **5c**, **5f**, **5h**, **5j**, and **5m** exhibited excellent activity against all the fungal strains. The compounds **5f** and **5h** showed ~ 90% activity

Table 2. *In-vitro* antibacterial activities of synthesized compounds **5a–o** in terms of zone of inhibition and percentage inhibition.

Comp.	Zone of inhibition% (mm)											
	S. aureus Conc. (μg/mL)			*V.-r. enteroccoccus* Conc. (μg/mL)			*E. coli* Conc. (μg/mL)			*S. dysentery* Conc. (μg/mL)		
	100	50	25	100	50	25	100	50	25	100	50	25
5a	34[a]	17	10	45	17	13	40	23	12	39	18	12
	61[b]	57	29	72	57	59	67	64	60	67	56	55
5b	37	16	10	39	16	11	37	20	Nil	34	15	Nil
	66	53	55	63	53	50	62	56	–	58	47	–
5c	49	23	14	54	26	17	52	31	16	46	26	17
	87	77	78	87	87	77	87	86	80	79	81	77
5d	30	16	Nil	42	20	14	44	20	11	35	17	12
	54	53	–	68	67	64	73	56	55	60	53	54
5e	32	16	10	46	18	13	40	20	Nil	31	20	13
	57	53	55	74	60	59	67	56	–	53	62	59
5f	45	19	12	52	23	16	56	26	15	34	17	11
	80	63	55	84	77	73	93	72	70	59	53	50
5g	38	15	08	43	17	13	39	21	12	35	15	Nil
	68	50	44	69	57	59	65	58	60	60	46	–
5h	46	24	13	56	22	17	52	30	16	52	24	16
	82	80	72	90	73	77	87	83	80	90	75	73
5i	34	18	10	44	19	Nil	41	20	10	45	17	13
	61	60	56	71	63	–	68	56	50	78	53	59
5j	44	22	13	52	25	19	50	29	14	52	26	18
	79	73	72	84	83	86	83	81	70	90	81	82
5k	36	19	10	38	17	12	47	19	Nil	46	22	14
	64	63	56	61	57	55	78	53	–	79	69	64
5l	35	20	11	39	15	Nil	49	20	11	49	19	13
	62	66	61	63	50	–	82	56	55	84	59	59
5m	45	22	14	49	21	15	54	31	16	54	25	16
	80	73	67	79	70	68	90	86	80	93	78	73
5n	29	14	Nil	37	18	Nil	46	23	Nil	34	15	Nil
	52	47	–	60	60	–	77	64	–	59	47	–
5o	34	15	09	39	16	13	44	22	12	36	20	11
	51	50	50	63	53	59	73	61	60	62	62	50
Standard	56	30	18	62	30	22	60	36	20	58	32	22
Control	–	–	–	–	–	–	–	–	–	–	–	–

Standard used: Ciprofloxacin; control: DMSO.

[a] Zone of inhibition (first row).

[b] Percentage inhibition (second row).

against *C. albicans* and *A. fumigatus*, respectively. However, compounds **5a**, **5b**, **5d**, **5n**, and **5o** exhibited low to moderate activity against the fungal strains.

In general, the introduction of chlorine substituents in the coumarin ring have elevated the antibacterial and antifungal activities to 90% as compared to other substituents (-CH_3, -OCH_3). The graphical representation of antibacterial and antifungal activities of azetidinone derivatives at a concentration of 100 μg/mL is depicted in the Figs. 1 and 2, respectively.

Minimum inhibitory concentration (MIC)

Compounds showing antibacterial and antifungal activity above 80% were selected for minimum inhibitory concentration (MIC) studies (Tables 4 and 5). The minimum inhibitory concentration was determined using the agar diffusion technique. Each of the test compounds and standards, Ciprofloxacin and Gentamycin, were dissolved in DMSO at an initial concentration of 250 μg/mL, and then, were serially diluted in culture medium as follows: 125, 62.5, 31.25, 16, 8, 4, 2, 1 μg/mL concentrations. The minimum inhibitory concentrations (MIC) were defined as the lowest concentrations of the compounds that prevented visible growth.

Cytotoxic activity

All the synthesized compounds **5a–o** were screened for their cytotoxicity (brine-shrimp bioassay) using the protocol of Meyer *et al*. [26]. From the data recorded in Table 6, it is evident that the compounds **5c**, **5f**, **5h**, **5j**, and **5m**

Table 3. *In-vitro* antifungal activities of synthesized compounds **5a – o** in terms of zone of inhibition and percentage inhibition.

Comp.	Zone of inhibition% (mm)								
	Aspergillus fumigates Conc. (μg/mL)			*Candida albicans* Conc. (μg/mL)			*Penicillium* Conc. (μg/mL)		
	100	50	25	100	50	25	100	50	25
5a	54[a)]	28	16	45	26	16	38	18	Nil
	77[b)]	67	57	70	65	57	59	50	–
5b	48	25	15	42	26	15	42	20	12
	69	59	54	66	65	54	66	56	46
5c	62	36	23	57	32	21	56	28	19
	89	85	82	89	80	75	87	78	73
5d	52	27	16	46	26	15	42	22	11
	74	64	57	71	65	54	66	61	42
5e	41	25	Nil	40	26	17	38	22	14
	59	50	–	62	65	61	59	61	54
5f	62	36	22	58	33	22	39	20	Nil
	89	85	78	90	82	79	61	56	–
5g	42	28	17	38	22	Nil	38	20	12
	60	67	61	59	55	–	59	56	46
5h	64	34	22	57	34	21	48	25	17
	91	81	78	89	85	75	75	69	65
5i	37	19	Nil	32	18	Nil	42	22	14
	53	45	–	50	40	–	66	61	54
5j	57	31	20	52	29	20	52	20	Nil
	81	74	71	81	72	71	81	56	–
5k	38	19	Nil	41	22	Nil	40	19	14
	54	45	–	64	55	–	62	53	54
5l	45	22	13	38	20	Nil	44	21	13
	64	52	46	59	50	–	69	58	50
5m	57	34	19	48	28	19	56	27	18
	81	81	67	75	70	68	87	75	69
5n	46	26	16	39	23	14	40	17	Nil
	66	62	57	61	57	50	62	47	–
5o	45	23	Nil	45	26	16	46	24	14
	64	55	–	70	65	57	72	67	54
Standard	70	42	28	64	40	28	64	36	26
Control	–	–	–	–	–	–	–	–	–

Standard used: Gentamycin; control: DMSO.

[a)] Zone of inhibition (first row).

[b)] Percentage inhibition (second row).

Table 4. *In-vitro* antibacterial activities of the selected compounds in MIC (μg/mL).

Compound	MIC (μg/mL)			
	S. aureus	*V.-r. enteroccoccus*	*E. coli*	S. dysentery
5c	04	04	04	08
5f	08	08	01	16
5h	04	01	08	01
5j	02	16	16	01
5m	04	02	01	01
Standard	0.78	0.70	0.19	0.19

Standard used: Ciprofloxacin.

displayed potent cytotoxic activity against *Artemia salina*, while the other compounds have shown moderate activity in this assay. Compound **5h** showed maximum activity (LD_{50} = 7.154×10^{-4} M) in the present series of compounds, whereas the other active compounds **5c**, **5f**, **5j**, and **5m** demonstrated slightly lower activity (LD_{50} = 5.231×10^{-4} M, 4.854×10^{-4} M, 5.616×10^{-4} M, and 5.782×10^{-4} M, respectively) than compound **5h**.

Conclusion

In conclusion, a series of 3-chloro-4-[4-(2-oxo-2*H*-chromen-4-ylmethoxy)-phenyl]-1-phenyl-azetidin-2-one derivatives **5a – o** were prepared and screened for their *in-vitro* antibacterial activity against four strains of bacteria and antifungal activity against three fungi strains, as well as cytotoxic studies. The bioassays indicated that the com-

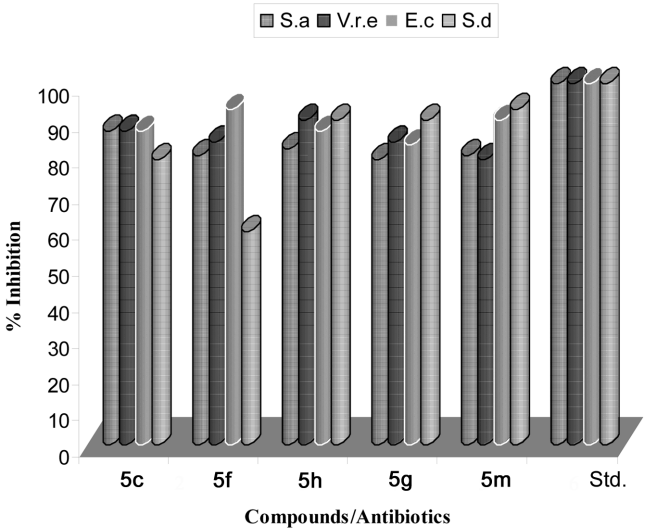


Figure 1. The antibacterial activities of selected azetidinone derivatives: **5c**, **5f**, **5h**, **5j**, and **5m** and Ciprofloxacin (Standard) at 100 μg/mL concentration.

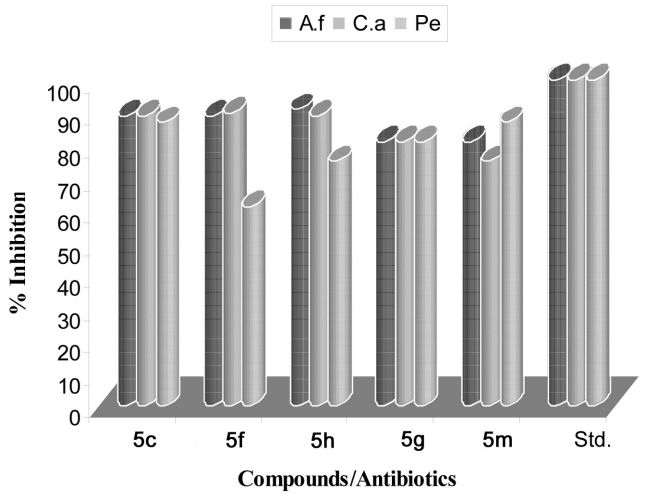


Figure 2. The antifungal activities of selected azetidinone derivatives: **5c**, **5f**, **5h**, **5j**, and **5m** and Gentamycin (Standard) at 100 μg/mL concentration.

Table 5. *In vitro* antifungal activity of the selected compounds in MIC (μg/mL).

Compound	MIC (μg/mL)		
	Aspergillus fumigates	*Candida albicans*	*Penicillium*
5c	04	04	08
5f	08	08	16.125
5h	01	04	08
5j	02	16	16
5m	08	08	16.125
Standard	2.0	2.0	2.0

Standard used: Gentamycin.

Table 6. Brine-shrimp bioassay data for the compounds **5a–o**.

Compound	LD_{50} (mM)
5a	3.753
5b	4.104
5c	0.5231
5d	2.967
5e	3.737
5f	0.4854
5g	2.749
5h	0.7154
5i	2.745
5j	0.5616
5k	2.531
5l	3.0564
5m	0.5782
5n	2.023
5o	2.349

pounds **5c**, **5f**, **5h**, **5j**, and **5m** are excellently active (up to 90%) against all the strains and the remaining compounds showed good to moderate activity comparable with the standard drugs. Compounds **5f**, **5h**, and **5m** displayed potent cytotoxic activity against *Artemia salina*. On the basis of structure-activity relationship study of the compounds, it can be concluded that, the presence of a chlorine group in the coumarin moiety enhanced the activity of the compounds. Henceforth, our findings may have a good impact on chemists and biochemists for further investigations in this field in search of chlorine-containing antimicrobial and cytotoxic agents.

Experimental

Materials and methods

All reagents and solvents were used as obtained from the supplier or recrystalized/redistilled as necessary. The melting points of the products were determined by open capillaries on a Büchi apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. The IR spectra were recorded on a Nicolet Impact-410 FT-IR spectrophotometer (Nicolet, Madison, WI, USA), using KBr pellets. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AC-300F 300 MHZ spectrometer (Bruker Bioscience, USA) in DMSO-d_6/$CDCl_3$ using TMSi as an internal standard with ^{1}H-resonant frequency of 300 MHZ and ^{13}C resonant frequency of 75 MHZ. D_2O exchange was applied to confirm the assignment of the signals of NH protons. The mass spectra were recorded on an Autospec EI-MS (Micromass, Waters). The elemental analysis was carried out using Heraeus CHN rapid analyzer (Heraeus, Gemany). All the compounds gave C, H, and N analysis within ± 0.4% of the theoretical values. The homogeneity of the compounds was described by TLC on aluminium silica gel 60 F254 (Merck, Germany) detected by UV light (254 nm) and iodine vapors. Compound's nomenclature was made using Chem. Draw Ultra version 6.0. *In-vitro* antibacterial and antifungal properties were studied at Luqman Pharmacy College, Gulbraga, Rajiv Gandhi University, India.

Chemistry

*General procedure for the preparation of 4-bromo-methyl coumarins **1a – e***

The present synthetic strategy begins with the generation of the required 4-bromo-methyl coumarins **1a – c**. These compounds were prepared by Pechmann cyclization of phenols with 4-bromoethyl acetoacetate [27, 28].

*General procedure preparation of 2-oxo-2H-chromen-4-ylmethoxy)benzaldehydes **2a – e***

p-Hydroxy benzaldehyde (10 mmol) and anhydrous K_2CO_3 (1.38 g, 10 mmol) were stirred in dry acetone 25 mL for 30 min. 4-Bromo-methyl coumarins (2.52 g, 10 mmol) were added and stirring was continued for 24 h. The reaction mixture was concentrated to one-fourth of the original volume and poured onto ice-cold water. The solid which separated was filtered and washed with 5% HCl (10 mL) to neutralize the excess of potassium carbonate, then washed with 100 mL of cold water and with dilute ethanol. The crude product was dried and recrystallized from DMF.

*4-(6-Methyl-2-oxo-2H-chromen-4-ylmethoxy)benzaldehyde **2a***

Colorless solid, m. p.: 218 – 219°C; IR (KBr, cm^{-1}) ν: 3046 (=CH-), 2778 (CH of CHO), 1718 (C=O of coumarin), 1690 (C=O of aldehyde), 1534 (C=C), 1022 (C-O-C); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 2.38 (s, 3H, CH_3), 5.31 (s, 2H, CH_2O), 6.64 (s, 1H, C_3H), 7.12 – 7.97 (m, 7H, Ar-H), 9.95 (s, 1H, CHO); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 21.1, 72.1, 114.0, 118.0, 123.0, 133.1, 133.3, 148.2, 152.4, 161.0, 192.0; ESI-MS: 294 [M^+]; Anal. calcd. for $C_{18}H_{14}O_4$: C, 73.45; H, 4.80. Found: C, 73.51; H, 4.87.

*4-(7-Methyl-2-oxo-2H-chromen-4-ylmethoxy)benzaldehyde **2b***

Colorless solid, m. p.: 223 – 225°C; IR (KBr, cm^{-1}) ν: 3038 (=CH-), 2788 (CH of CHO), 1707 (C=O of coumarin), 1697 (C=O of aldehyde), 1562 (C=C), 1016 (C-O-C); ^{1}H-NMR (300 MHZ, $CDCl_3$, ppm) δ: 2.48 (s, 3H, CH_3), 5.33 (s, 2H, CH_2O), 6.60 (s, 1H, C_3H), 7.15 – 7.91 (m, 7H, Ar-H), 9.91 (s, 1H, CHO); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 20.6, 78.0, 113.5, 117.8.0, 121.3, 134.1, 148.2, 155.4, 167.6, 188.0; ESI-MS: 294 [M^+]. Anal. calcd. for $C_{18}H_{14}O_4$: C, 73.44; H, 4.81. Found: C, 73.48; H, 4.86.

*4-(6-Chloro-2-oxo-2H-chromen-4-ylmethoxy)benzaldehyde **2c***

Colorless solid, m. p.: 220 – 222°C; IR (KBr, cm^{-1}) ν: 3062 (=CH-), 2808 (CH of CHO), 1722 (C=O of coumarin), 1698 (C=O of aldehyde), 1571 (C=C), 1021 (C-O-C); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 5.25 (s, 2H, CH_2O), 6.73 (s, 1H, C_3-H), 7.21 – 7.91 (m, 7H, Ar-H), 9.90 (s,1H, CHO); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 83.4, 110.0, 116.0, 126.0, 135.1, 138.3, 148.2, 158.4, 161.0, 167.7, 192.0; ESI-MS: 315 [M^+ + 1]. Anal. calcd. for $C_{17}H_{11}ClO_4$: C, 64.87; H, 3.57; Cl, 11.28. Found: C, 64.91; H, 3.62; Cl, 11.32.

*4-(3-Oxo-3H-benzochromen-1-ylmethoxy)benzaldehyde **2d***

Light yellow solid, m. p.: 222 – 224°C; IR (KBr, cm^{-1}) ν: 3080 (=CH-), 2836 (CH of CHO), 1712 (C=O of coumarin), 1695 (C=O of aldehyde), 1562 (C=C), 1031 (C-O-C); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 5.75 (s, 2H, CH_2O), 6.94 (s, 1H,C_3-H), 7.13 – 8.15 (m, 10H, Ar-H), 9.96 (s,1H, CHO); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 81.4, 106.0, 114.0, 123.0, 126.8, 129.1, 130.3, 148.7, 163.5, 169.3, 193.2; ESI-MS: 330 [M^+]. Anal. calcd. for $C_{21}H_{14}O_4$: C, 76.38; H, 4.27. Found: C, 76.42; H, 4.32.

*4-(2-Oxo-2H-benzochromen-4-ylmethoxy)benzaldehyde **2e***

Light redish solid, m. p.: 248 – 249°C; IR (KBr, cm^{-1}) ν: 3052 (=CH-), 2842 (-CH of CHO), 1718 (C=O of coumarin), 1707 (C=O of aldehyde), 1569 (C=C), 1028 (C-O-C); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 5.48 (s, 2H, CH_2O), 6.80 (s, 1H, C_3-H), 7.08 – 8.89 (m, 10H, Ar-H), 9.92 (s, 1H, CHO); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 80.8, 107.5, 113.1, 122.1, 127.6, 129.8, 133.0, 146.6, 164.4, 168.8, 191.5; ESI-MS: 330 [M^+]. Anal. calcd. for $C_{21}H_{14}O_4$: C, 76.39; H, 4.26. Found: C, 76.45; H, 4.30.

*Preparation of 4-(4-phenyliminomethyl-phenoxymethyl)chromen-2-ones **4a – o***

4-Phenyliminomethyl-phenols **3a – c** (10 mmol) and anhydrous K_2CO_3 (1.38 g, 10 mmol) were stirred in dry acetone (25 mL) for 30 min. 4-Bromo-methyl coumarins **1a – e** (2.52 g, 10 mmol) were added and stirring was continued for 24 h. The reaction mixture was concentrated and poured into crushed ice (100 g). The solid which separated was filtered and washed with 5% HCl (10 mL) to neutralize the excess of potassium carbonate, then washed with 100 mL of cold water and with dilute ethanol. The crude product was dried and recrystallized from DMF.

*6-Methyl-4-(4-phenyliminomethyl-phenoxymethyl)chromen-2-one **4a***

Yield: 81%, colorless crystals from DMF, m. p.: 215 – 217°C; IR (KBr, cm^{-1}) ν: 1718 (C=O of coumarin), 1605 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 2.32 (s, 3H, C_6-CH_3), 5.31 (s, 2H, CH_2-O), 6.43 (s, 1H, C_3-H), 7.10 – 8.32 (m, 12H, Ar-H), 9.83 (s, 1H, -CH-N); ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 18.8, 80.03, 105.06, 112.20, 120.12, 122.20, 125.6, 127.56, 129.40, 132.10, 135.8, 149.12, 155.0, 162.02, 164.12, 167.42; ESI-MS: 370 [M^+ + 1]. Anal. calcd. for $C_{24}H_{19}NO_3$: C, 78.03; H, 5.18; N, 3.79. Found: C, 78.04; H, 5.21; N, 3.81.

*7-Methyl-4-(4-phenyliminomethyl-phenoxymethyl)chromen-2-one **4b***

Yield: 77%, colorless crystals from DMF, m. p.: 187 – 188°C; IR (KBr, cm^{-1}) ν: 1710 (C=O of coumarin), 1598 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 1.91 (s, 3H, C_7-CH_3), 4.99 (s, 2H, CH_2-O), 6.31 (s, 1H, C_3-H), 6.89 – 7.79 (m, 12H, Ar-H), 9.71 (s, 1H, -CH-N); ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 21.31, 77.12, 108.12, 111.00, 119.91, 121.71, 123.43, 126.71, 130.02, 134.31, 138.82, 146.54, 153.07, 157.76, 160.01, 162.17; ESI-MS: 370 [M^+ + 1]. Anal. calcd. for $C_{24}H_{19}NO_3$: C, 78.03; H, 5.18; N, 3.79. Found: C, 78.06; H, 5.19; N, 3.77.

*6-Chloro-4-(4-phenyliminomethyl-phenoxymethyl)chromen-2-one **4c***

Yield: 59%, light yellow crystals from DMF, m. p.: 232 – 234°C; IR (KBr, cm^{-1}) ν: 1722 (C=O of coumarin), 1612 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 4.83 (s, 2H, CH_2-O), 6.36 (s, 1H, C_3-H), 6.88 – 7.49 (m, 12H, Ar-H), 9.05 (s, 1H, -CH-N); ^{13}C-NMR (75 MHz,

DMSO-d_6, ppm) δ: 77.56, 104.97, 115.10, 119.03, 121.78, 123.86, 126.1, 127.23, 129.55, 130.02, 146.87, 153.56, 158.23, 161.23, 163.83, 164.17; ESI-MS: 390 [M^+ + 1]. Anal. calcd. for $C_{23}H_{16}ClNO_3$: C, 70.86; H, 4.14; N, 3.59. Found: C, 70.85; H, 4.17; N, 3.56.

1-(4-Phenyliminomethyl-phenoxymethyl)benzo[f]-chromen-3-one ***4d***

Yield: 72%, reddish-colored crystals from DMF, m. p.: 232 – 233°C; IR (KBr, cm^{-1}) ν: 3013 (=CH) 1724 (C=O of coumarin), 1612 (C=N), 1506 (C=C), 1115 (C-O-C); ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 4.10 (s, 2H, CH_2-O), 6.31 (s, 1H, C_3-H), 6.98 – 7.99 (m, 15H, Ar-H), 9.91 (s, 1H, -CH=N); ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 73.56, 103.65, 112.36, 114.76, 118.78, 120.03, 121.87, 122.68, 123.01, 124.26, 125.87, 127.98, 129.76, 132.65, 137.87, 143.76, 151.21, 154.65, 155.54, 158.41, 161.21; ESI-MS: 406 [M^+ + 1]. Anal. calcd. for $C_{27}H_{19}NO_3$: C, 79.97; H, 4.68; N, 3.45. Found: C, 80.00; H, 4.69; N, 3.44.

1-(4-Phenyliminomethyl-phenoxymethyl)benzo[h]-chromen-2-one ***4e***

Yield: 83%, yellow-colored crystals from DMF, m. p.: 203 – 204°C; IR (KBr, cm^{-1}) ν: 3034 (=CH), 1719 (C=O of coumarin), 1607 (C=N), 1478 (C=C), 1122 (C-O-C); ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 4.94 (s, 2H, CH_2-O), 6.19 (s, 1H, C_3-H), 6.88 – 8.23 (m, 15H, Ar-H), 8.76 (s, 1H, -CH=N); ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 70.21, 100.95, 110.20, 116.07, 119.67, 121.05, 121.37, 122.18, 123.32, 124.00, 125.65, 126.54, 128.54, 131.45, 133.65, 144.45, 152.31, 157.43, 160.06, 163. 23, 167.18; ESI-MS: 406 [M^+ + 1]. Anal. calcd. for $C_{27}H_{19}NO_3$: C, 79.97; H, 4.68; N, 3.45. Found: C, 79.99; H, 4.71; N, 3.47.

6-Methyl-4-(4-(p-tolylimino-methyl)phenoxymethyl)-chromen-2-one ***4f***

Yield: 71%, colorless crystals from DMF, m. p.: 177 – 178°C; IR (KBr, cm^{-1}) ν: 3019 (=CH), 1715 (C=O coumarin), 1589 (C=N), 1501 (C=C), 1099 (C-O-C); ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 2.31 (s, 3H, C_6-CH_3), 2.41 (s, 3H, CH_3), 5.22 (s, 2H, CH_2-O), 6.32 (s, 1H, C_3-H), 6.76 – 7.98 (m, 11H, Ar-H), 9.78 (s, 1H, -CH=N); ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 17.08, 21.87, 76.19, 107.89, 111.87, 120.92, 122.76, 123.59, 124.12, 125.07, 127.87,128.91, 133.57, 135.67, 139.91, 144.02, 149.94, 155.61, 158.52, 160.02, 163.01; ESI-MS: 384 [M^+ + 1]. Anal. calcd. for $C_{25}H_{21}NO_3$: C, 78.29; H, 5.48; N, 3.65. Found: C, 78.27; H, 5.52; N, 3.63.

7-Methyl-4-(4-(p-tolylimino-methyl)phenoxymethyl)-chromen-2-one ***4g***

Yield: 78%, colorless crystals from DMF, m. p.: 233 – 235°C; IR (KBr, cm^{-1}) ν: 3067 (=CH), 1709 (C=O coumarin), 1611 (C=N), 1507 (C=C), 1109 (C-O-C); ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 1.87 (s, 3H, C_7-CH_3), 2.34 (s, 3H, CH_3), 5.07 (s, 2H, CH_2-O), 6.23 (s, 1H, C_3-H), 6.89 – 7.66 (m, 11H, Ar-H), 9.03 (s, 1H, -CH=N); ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 15.9, 22.5, 78.12, 104.65, 113.45, 119.65, 121.45, 122.32, 124.56, 125.32, 126.54,129.32, 130.5, 136.65, 137.54, 149.32, 150.12, 156.45, 159.65, 162.32, 165.43; ESI-MS: 384 [M^+ + 1]. Anal. calcd. for $C_{25}H_{21}NO_3$: C, 78.29; H, 5.48; N, 3.65. Found: C, 78.32; H, 5.50; N, 3.67.

6-Chloro-4-(4-(p-tolylimino-methyl)phenoxymethyl]-chromen-2-one ***4h***

Yield: 66%, colorless crystals from DMF, m. p.: 201 – 202°C; IR (KBr, cm^{-1}) ν: 1705 (C=O of coumarin), 1603 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 2.12 (s, 1H, CH_3), 4.91 (s, 2H, CH_2-O), 6.27 (s, 1H, C_3-H), 6.92 – 7.79 (m, 11H, Ar-H), 9.57 (s, 1H, -CH=N); ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 17.83, 80.13, 106.79, 112.72, 117.92, 120.29, 124.31, 125.68, 126.79, 129.87, 133.46, 144.65, 151.23, 155.65, 159.01, 160.43, 167.15; ESI-MS: 404 [M^+ + 1]. Anal. calcd. for $C_{24}H_{18}ClNO_3$: C, 71.38; H, 4.49; N, 3.47. Found: C, 71.40; H, 4.53; N, 3.50.

1-[4-(p-Tolylimino-methyl)phenoxymethylbenzo[f]-chromen-3-one ***4i***

Yield: 72%, reddish crystals from DMF, m. p.: 283 – 284°C; IR (KBr, cm^{-1}) ν: 1735 (C=O of coumarin), 1615 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 2.28 (s, 3H, CH_3), 4.72 (s, 2H, CH_2-O), 6.56 (s, 1H, C_3-H), 6.88 – 7.49 (m, 14H, Ar-H), 8.89 (s, 1H, -CH=N); ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 20.22, 80.13, 106.23, 113.43, 117.32, 120.76, 122.76, 123.97, 126.32, 129.34, 130.67, 137.12, 151.03, 156.97, 162.34, 165.10, 166.54; ESI-MS: 420 [M^+ + 1]. Anal. calcd. for $C_{28}H_{21}NO_3$: C, 80.16; H, 5.05; N, 3.34. Found: C, 80.17; H, 5.02; N, 3.32.

4-[4-(p-Tolylimino-methyl)phenoxymethylbenzo[h]-chromen-2-one ***4j***

Yield: 57%, yellow crystals from DMF, m. p.: 256 – 257°C; IR (KBr, cm^{-1}) ν: 1727 (C=O of coumarin), 1600 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 2.07 (s, 3H, CH_3), 4.41 (s, 2H, CH_2-O), 6.41 (s, 1H, C_3-H), 6.67 – 7.87(m, 14H, Ar-H), 9.87 (s, 1H, -CH=N); ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 17.65, 77.92, 103.80, 111.09, 114.78, 119.71, 121.32, 124.05, 127.46, 128.91, 132.54, 139.07, 145.67, 154.43, 157.43, 159.92, 162.03; ESI-MS: 420 [M^+ + 1]. Anal. calcd. for $C_{28}H_{21}NO_3$: C, 80.16; H, 5.05; N, 3.34. Found: C, 80.19; H, 5.06; N, 3.36.

4-{4-[(4-Methoxy-phenylimino)methyl]phenoxymethyl)-6-methylchromen-2-one ***4k***

Yield: 69%, colorless crystals from DMF, m. p.: 205 – 206°C; IR (KBr, cm^{-1}) ν: 3024 (=CH), 1712 (C=O coumarin), 1613 (C=N), 1512 (C=C), 1114 (C-O-C); ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 2.32 (s, 3H, C_6-CH_3), 3.13 (s, 3H, OCH_3), 4.87 (s, 2H, CH_2-O), 6.23 (s, 1H, C_3-H), 6.83 – 7.65 (m, 11H, Ar-H), 8.65 (s, 1H, -CH=N); ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 19.14, 51.23, 79.34, 101.32, 111.43, 115.55, 119.23, 121.32, 123.13, 125.78, 126.84, 128.76, 130.32, 133.13, 143.23, 146.67, 156.32, 159.54, 161.23, 165.31, 166.43; ESI-MS: 400 [M^+ + 1]. Anal. calcd. for $C_{25}H_{21}NO_4$: C, 75.15; H, 5.26; N, 3.50. Found: C, 75.18; H, 5.29; N, 3.53.

4-{4-[(4-Methoxy-phenylimino)methyl]phenoxymethyl)-7-methylchromen-2-one ***4l***

Yield: 74%, colorless crystals from DMF, m. p.: 231 – 232°C; IR (KBr, cm^{-1}) ν: 3109 (=CH), 1709 (C=O coumarin), 1621 (C=N), 1501 (C=C), 1109 (C-O-C); ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 1.85 (s, 3H, C_7-CH_3), 3.68 (s, 3H, OCH_3), 4.54 (s, 2H, CH_2-O), 6.09 (s, 1H, C_3-H), 6.77 – 8.09 (m, 11H, Ar-H), 9.92 (s, 1H, -CH=N); ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 22.61, 59.04, 81.73, 103.09, 112.00, 117.03, 118.07, 122.05, 124.10, 126.90, 127.23, 129.03, 131.68, 137.57, 144.04, 148.59, 155.56, 157.05, 159.45, 160.89, 163.12;

ESI-MS: 400 [M^+ + 1]. Anal. calcd. for $C_{25}H_{21}NO_4$: C, 75.15; H, 5.26; N, 3.50. Found: C, 75.13; H, 5.25; N, 3.52.

6-Chloro-4-{4-[(4-methoxy-phenyliminomethyl]-phenoxymethyl]chromen-2-one ***4m***

Yield: 71%, colorless crystals from DMF, m. p.: 167 – 168°C; IR (KBr, cm^{-1}) ν: 1721 (C=O of coumarin), 1609 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 3.45 (s, 1H, OCH_3), 4.39 (s, 2H, CH_2-O), 6.26 (s, 1H, C_3-H), 6.67 – 7.89 (m, 11H, Ar-H), 9.73 (s, 1H, -CH=N); ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 56.32, 77.52, 104.99, 110.59, 114.36, 119.17, 122.80, 124.24, 127.89, 130.09, 137.89, 142.89, 148.17, 156.32, 157.03, 159.04, 161.38; ESI-MS: 420 [M^+ + 1]. Anal. calcd. for $C_{24}H_{18}ClNO_4$: C, 68.66; H, 4.32; N, 3.34. Found: C, 68.69; H, 4.35; N, 3.32.

1-{4-[(4-Methoxy-phenylimino)methyl]phenoxymethyl)-benzo[f]chromen-3-one ***4n***

Yield: 59%, reddish crystals from DMF, m. p.: 232 – 234°C; IR (KBr, cm^{-1}) ν: 1721 (C=O of coumarin), 1617 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 3.34 (s, 3H, OCH_3), 4.94 (s, 2H, CH_2-O), 6.26 (s, 1H, C_3-H), 6.89 – 7.87 (m, 14H, Ar-H), 9.69 (s, 1H, -CH=N); ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 59.76, 75.69, 107.82, 112.69, 114.70, 118.32, 119.07, 120.29, 123.06, 125.99, 128.73, 132.18, 138.79, 143.77, 148.67, 150.78, 155.09, 158.89, 160.03, 162.43; ESI-MS: 436 [M^+ + 1]. Anal. calcd. for $C_{28}H_{21}NO_4$: C, 77.23; H, 4.86; N, 3.21. Found: C, 77.20; H, 4.87; N, 3.22.

4-{4-[(4-Methoxy-phenylimino)methyl]phenoxymethyl)-benzo[h]chromen-2-one ***4o***

Yield: 65%, colorless crystals from DMF, m. p.: 203 – 204°C; IR (KBr, cm^{-1}) ν: 1728 (C=O of coumarin), 1603 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 3.59 (s, 3H, OCH_3), 4.81 (s, 2H, CH_2-O), 6.51 (s, 1H, C_3-H), 6.72 – 7.83 (m, 14H, Ar-H), 8.97 (s, 1H, -CH=N); ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ: 54.04, 77.19, 104.55, 114.24, 115.54, 117.88, 120.45, 121.46, 122.92, 124.58, 127.43, 130.05, 134.65, 145.65, 150.54, 157.33, 159.63, 160.04, 163.45, 164.23; ESI-MS: 436 [M^+ + 1]. Anal. calcd. for $C_{28}H_{21}NO_4$: C, 77.23; H, 4.86; N, 3.21. Found: C, 77.26; H, 4.88; N, 3.24.

Preparation of 3-chloro-4-[4-(2-oxo-2H-chromen-4-ylmethoxy)phenyl]-1-phenylazetidin-2-ones ***5a – o***

The mixture of 4-(4-phenyliminomethyl-phenoxymethyl)-chromen-2-ones (0.01 mol) and triethyl amine (0.01 mol) was dissolved in DMF/benzene (50 mL), cooled and stirred. To this well-stirred cold solution of chloro acetyl chloride (0.01 mmol) was added dropwise within a period of 20 min. The reaction mixture was then stirred for an additional 3 h and left at room temperature for 48 h. The resultant mixture was concentrated, cooled, poured into ice cold water, filtered, and then dried. The product thus obtained was purified by column chromatography over silica gel using 30% ethyl acetate: 70% benzene as an eluent. Recrystallization was done from a suitable solvent which yielded the 2-azetidinones **5a – o**.

3-Chloro-4-[4-(6-methyl-2-oxo-2H-chromen-4-ylmethoxy)phenyl]-1-phenyl-azetidin-2-one ***5a***

Colorless shiny crystals from DMF; IR (KBr, cm^{-1}) ν: 3089 (=CH-), 1768 (>C=O of β-lactam), 1722 (>C=O of coumarin), 1520 (C=C), 782 (-C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 2.38 (s, 3H, C_6-CH_3), 4.98 (s, 2H, CH_2-O), 5.05 (s, 1H, -N-CH), 5.34 (s, 1H, -CH-Cl), 6.65 (s, 1H, C_3-H), 6.88 – 7.96 (m, 12H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 21.0, 53.3, 62.0, 80.8, 108.4, 115.2, 121.2, 125.2, 128.1, 134.4, 140.2, 148.4, 158.2, 162.1, 184.4; ESI-MS: 446 [M^+ + 1].

3-Chloro-4-[4-(7-methyl-2-oxo-2H-chromen-4-ylmethoxy)phenyl]-1-phenyl-azetidin-2-one ***5b***

Colorless crystals from DMF; IR (KBr, cm^{-1}) ν: 3109 (=CH-), 1772 (>C=O of β-lactam), 1718 (>C=O of coumarin), 1506 (C=C), 778 (-C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 2.26 (s, 3H, C_7-CH_3), 4.67 (s, 2H, CH_2-O), 4.98 (s, 1H, -N-CH), 5.40 (s, 1H, -CH-Cl), 6.56 (s, 1H, C_3-H), 6.70-8.02 (m, 12H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 23.4, 56.7, 60.4, 82.0, 106.7, 114.0, 122.0, 126.8, 128.8, 132.8, 142.0, 149.0, 157.7, 160.6, 173.7; ESI-MS: 446 [M^+ + 1].

3-Chloro-4-[4-(6-chloro-2-oxo-2H-chromen-4-ylmethoxy)phenyl]-1-phenyl-azetidin-2-one ***5c***

Colorless crystals from DMF; IR (KBr, cm^{-1}) ν: 3088 (=CH-), 1765 (>C=O of β-lactam), 1722 (>C=O of coumarin), 1500 (C=C), 782 (-C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 4.79 (s, 2H, CH_2-O), 5.20 (s, 1H, -N-CH), 5.47 (s, 1H, -CH-Cl), 6.40 (s, 1H, C_3-H), 7.12 – 7.92 (m, 12H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 60.2, 63.6, 79.6, 108.3, 113.2, 121.1, 123.6, 126.4, 136.6, 145.8, 150.3, 155.9, 164.4, 178.9; ESI-MS: 466 [M^+ + 1].

3-Chloro-4-[4-(3-oxo-3H-benzo[f]chromen-1-ylmethoxy)phenyl]-1-phenyl-azetidin-2-one ***5d***

Light yellow crystals from DMF; IR (KBr, cm^{-1}) ν: 3122 (=CH-), 1782 (>C=O of β-lactam), 1730 (>C=O of coumarin), 1521 (C=C), 792 (-C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 4.57 (s, 2H, CH_2-O), 5.08 (s, 1H, -N-CH), 5.40 (s, 1H, -CH-Cl), 6.36 (s, 1H, C_3-H), 6.86 – 8.35 (m, 15H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 56.7, 64.9, 82.1, 102.6, 110.8, 116.7, 120.0, 122.6, 126.4, 129.7, 134.0, 140.2, 149.6, 157.3, 160.1, 170.2; ESI-MS: 482 [M^+ + 1].

3-Chloro-4-[4-(3-oxo-2H-benzo[h]chromen-4-ylmethoxy)phenyl]-1-phenyl-azetidin-2-one ***5e***

Reddish crystals from DMF; IR (KBr, cm^{-1}) ν: 3122 (=CH-), 1777 (>C=O of β-lactam), 1726 (>C=O of coumarin), 1507 (C=C), 781 (-C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 4.62 (s, 2H, CH_2-O), 5.32 (s, 1H, -N-CH), 5.44 (s, 1H, -CH-Cl), 6.20 (s, 1H, C_3-H), 6.70 – 7.97 (m, 15H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 60.0, 63.5, 80.0, 99.8, 107.2, 113.1, 122.4, 125.0, 127.7, 130.3, 135.3, 142.0, 150.8, 158.7, 163.2, 172.0; ESI-MS: 482 [M^+ + 1].

3-Chloro-4-[4-(6-methyl-2-oxo-2H-chromen-4-ylmethoxy)phenyl]-1-p-tolyl-azetidin-2-one ***5f***

Colorless shiny crystals from DMF; IR (KBr, cm^{-1}) ν: 3089 (=CH-), 1768 (>C=O of β-lactam), 1722 (>C=O of coumarin), 1520 (C=C), 782 (-C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 2.17 (s, 3H, C_6-CH_3), 2.38 (s, 3H, CH_3), 4.57 (s, 2H, CH_2-O), 5.12 (s, 1H, -N-CH), 5.49 (s, 1H, -CH-Cl), 6.55 (s, 1H, C_3-H), 6.78 – 7.75 (m, 11H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 19.2, 23.2, 59.1, 63.6, 82.5, 103.6, 110.0, 121.2, 125.2, 128.1, 134.4, 140.2, 148.4, 158.2, 162.1, 184.4; ESI-MS: 460 [M^+ + 1].

3-Chloro-4-[4-(7-methyl-2-oxo-2H-chromen-4-ylmethoxy)phenyl]-1-p-tolyl-azetidin-2-one ***5g***

Colorless shiny crystals from DMF; IR (KBr, cm^{-1}) ν: 3102 (=CH-), 1772 (>C=O of β-lactam), 1717 (>C=O of coumarin), 1501 (C=C), 766 (-C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 2.26 (s, 3H, C_7-CH_3), 2.41 (s, 3H, CH_3), 4.62 (s, 2H, CH_2-O), 5.06 (s, 1H, -N-CH), 5.51 (s, 1H, -CH-Cl), 6.40 (s, 1H, C_3-H), 6.68–7.56 (m, 11H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 20.0 22.7, 60.3, 63.9, 80.8, 106.0, 112.3, 119.0, 122.2, 124.5, 125.5, 126.5, 133.0, 136.5, 138.3, 150.3, 154.5, 157.7, 160.0, 171.3; ESI-MS: 460 [M^+ + 1].

3-Chloro-4-[4-(6-chloro-2-oxo-2H-chromen-4-ylmethoxy)phenyl]-1-p-tolyl-azetidin-2-one ***5h***

Colorless shiny crystals from DMF; IR (KBr, cm^{-1}) ν: 2993 (=CH-), 1768 (>C=O of β-lactam), 1709 (>C=O of coumarin), 1487 (C=C), 804 (-C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 2.20 (s, 3H, CH_3), 4.32 (s, 2H, CH_2-O), 4.78 (s, 1H, -N-CH), 5.37 (s, 1H, -CH-Cl), 6.26 (s, 1H, C_3-H), 6.82–7.68 (m, 11H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 17.9, 56.0, 61.6, 77.9, 109.1, 113.0, 120.2, 126.0, 128.5, 130.0, 134.6, 138.0, 150.0, 158.8, 163.3, 169.7; ESI-MS: 481 [M^+ + 1].

3-Chloro-4-[4-(3-oxo-3H-benzo[f]chromen-1-ylmethoxy)phenyl]-1-p-tolyl-azetidin-2-one ***5i***

Yellow crystals from DMF; IR (KBr, cm^{-1}) ν: 3102 (=CH-), 1777 (>C=O of β-lactam), 1726 (>C=O of coumarin), 1507 (C=C), 779 (-C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 2.17 (s, 3H, CH_3), 4.47 (s, 2H, CH_2-O), 5.07 (s, 1H, -N-CH), 5.45 (s, 1H, -CH-Cl), 6.42 (s, 1H, C_3-H), 6.65–8.06 (m, 14H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 19.4, 52.3, 59.2, 78.6, 106.9, 114.2, 117.4, 120.0, 123.3, 127.0, 129.9, 136.2, 143.4, 148.2, 153.1, 158.7, 160.5, 165.3, 173.0; ESI-MS: 496 [M^+ + 1].

3-Chloro-4-[4-(2-oxo-2H-benzo[h]chromen-4-ylmethoxy)phenyl]-1-p-tolyl-azetidin-2-one ***5j***

Reddish crystals from DMF; IR (KBr, cm^{-1}) ν: 3115 (=CH-), 1782 (>C=O of β-lactam), 1722 (>C=O of coumarin), 1495 (C=C), 787 (-C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 2.25 (s, 3H, CH_3), 4.55 (s, 2H, CH_2-O), 5.12 (s, 1H, -N-CH), 5.52 (s, 1H, -CH-Cl), 6.30 (s, 1H, C_3-H), 6.70–7.91 (m, 14H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 21.0, 60.1, 64.1, 80.2, 107.0, 112.9, 120.1, 121.4, 122.8, 124.5, 127.7, 130.2, 133.2, 135.0,138.6, 150.2, 155.3, 159.1, 160.7, 163.8, 169.6; ESI-MS: 496 [M^+ + 1].

3-Chloro-1-(4-methoxyphenyl)-4-[4-(6-methyl-2-oxo-2H-chromen-4-ylmethoxy)phenyl]azetidin-2-one ***5k***

Colorless shiny crystals from DMF; IR (KBr, cm^{-1}) ν: 3089 (=CH-), 1773 (>C=O of β-lactam), 1716 (>C=O of coumarin), 1479 (C=C), 779 (-C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 2.09 (s, 3H, C_6-CH_3), 3.56 (s, 3H, OCH_3), 4.74 (s, 2H, CH_2-O), 5.05 (s, 1H, -N-CH), 5.51 (s, 1H, -CH-Cl), 6.37 (s, 1H, C_3-H), 6.70–7.88 (m, 11H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 20.3, 51.9, 60.2, 63.7, 80.8, 107.0, 111.3, 113.8, 120.7, 127.0, 128.6, 140.4, 146.9, 155.3, 158.1, 160.5, 163.0, 171.0; ESI-MS: 477 [M^+ + 1].

3-Chloro-1-(4-methoxyphenyl)-4-[4-(7-methyl-2-oxo-2H-chromen-4-ylmethoxy)phenyl]azetidin-2-one ***5l***

Colorless shiny crystals from DMF; IR (KBr, cm^{-1}) ν: 3105 (=CH-), 1766 (>C=O of β-lactam), 1710 (>C=O of coumarin), 1501 (C=C), 782 (-C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 2.21 (s, 3H, C_7-CH_3), 3.71 (s, 3H, OCH_3), 4.53 (s, 2H, CH_2-O), 4.93 (s, 1H, -N-CH), 5.33 (s, 1H, -CH-Cl), 6.30 (s, 1H, C_3-H), 6.66–7.70 (m, 11H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 21.0, 47.7, 59.1, 62.0, 83.1, 105.9, 112.1, 115.2, 121.1, 122.5, 124.2, 126.3, 127.2, 128.8,132.1, 134.7, 138.3, 150.1, 156.0, 159.0, 160.7, 162.3, 169.3; ESI-MS: 477 [M^+ + 1].

3-Chloro-4-[4-(6-chloro-2-oxo-2H-chromen-4-ylmethoxy)phenyl]-1-(4-methoxyphenyl)azetidin-2-one ***5m***

Colorless shiny crystals from DMF; IR (KBr, cm^{-1}) ν: 3091 (=CH-), 1777 (>C=O of β-lactam), 1716 (>C=O of coumarin), 1487 (C=C), 779 (-C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 3.50 (s, 3H, OCH_3), 4.61 (s, 2H, CH_2-O), 5.04 (s, 1H, -N-CH), 5.41 (s, 1H, -CH-Cl), 6.51(s, 1H, C_3-H), 6.76–7.88 (m, 11H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 50.3, 60.2, 63.6, 80.6, 105.9, 113.5, 116.2, 120.4, 121.9, 127.1, 128.5, 130.1,133.0, 135.1, 145.7, 155.3, 159.2, 160.0, 163.1, 170.7; ESI-MS: 497 [M^+ + 1].

3-Chloro-1-(4-methoxyphenyl)-4-[4-(3-oxo-3H-benzo[f]chromen-1-ylmethoxy)phenyl]-1-p-tolyl-azetidin-2-one ***5n***

Yellow crystals from DMF; IR (KBr, cm^{-1}) ν: 3118 (=CH-), 1770 (>C=O of β-lactam), 1731 (>C=O of coumarin), 1515 (C=C), 792 (-C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 3.41 (s, 3H, OCH_3), 4.53 (s, 2H, CH_2-O), 5.16(s, 1H, -N-CH), 5.51 (s, 1H, -CH-Cl), 6.30 (s, 1H, C_3-H), 6.85–7.91 (m, 14H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ: 53.1, 60.0, 63.3, 80.4, 108.0, 112.7, 114.7, 121.2, 123.0, 126.6, 127.3, 128.9, 133.4, 137.0, 145.3, 150.2, 157.1, 159.7, 163.7, 168.2; ESI-MS: 512 [M^+ + 1].

3-Chloro-1-(4-methoxyphenyl)-4-[4-(2-oxo-2H-benzo[h]chromen-4-ylmethoxy)phenyl]azetidin-2-one ***5o***

Pale red crystals from DMF; IR (KBr, cm^{-1}) ν: 3104 (=CH-), 1782 (>C=O of β-lactam), 1722 (>C=O of coumarin), 1506 (C=C), 786 (-C-Cl); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ: 3.63 (s, 3H, OCH_3), 4.67 (s, 2H, CH_2-O), 4.78 (s, 1H, -N-CH), 5.23 (s, 1H, -CH-Cl), 6.23 (s, 1H, C_3-H), 6.76–7.83 (m, 14H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm): 47.7, 56.3, 61.2, 82.1, 107.3, 113.0, 115.8, 118.2, 121.6, 123.4, 126.3, 127.7, 129.2, 133.7, 135.3, 148.3, 156.0, 159.1, 162.3, 170.3; ESI-MS: 512 [M^+ + 1].

Biological evaluation

Antibacterial assay

The synthesized compounds **5a–o** were screened *in vitro* for their antibacterial activity against two Gram-positive [*Staphylococcus aureus* (ATCC-29213) and *Vancomycin*-resistant *enteroccoccus* (ATCC-51299)] and two Gram-negative [*Escherichia coli* (ATCC-25922) and *Shigella dysentery* (natural isolates)] bacterial strains by the agar-well diffusion method [25]. Ciprofloxacin was used as reference standards to compare antibacterial activities. The wells (6 mm in diameter) were dug in the media with the help of a sterile metallic borer with centers at least 24 mm apart. Two to eight hours old bacterial inocula containing approximately 10^4–10^6 colony-forming units (CFU/mL) were spread on the surface of the nutrient agar with the help of a sterile cotton swab. The tests were carried out at a concentration of 100 μg/mL, 50 μg/mL, and 25 μg/mL. After 48 h of incubation at 37°C, the zone of inhibition was measured in millimeters. The percentage

inhibition of test compounds was related to the standard whose zone of inhibition was taken as 100%. In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions alone of DMSO and they showed no activity against any bacterial strains.

Antifungal assay

Antifungal activities of compounds **5o – e** were evaluated against *Aspergillus fumigatus* (36607), *Candida albicans* (10231), and *Penicillium* (natural isolates). Sabouraud dextrose agar (Oxoid, Hampshire, England) was seeded with 10^5 (CFU/mL) fungal spore suspensions and transferred to petri plates. Discs soaked in 20 mL (100 mg/mL in DMSO) of all compounds were placed at different positions on the agar surface. The plates were incubated at 37°C for seven days. Dimethyl sulphoxide (DMSO) was used as a solvent control and Gentamycin used as a standard drug to compare antifungal activity. The tests were carried out at a concentration of 100 μg/mL, 50 μg/mL, and 25 μg/mL and the zone of inhibition was measured in millimeters. The percentage inhibition of test compounds was related to the standard whose zone of inhibition was taken as 100%.

Cytotoxic activity

Brine shrimp (*Artemia salina* leach) eggs were hatched in a shallow rectangular plastic dish (22 6 32 cm), filled with artificial sea water, which was prepared with commercial salt mixture and double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the other compartment was opened to ordinary light. After two days, nauplii were collected by a pipette from the lighted side. A sample of the test compound was prepared by dissolving 20 mg of each compound in 2 mL of DMF. From this stock solutions 500, 50, and 5 μg/mL were transferred to nine vials (three for each dilutions were used for each test sample and LD_{50} is the mean of three values) and one vial was kept as control having 2 mL of DMF only. The solvent was allowed to evaporate overnight. After two days, when shrimp larvae were ready, 1 mL of sea water and ten shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with sea water to 5 mL per vial. After 24 h, the number of survivors were counted [26]. Data were analyzed by a Finney computer program to determine the LD_{50} values [29].

This research work is financially supported by the Council of Scientific & Industrial Research (CSIR), New Delhi 110 012. (Ref: No. 01(2301)/09/EMR-II dated 19-03-2009).

The authors have declared no conflict of interest.

References

[1] J. A. Heinemann, R. G. Ankenbauer, C. F. Amabile-Cuevas, *Drug Discov. Today* **2000**, *5 (5)*, 195 – 204.

[2] M. J. Rybak, R. L. Akins, *Drugs* **2001**, *61*, 1 – 7.

[3] G. I. Georg (Ed.) *The Organic Chemistry of* β*-Lactams, VCH, Weinheim,* **1993**.

[4] G. S. Singh, B. J. Mmolotsi, *Il Farmaco* **2005**, *60 (9)*, 727 – 730.

[5] A. Kumar, C. S. Rajput, S. K. Bhati, *Bio. Med. Chem.* **2007**, *15*, 3089 – 3096.

[6] J. S. Biradar, S. Y. Manjunath, *Indian J. Chem. (B)* **2004**, *43B*, 141 – 143.

[7] J. M. Desai, V. H. Shah, *Indian J. Chem. (B)* **2003**, *42B*, 631 – 635.

[8] B. K. Banik, F. F. Becker, I. Banik, *Bioorg. Med. Chem.* **2004**, *12 (10)*, 2523 – 2528.

[9] J. Anaya, D. S. Gero, M. Grande, J. I. M. Hernando, N. M. Laso, *Bioorg. Med. Chem.* **1999**, *7 (5)*, 837 – 850.

[10] G. Gerona-Navarro, M. J. Perez de Vega, M. T. Garcia-Lopez, G. Andrei, R. Snoeck, *et al*., *J. Med.Chem.* **2005**, *48 (7)*, 2612 – 2621.

[11] C. Yoakim, W. W. Ogilvie, D. R. Cameron, C. Chabot, *et al*., *J. Med. Chem.* **1998**, *41*, 2882 – 2891.

[12] J. C. Powers, J. L. Asgian, O. D. Ekici, K. B. E. James, *Chem. Rev.* **2002**, *102*, 4639 – 4750.

[13] M. Bengalia, R. Annunziata, M. Clinquini, F. Cozzi, *J. Org. Chem.* **2003**, *68*, 2952 – 2955.

[14] C. M. L. Delpiccolo, E. G. Mata, *Tetrahedron Lett.* **2004**, *45*, 4085 – 4088.

[15] S. Gerard, G. Dive, B. Clamet, R. Touillaux, J. Marchand-Brynaert, *Tetrahedron* **2002**, *58*, 2423 – 2433.

[16] W. Bode, E. F. Meyer, J. C. Powers, *Biochemistry* **1989**, *28*, 1951 – 1963.

[17] J. Tozsera, T. Sperka, J. Pitlik, P. Bagossia, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3086 – 3090.

[18] W. Wang, P. Devasthale, D. Farrelly, G. Liqun, *Bio. Med. Chem. Lett.* **2008**, *18*, 1939 – 1944.

[19] B. Alcaide, P. Almendros, *Synlett.* **2002**, 381 – 393.

[20] D. Castagnolo, S. Armaroli, F. Corelli, M. Botta, *Tetrahedron Asymmetry* **2004**, *15 (6)*, 941 – 949.

[21] M. V. Kulkarni, G. M. Kulkarni, C. H. Lin, C. M. Sun, *Curr. Med. Chem.* **2006**, *13 (23)*, 2795 – 2818.

[22] R. V. Shingalapur, K. M. Hosamani, R. S. Keri, *Eur. J. Med. Chem.* **2009**, *44*, 4244 – 4248.

[23] R. S. Harisha, K. M. Hosamani, R. S. Keri, *Arch. Pharm. Chem. Life Sci.* **2009**, *342*, 412 – 419.

[24] K. M. Hosamani, R. S. Harisha, R. S. Keri, S. H. Manohar, M. G. Moloney, *J. Enzyme Inhib. Med. Chem.* **2009**, *24 (5)*, 1095 – 1100.

[25] A.-U. Rahman, M. I. Choudhary, W. J. Thomsen, *Bioassay Techniques for Drug Development*, Harwood Academic Publishers, Amsterdam, The Netherlands, **2001**, p. 16.

[26] B. N. Meyer, N. R. Ferrigni, J. E. Putnam, L. B. Jacobsen, *et al*., *Planta Med.* **1982**, *45*, 31 – 34.

[27] M. V. Kulkarni, B. G. Pujar, V. D. Patil, *Arch. Pharm.* **1983**, *316 (2)*, 15 – 21.

[28] A. Burger, G. E. Ullyot, *J. Org. Chem.* **1947**, *12*, 346 – 355.

[29] D. J. Finney, *Probit. Analysis*, 3rd ed., Cambridge University Press, Cambridge, UK, **1971**.

