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Original article

Analgesic, anti-pyretic and DNA cleavage studies of novel pyrimidine derivatives of coumarin moiety

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ABSTRACT

A novel series of 4-[4-(6-phenyl-pyrimidin-4-yl)-phenoxymethyl]-chromen-2-ones [**5**–**7**(**a**–**e**)] were synthesized from various 4-bromomethyl coumarins **1**(**a**–**e**). The synthesized compounds were screened for *in-vivo* analgesic and anti-pyretic activities at a dose of 25 and 100 mg/kg body weight (b.w), respectively. Among them, compounds **5**(**d**), **6**(**c**) and **7**(**d**) exhibited significant analgesic activity comparable with standard drug analgin using Tail-flick model. Compounds **5**(**a**) and **7**(**a**–**d**) showed significant anti-pyretic activities comparable with standard drug aspirin using yeast-induced pyrexia model. DNA cleavage study by agarose gel electrophoresis method was also studied. Qualitative SAR studies indicate that, compounds with amino group at 2-position of pyrimidine ring enhances analgesic and anti-pyretic activities and compounds with hydroxyl and thio group at 2-position of pyrimidine ring increase DNA cleavage activities.

1. Introduction

Pain is an unpleasant and subjective sensation resulting from a harmful sensorial stimulation that alerts the body about a current or potential damage to its tissues and organs [1]. It is estimated that more than 75 million people refer to health services annually, presenting some form of recurrent or persistent pain [2]. In spite of painful sensation that can be solved most efficiently by removal of the underlying cause, the pain-causing stimulus cannot always be either easily defined or quickly removed. Therefore, the health professionals are usually faced with the necessity to manage the symptomatology of the pain [1].

Fever is a complex physiologic response triggered by infections or aseptic stimuli. Elevation in body temperature occurs when the concentration of prostaglandin E_2 (PGE_2) increases within parts of the brain. Such an elevation contributes to a considerable alteration in the firing rate of neurons that control the thermoregulation process in the hypothalamus. It is now evident that most anti-pyretics exert their action by inhibiting the enzymatic activity of cyclooxygenase and consequently reducing the levels of PGE_2 within the hypothalamic region. In order to combat these diseases caused by pathogens, it is usual that chemotherapeutic, analgesic and anti-pyretic agents are prescribed separately in clinical practices [3].

The pyrimidine entity is one of the most prominent structures found in nucleic acid chemistry. Pyrimidine derivatives including uracil, thymine, cytosine, adenine, and guanine are fundamental building blocks for deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Vitamin B_1 (thiamine) is a well-known example of a naturally occurring pyrimidine that is encountered in our daily lives. They also play an essential role in several biological processes, found in nucleoside antibiotics, anti-bacterials, cardio-vascular as well as considerable chemical reactions and also occupy a prominent place in the pharmaceutical arena [4]. Pyrimidine derivatives form a component in a number of useful drugs and are associated with many biological, pharmaceutical and therapeutical activities [5]. Condensed pyrimidine derivatives have been reported as anti-microbial [6], analgesic, anti-viral, anti-inflammatory [7], anti-HIV [8], anti-tubercular [9], anti-tumor [10], anti-neoplastic [11], anti-malarial [12], diuretic [13], cardiovascular [14] agents. Pyrimidine compounds are also used as hypnotic drugs for the nervous system [15], calcium-sensing receptor antagonists [16] and also for antagonists of the human A2A adenosine receptor [17]. Like pyrimidine, coumarin also exhibits diverse biological properties [18].

It was envisaged that these two active pharmacophores, if linked together would generate novel molecular templates which are likely to exhibit interesting biological properties in animal models. The above-cited applications prompted us to synthesize a series of new compounds reported in this article. Owing to the importance and in continuation of our work on synthesis of

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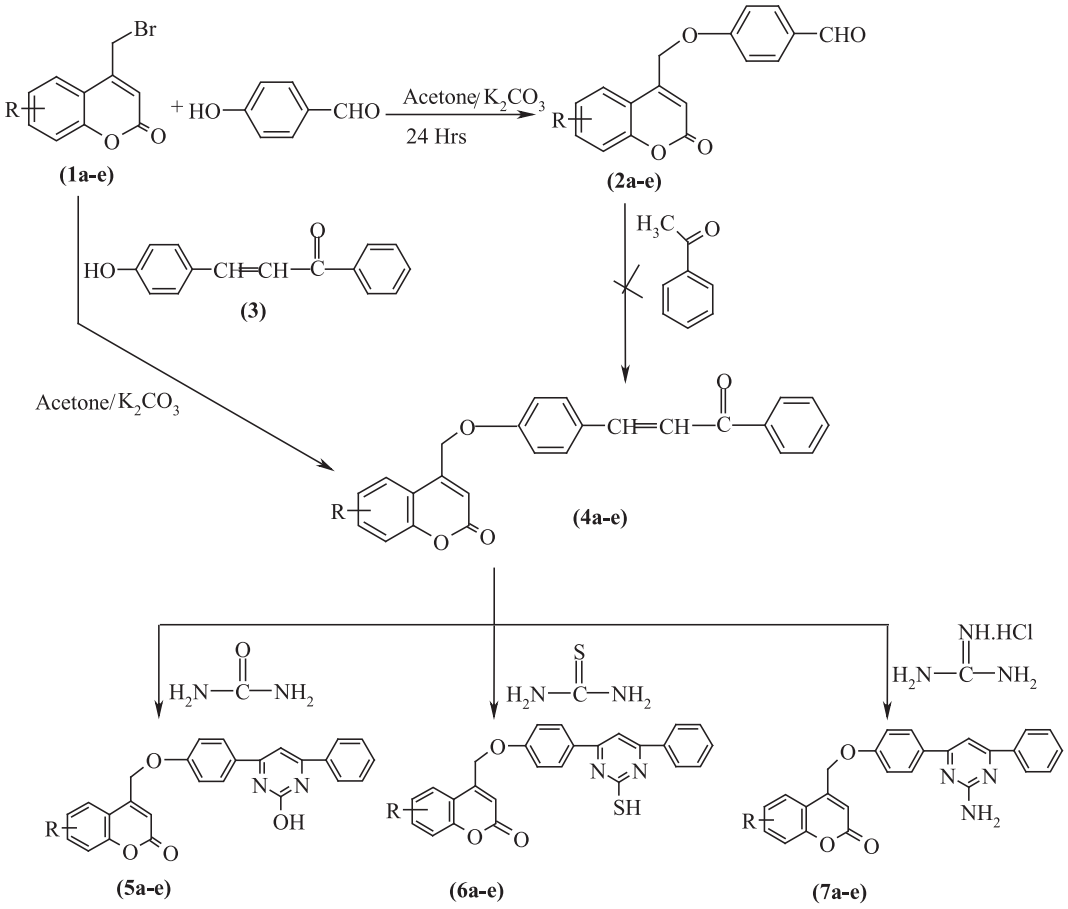
biologically active compounds [19–22], now we wish to describe the synthesis of new pyrimidine derivatives from 4-aryloxymethylcoumarins (Scheme 1). The compounds were screened for their *in-vivo* analgesic, anti-pyretic and DNA cleavage study. Thus, we have created new avenues to explore the potent heterocyclic moieties for the pharmacological activities in medicinal chemistry.

2. Chemistry

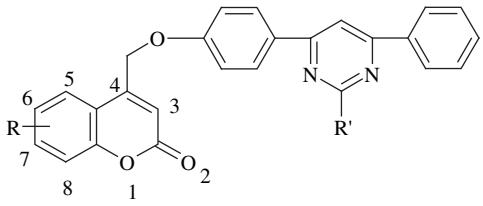
For the synthesis of target compounds, the reaction sequence outlined in Scheme 1, were followed. Synthesis of various 4-bromomethyl coumarins (**1a**–**e**) was brought about by the Pechmann cyclisation of phenols with 4-bromoethylacetoacetate. 4-bromomethyl coumarins **1**(**a**–**e**) were reacted with 4-hydroxy benzaldehyde in the presence of anhydrous K_2CO_3 in dry acetone at room temperature to give the corresponding ethers **2**(**a**–**e**). In the IR spectrum of 4-(2-oxo-2H-chromen-4-yl-methoxy)-benzaldehyde **2** (**a**), the lactone carbonyl stretching frequency was observed at 1718 cm^{-1}, whereas the aldehydic carbonyl stretching appeared at 1690 cm^{-1}. In the ^1H NMR spectrum of compound **2**(**a**), 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 9.95 ppm. Attempted condensation of **2**(**a**–**e**) with acetophenone in 40% NaOH did not result in the formation of chalcones **4**(**a**–**e**). A plausible explanation for this could be competition between the activated C_4–CH_2 and $COCH_3$ group for carbanion formation. Compounds **4**(**a**–**e**) were prepared by a different method, in which the chalcone (**3**) was prepared separately by the reaction of *p*-hydroxy benzaldehyde and acetophenone in presence of 20% alcoholic NaOH solution. This was then treated with 4-bromomethyl coumarins **1**(**a**–**e**) in the presence of anhydrous K_2CO_3 in dry acetone at room temperature. In the IR spectrum of 6-methyl-4-[4-3-oxo-3-phenyl-propenyl)-phenoxymethyl]-chromem-2-one **4**(**a**), the lactone carbonyl stretching frequency was observed at 1722 cm^{-1} and α, β-unsaturated keto group at 1660 cm^{-1}. In the ^1H NMR spectrum of compound **4**(**a**), the olefinic protons (cis) were observed at δ 7.43 and 7.92 ppm as two separate doublets. Further the compounds **4** (**a**–**e**) were treated with urea, thiourea and guanidine hydrochloride in DMF to yield the corresponding pyrimidine derivatives. The IR spectrum of 4-[4-2-hydroxy-6-phenyl-pyrimidin-4-yl-phenoxymethyl]-6-methyl-chromem-2-one **5**(**a**) shows lactone carbonyl stretching frequency at 1718 cm^{-1} and hydroxyl group of pyrimidine at 3498 cm^{-1}. In the PMR spectrum of compound **5**(**a**), a singlet was observed at δ 2.16 ppm due to C_6–CH_3 protons. The C_4-CH_2 protons were observed downfield as a singlet at δ 5.47 ppm. The pyrimidine proton was observed downfield as a singlet at δ 7.03 ppm and hydroxyl proton at δ 10.7 ppm. The aromatic protons resonated as multiplet in the region of δ 6.88–8.15 ppm. All the physical and analytical data of the synthesized compounds are presented in Table 1.



Where R = 6-CH_3, 7-CH_3, 6-Cl, 5,6-Benzo, 7,8-Benzo

Scheme 1. Schematic representation for the synthesis of pyrimidines derivatives from 4-aryloxymethylcoumarin.

Table 1
Physical and analytical data of pyrimidine derivatives [**5**–**7**(**a**–**e**)].



Entry	Product[a]	R	R′	Yield (%)[b]	m. p (°C)[c]	Mol. Formula/Mol. Wt	Elem. Analysis (Cal./Found)		
							C	H	N
1	**5a**	6-CH_3	OH	61.0	265–266	$C_{27}H_{20}N_2O_4$/436	74.30	4.62	6.42
							74.32	4.65	6.45
2	**5b**	7-CH_3	OH	57.0	203–204	$C_{27}H_{20}N_2O_4$/436	74.30	4.62	6.42
							74.29	4.60	6.45
3	**5c**	6-Cl	OH	53.0	176–177	$C_{26}H_{17}ClN_2O_4$/457	68.35	3.75	6.13
							68.37	3.78	6.10
4	**5d**	5,6-Benzo	OH	64.0	165–166	$C_{30}H_{20}N_2O_4$/472	76.26	4.27	5.93
							76.30	4.30	5.97
5	**5e**	7,8-Benzo	OH	58.0	210–211	$C_{30}H_{20}N_2O_4$/472	76.26	4.27	5.93
							76.39	4.25	5.95
6	**6a**	6-CH_3	SH	73.2	216–218	$C_{27}H_{20}N_2O_3S$/452	71.66	4.45	6.19
							71.68	4.47	6.20
7	**6b**	7-CH_3	SH	61.0	168–169	$C_{27}H_{20}N_2O_3S$/452	71.66	4.45	6.19
							71.69	4.43	6.17
8	**6c**	6-Cl	SH	64.0	137–138	$C_{26}H_{17}ClN_2O_3S$/473	66.03	3.62	5.92
							66.06	3.66	5.96
9	**6d**	5,6-Benzo	SH	58.0	243–244	$C_{30}H_{20}N_2O_3S$/488	73.75	4.13	5.73
							73.78	4.16	5.70
10	**6e**	7,8-Benzo	SH	68.0	221–222	$C_{30}H_{20}N_2O_3S$/488	73.75	4.13	5.73
							73.73	4.10	5.75
11	**7a**	6-CH_3	NH_2	64.0	274–275	$C_{27}H_{21}N_2O_3$/435	74.47	4.86	9.65
							74.51	4.89	9.62
12	**7b**	7-CH_3	NH_2	73.0	227–228	$C_{27}H_{21}N_2O_3$/435	74.47	4.86	9.65
							74.45	4.85	9.67
13	**7c**	6-Cl	NH_2	68.0	176–177	$C_{26}H_{18}ClN_3O_3$/456	68.50	3.98	9.22
							68.53	3.96	9.19
14	**7d**	5,6-Benzo	NH_2	67.0	142–144	$C_{30}H_{22}ClNO_5$/471	70.42	4.49	8.91
							70.45	4.52	8.93
15	**7e**	7,8-Benzo	NH_2	59.0	227–229	$C_{30}H_{22}ClNO_5$/471	70.42	4.49	8.91
							70.46	4.50	8.88

[a] Products were characterized by IR, NMR, MS and elemental analysis.
[b] Isolated yields.
[c] Melting points are uncorrected.

3. Pharmacology

All the synthesized compounds [**5**–**7**(**a**–**e**)] were screened for analgesic activity by tail-flick method in rats, used by D'Amour and Smith [23]. The reaction time was measured at the end of 0, 30, 60 and 90 min after the administration of the compound and the standard drug employed was analgin. The anti-pyretic activity of the newly synthesized compounds [**5**–**7**(**a**–**e**)] was screened by using the yeast-induced pyrexia method [24]. Rectal temperature was recorded by using clinical thermometer before (−18 h) and 18 h after (0 h) brewer's yeast injection. Aspirin (300 mg/kg, p.o.) was used as standard drug for comparing the anti-pyretic activity of the synthesized compounds. The DNA cleavage of newly synthesized compounds [**5**–**6**(**a**–**e**)] and **7**(**a**–**b**) pyrimidine derivatives were studied by agarose gel electrophoresis method [25].

4. Results and discussion

4.1. Acute toxicity studies

All the compounds have shown good safety profile till the highest dose. No adverse effect or mortality was detected in albino rats up to 3 g kg^{-1}, p.o. during the 24 h observation period. There was no sedation, convulsions and tremors upon inspection, no ulceration and no haemorrhagic spots were observed. Postmortem examination of the stomach, and intestine did not reveal any ulcer-haemorrhagic spots.

4.2. Analgesic activity

All the synthesized compounds were screened for analgesic activity by tail-flick method used by D'Amour and Smith [23]. The reaction time was measured at the end of 0, 30, 60 and 90 min after the administration of the compound and the standard drug employed was analgin. The analgesic screening results revealed that the entire tested compounds exhibited moderate to good analgesic activity (Table 2) compared to the reference drug. The compounds **5**(**b**), **5**(**d**), **5**(**e**), **6**(**b**), **6**(**c**), **7**(**c**) and **7**(**d**) showed excellent analgesic activity at 60 and 90 min. Whereas compounds **5**(**a**), **6**(**a**), **6**(**e**), **7**(**a**) and **7**(**b**) showed moderate to good analgesic activity comparable with standard drug analgin at 60 and 90 min. Compounds **5**(**c**), **6**(**d**) and **7**(**e**) showed lesser degree of activity. Compounds **5**(**d**), **6**(**c**) and **7**(**d**) showed nearly comparable activity to that of reference drug analgin in peripheral analgesic activity.

Table 2
Analgesic activity of synthesized compounds [**5**–**7**(**a**–**e**)] by tail-flick method in rats.

Compound	Dose (mg/kg) Body weight	Average (±) reaction time (s). Time after drugs treatment (min)			
		0 (min)	30 (min)	60 (min)	90 (min)
Control 1% Tween 80	–	3.72 (± 0.358)	3.97 (± 0.287)	4.00 (± 0.360)	3.87 (± 0.355)
Standard Analgin	25	4.22 (± 0.410)	5.77 (± 0.250)*	8.76 (± 0.252)**	9.02(± 0.000)**
5a	25	3.94 (± 0.410)	4.48 (± 0.409)	5.46 (± 0.577)*	6.20 (± 0.252)*
5b	25	4.00(± 0.408)	4.50 (± 0.577)	6.25 (± 0.410)**	7.75 (± 0.248)**
5c	25	3.80(± 0.000)	4.02(± 0.108)	4.04(± 0.208)	4.25(± 0.200)
5d	25	3.74(± 0.249)	5.00(± 0.517)	8.25(± 0.456)**	8.87(± 0.450)**
5e	25	4.00(± 0.408)	4.75(± 0.50)	6.25(± 0.353)**	7.25(± 0.55)**
6a	25	3.85(± 0.249)	4.50(± 0.219)	6.50(± 0.108)*	7.45(± 0.330)**
6b	25	3.80(± 0.408)	4.50(± 0.409)	7.25(± 0.050)**	7.50(± 0.408)**
6c	25	4.00(± 0.353)	5.10(± 0.00)	7.54(± 0.408)**	8.50(± 0.408)**
6d	25	3.76(± 0.540)	4.25(± 0.118)	4.50(± 0.208)	4.50(± 0.20)
6e	25	3.84(± 0.353)	4.52(± 0.408)	5.25(± 0.300)*	6.25(± 0.408)**
7a	25	3.94(± 0.249)	4.75(± 0.353)	6.75(± 0.500)*	7.25(± 0.288)**
7b	25	4.00(± 0.353)	5.10(± 0.000)	6.75(± 0.500)*	7.50(± 0.408)**
7c	25	3.80(± 0.408)	5.02(± 0.408)	7.00(± 0.572)*	7.10(± 0.354)**
7d	25	3.28(± 0.353)	4.00(± 0.201)	7.25(± 0.500)*	8.00(± 0.408)**
7e	25	3.90(± 0.408)	4.04(± 0.408)	4.75(± 0.249)	5.00(± 0.577)**

Method: tail-flick method; test animals: albino rats; number of animals per group: 6; route of administration: oral; standard: Analgin (25 mg/kg); $^{*}P < 0.05$, $^{**}P < 0.01$, when compared to control.
Statistical analysis: the statistical analysis was performed by one-way ANOVA followed Dunnet's test.

4.3. Anti-pyretic activity

All the synthesized compounds [**5**–**7**(**a**–**e**)] were screened for anti-pyretic activity by using the brewer's yeast-induced pyrexia method [24]. Aspirin is used as a reference drug. Fever was induced by injecting 20 ml/kg (s.c.) of 20% aqueous suspension of brewer's yeast in normal saline below the nape of the neck and rectal temperature was recorded by clinical thermometer immediately before (−18 h) and 18 h after (0 h). The anti-pyretic screening results revealed that (Table 3), the compounds **5**(**a**), **5**(**c**), **6**(**c**), **6**(**d**), **7**(**a**), **7**(**b**), **7**(**c**) and **7**(**d**) significantly decrease the temperature of pyretic rats at 1, 3 and 6 h after compound administration (Table 3). The maximum mean rectal temperatures produced by brewer's yeast in the presence of compounds **7**(**a**) and **7**(**c**) were (31.68, 32.45 °C), respectively. In addition, compounds **6**(**c**), **6**(**d**), **6**(**e**), **7**(**b**) and **7**(**c**), showed a decrease in the rectal temperature after 3 h were (32.61, 31.98, 32.00, 32.60, 32.45 °C), respectively compared to 34.68 °C in the control group.

4.4. Electrophoretic analysis

The DNA cleavage of some pyrimidine derivatives [**5**–**6**(**a**–**e**)] and **7**(**a**–**b**) were studied by agarose gel electrophoresis method and are presented in Figs. 1 and 2. The gel after electrophoresis clearly revealed that, the compound **5**(**a**) and **5**(**d**) shown more intense streak, indicating the activity. Significant activity was also seen in compound **5**(**b**), **5**(**c**), **5**(**e**), **6**(**b**), **6**(**d**), **6**(**e**) and **7**(**b**). The difference was observed in the bands of compounds (Lanes 1–12) compared to the control DNA of *E. coli*. This shows that the control DNA alone does not show any apparent cleavage as the compounds did. However, the nature of reactive intermediates involved in the DNA cleavage by the compounds has not been clear. The results indicate the importance of pyrimidine derivatives in these isolated DNA cleavage reactions. As the compounds **5**(**a**) and **5**(**d**) were observed to cleave the DNA, it can be concluded that the compound inhibits the growth of the pathogenic organism by cleaving the genome.

Table 3
Anti-pyretic activity of synthesized compounds [**5**–**7**(**a**–**e**)] on brewer's yeast-induced pyrexia in rats.

Compound	Dose (mg/kg) Body weight	Rectal temperature in °C at time (h)					
		−18[a]	0[b]	1	3	5	6
Control malsaline	–	34.68(± 0.18)	33 ± 0.08 (± 0.68)[c]	33.78 (± 0.18)	32.80(± 0.09)	34.36(± 0.14)	33.44(± 0.18)
Standard Aspirin	300	34.70(± 0.12)	33.08 ± 0.26 (± 0.84)[c]	32.82 (± 0.14)***	32.00 (± 0.11)***	32.30(± 0.11)***	32.38(± 0.12) ***
5a	100	34.40(± 0.18)	33.14 ± 0.32 (± 0.90)[c]	33.18 (± 0.16)*	32.31(± 0.22)	33.12(± 0.26)**	32.97(± 0.24)
5b	100	34.71(± 0.15)	33.08 ± 0.44 (± 0.84)[c]	32.40 (± 0.15)	32.84(± 0.08)**	33.10(± 0.26)	32.40(± 0.24)
5c	100	34.52(± 0.11)	33.82 ± 0.60 (± 0.95)[c]	33.18 (± 0.17)**	32.64(± 0.21)*	33.28(± 0.29)	32.61(± 0.28)
5d	100	34.68(± 0.09)	33.43 ± 0.52 (± 0.83)[c]	32.22 (± 0.18)	32.72(± 0.18)	32.82(± 0.24)	32.68(± 0.08)***
5e	100	34.75(± 0.06)	33.54 ± 0.44 (± 0.75)[c]	32.25 (± 0.20)	32.68(± 0.20)*	33.44(± 0.11)**	32.74(± 0.21)
6a	100	34.80(± 0.11)	33.64 ± 0.20 (± 0.95)[c]	32.55 (± 0.22)*	32.77(± 0.24)	32.80(± 0.08)**	31.98(± 0.18)
6b	100	34.82(± 0.09)	33.78 ± 0.36 (± 0.88)[c]	32.81 (± 0.26)*	32.66(± 0.26)	32.90(± 0.12)**	31.82(± 0.20)
6c	100	34.44(± 0.12)	33.80 ± 0.50 (± 0.82)[c]	32.50 (± 0.32)*	32.61(± 0.28)**	32.84(± 0.36)	32.84(± 0.22)**
6d	100	34.46(± 0.18)	33.78 ± 0.62 (± 0.91)[c]	32.92 (± 0.18)*	31.98(± 0.32)	33.01(± 0.14)**	32.66(± 0.24)
6e	100	34.68(± 0.22)	33.88 ± 0.58 (± 0.83)[c]	33.01 (± 0.21)*	32.00(± 0.16)**	33.09(± 0.19)	32.50(± 0.32)
7a	100	34.36(± 0.36)	33.44 ± 0.66 (± 0.82)[c]	32.66 (± 0.35)*	31.68 (± 0.07)***	33.30(± 0.18)	33.00(± 0.08)***
7b	100	34.54(± 0.32)	33.38 ± 0.55 (± 0.80)[c]	32.74 (± 0.33)*	32.60(± 0.09)**	33.80(± 0.13)**	32.80(± 0.11)**
7c	100	34.72(± 0.18)	33.62 ± 0.44 (± 0.88)[c]	32.74 (± 0.33)*	32.45 (±0.08)***	32.09(± 0.15)	32.54(± 0.12)
7d	100	34.48(± 0.14)	33.77 ± 0.36 (± 0.75)[c]	32.80 (± 0.26)	31.84 (± 0.11)**	32.22(± 0.17)	31.88(± 0.14)
7e	100	34.82(± 0.15)	33.64 ± 0.55 (± 0.72)[c]	32.68 (± 0.30)*	31.40 (± 0.18)	32.51(± 0.23)	32.09(± 0.18)**

n = Six animals in each group, values are mean ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ when compared to control.
[a] Temperature just before yeast injection.
[b] Temperature just before drug administration.
[c] Change in temperature following yeast injection.

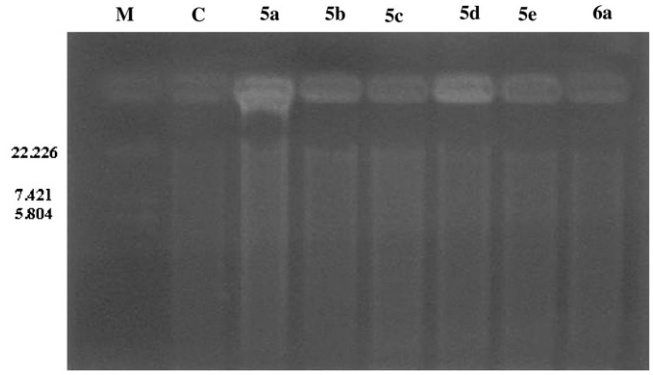


Fig. 1. DNA cleavage analysis of pyrimidine derivatives [**5**(**a**–**e**) and **6a**].

4.5. Structure activity relationship (SAR) study

The effect of varying and introducing new substituents in pyrimidine moiety has been explored in synthetic organic chemistry for significant biological activity in medicinal chemistry (Table 1). Thus, a variety of 2-substituted pyrimidine analogues containing –OH, –SH and $-NH_2$ groups have been exploited for their analgesic and anti-pyretic activities. These compounds with $-NH_2$ functional group at 2-position of the pyrimidine moiety have shown significant analgesic and anti-pyretic activities compared to the –SH and –OH groups. The synthesized compounds with –SH functional group at 2-position of pyrimidine ring showed moderate analgesic and anti-pyretic activities compared to that of -OH functional group. Whereas, the title compounds **5**(**b**), **5**(**c**), **5**(**e**), **6**(**b**), **6**(**d**) and **6**(**e**) showed significant DNA cleavage activity. Therefore, such compounds containing –OH and –SH substitutents at 2-position of the pyrimidine moiety enhance the DNA cleavage which could be exploited in medicinal chemistry for significant biological activities. Hence, we concluded that amongst the tested compounds, those containing $-NH_2$ functional group at 2-position of pyrimidine ring increases their analgesic and anti-pyretic activities. But, in case of DNA cleavage study, it is regarded as exactly reverse of analgesic and anti-pyretic activities.

5. Conclusion

A new series of 4-[4-(6-phenyl-pyrimidin-4-yl)-phenoxymethyl]-chromen-2-ones [**5**–**7**(**a**–**e**)] analogues were designed, synthesized and characterized. The synthesized compounds screened for their *in-vivo* analgesic, anti-pyretic and DNA cleavage studies. Some of the synthesized compounds *viz.*, **5**(**d**), **6**(**c**) and **7**(**d**) exhibited significant and the remaining compounds showed good to moderate analgesic activity comparable to that of standard drug analgin in the tail-flick model at 25 mg/kg body weights of the animals. The compounds **5**(**a**), **7**(**a**), **7**(**b**), **7**(**c**) and **7**(**d**) have significant anti-pyretic activity comparable with standard drug aspirin in yeast-induced pyrexia model at 100 mg/kg. The DNA cleavage studies revealed that, pyrimidine derivatives **5**(**a**) and **5**(**d**) showed non-specific cleavage of DNA. In general, test compounds are non-toxic up to 3000 mg kg^{-1} body weights of the animals and showed significant *in-vivo* analgesic and anti-pyretic activities. Qualitative SAR studies indicate that the compounds containing amino group at the 2-position of pyrimidine ring increases their analgesic and anti-pyretic activities and the compounds with hydroxyl and thio group at the 2-position of pyrimidine ring increases DNA cleavage activities.

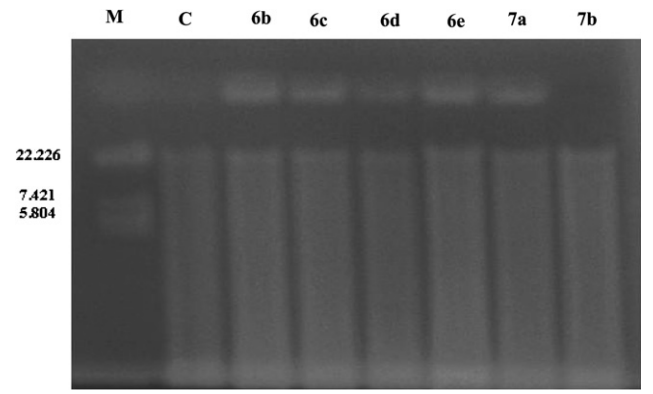


Fig. 2. DNA cleavage analysis of pyrimidine derivatives [**6**(**b**–**e**) and **7**(**a**–**b**)].

6. Experimental protocols

6.1. Chemistry

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 Buchi apparatus and are uncorrected. The IR spectra were recorded on a Nicolet Impact-410 FT-IR spectrophotometer, using KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC-300F 300 MHz spectrometer in DMSO/$CDCl_3$ using TMS 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/OH protons. Mass spectra were recorded on an Autospec EI-MS. The elemental analysis was carried out using Heraus CHN rapid analyzer. 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 alumina silica gel 60 F_{254} (Merck) detected by U.V light (254 nm) and iodine vapours. The *in-vivo* analgesic and anti-pyretic activities were screened at Luqman Pharmacy College, Gulbarga, Rajiv Gandhi University, India.

6.2. General procedure for the preparation of compounds

6.2.1. Preparation of 4-bromomethyl coumarins *1*(*a*–*e*)

The present synthetic strategy begins with the generation of the required 4-bromomethylcoumarins. These compounds are prepared by the Pechmann cyclisation of phenols with 4-bromoethylacetoacetate [26].

6.2.2. Preparation of 2-oxo-2H-chromen-4-ylmethoxy-benzaldehydes *2*(*a*–*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-Bromomethylcoumarins (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 separated was filtered and washed with 5% HCl (10 ml) to neutralize excess of K_2CO_3, then washed with 100 ml of cold water and with dilute ethanol. The crude product was dried and recrystalized from DMF.

*6.2.2.1. 4-(6-Methyl-2-oxo-2H-chromen-4-ylmethoxy)-benzaldehyde **2**(**a**).* Colourless solid, m.p. 218–219 °C, IR (KBr, υ cm^{-1}): 3046 (=CH–), 2778 (CH of CHO), 1718 (C=O of coumarin), 1690 (C=O of aldehyde), 1534 (C=C) cm^{-1},1022 (C–O–C); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 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,

$CDCl_3$, δ ppm): 21.1, 72.1, 109.5, 112.7, 114.0, 118.0, 123.0, 128.5, 133.1, 133.3, 136.5, 148.2, 152.4, 158.2, 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, 73.46; H, 4.83%.

*6.2.2.2. 4-(7-Methyl-2-oxo-2H-chromen-4-ylmethoxy)-benzaldehyde **2**(**b**).* Colourless solid, m.p.223–225 °C, IR (KBr, υ cm^{-1}): 3038 (=CH–), 2788 (CH of CHO), 1707 (C=O of coumarin), 1697 (C=O of aldehyde), 1562 (C=C) cm^{-1}, 1016 (C–O–C); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 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, $CDCl_3$, δ ppm): 20.6, 78.0, 108.6, 113.5, 115.6, 117.8, 121.3, 124.6, 128.4, 130.3, 132.6, 134.1, 148.2, 155.4, 158.6, 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, 73.47; H, 4.85%.

*6.2.2.3. 4-(6-Chloro-2-oxo-2H-chromen-4-ylmethoxy)-benzaldehyde **2**(**c**).* Colourless solid, m.p.220–222 °C, IR (KBr, υ cm^{-1}) : 3062 (=CH–), 2808 (CH of CHO), 1722 (C=O of coumarin), 1698 (C=O of aldehyde), 1571 cm^{-1} (C=C), 1021 (C–O–C); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 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, $CDCl_3$, δ ppm): 83.4, 110.0, 113.5, 116.0, 119.8, 126.0, 127.9, 130.1, 131.6, 135.1, 138.3, 148.2, 151.6, 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; Found C, 64.90; H, 3.60%.

*6.2.2.4. 4-(3-Oxo-3H-benzochromen-1-ylmethoxy)-benzaldehyde **2**(**d**).* Pale yellow solid, m.p.222–224 °C, IR (KBr, υ cm^{-1}): 3080 (=CH–), 2836 (CH of CHO), 1712 (C=O of coumarin), 1695(C=O of aldehyde), 1562 cm^{-1} (C=C), 1031 (C–O–C); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 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, $CDCl_3$, δ ppm): 81.4, 106.0, 113.5, 114.0, 117.8, 120.5, 121.6, 123.0, 126.8, 129.1, 129.9, 130.3, 133.5, 135.2, 148.7, 149.6, 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, 76.41; H, 4.30%.

*6.2.2.5. 4-(2-Oxo-2H-benzochromen-4-ylmethoxy)-benzaldehyde **2**(**e**).* Pale red solid, m.p.248–249 °C, IR (KBr, υ cm^{-1}): 3052 (=CH–), 2842 (–CH of CHO), 1718 (C=O of coumarin), 1707 (C=O of aldehyde), 1569 cm^{-1} (C=C), 1028 (C–O–C); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 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, $CDCl_3$, δ ppm): 80.8, 107.5, 112.6, 113.1, 116.3, 122.1, 125.6, 127.6, 128.2, 129.8, 131.6, 132.6, 133.0, 135.3, 146.6, 149.6, 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, 76.42; H, 4.23%.

*6.2.3. Preparation of 4-[4-3-Oxo-3-phenyl-propenyl-phenoxymethyl]-chromen-2-one **4**(**a**–**e**)*

Compound (**3**) (0.01 mol) and anhydrous K_2CO_3 (0.01 mol) were stirred in anhydrous acetone for half an hour. To this substituted 4-bromomethyl coumarins **1**(**a**–**e**) (0.01 mol) were added and the stirring was continued for 24 h. The reaction mixture was added to the crused ice and neutralized with 1:1 HCl. The solid separated was filtered and washed with water, dried and recrystalized from dioxan-ethanol mixture.

*6.2.3.1. 6-Methyl-4-[4-(3-Oxo-3-phenyl-propenyl)-phenoxymethyl]-chromen-2-one **4**(**a**).* Colourless shiny crystals, yield 70%, m.p.232–233 °C, IR (KBr, υ cm^{-1}): 3059 (=CH–), 1722 (C=O of coumarin), 1660 (α, β-unsaturated keto group), 1527 (C=C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 2.26 (s, 3H, CH_3), 5.42 (s, 2H, CH_2O), 6.60 (s, 1H, C_3H), 7.43 (d, $J = 15.7$ Hz, 1H, –CH=CH), 7.92 (d, $J = 15.8$ Hz, 1H, –CH=CH), 6.76–7.81 (m, 12H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 20.0, 74.6, 103.8, 112.6, 113.9, 116.8, 119.2, 122.5, 126.5, 127.6, 128.3, 129.8, 130.6, 131.6, 132.9, 135.0, 140.1, 147.2, 157.7, 160.2, 162.3, 179.0; ESI-MS: 396 (M^+); Anal. Calcd for $C_{26}H_{20}O_4$: C, 78.77; H, 5.09; Found C, 78.79; H, 5.12%.

*6.2.3.2. 7-Methyl-4-[4-(3-Oxo-3-phenyl-propenyl)-phenoxymethyl]-chromen-2-one **4**(**b**).* Colourless shiny crystals, Yield 58%, m.p.205–206 °C, IR (KBr, υ cm^{-1}): 3079 (=CH–), 1717 (C=O of coumarin), 1655 (α, β-unsaturated keto group), 1520 (C=C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 2.15 (s, 3H, CH_3), 5.33 (s, 2H, CH_2O), 6.67 (s, 1H, C_3H), 7.47 (d, $J = 15.5$ Hz, 1H, –CH=CH), 7.87 (d, $J = 15.5$ Hz 1H,–CH=CH), 6.88–8.13 (m, 12H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 22.6, 79.3, 106.3, 110.3, 112.6, 117.8, 120.7, 122.0, 125.9, 126.9, 128.3, 129.8, 130.6, 131.1, 131.9, 132.8, 134.0, 135.8, 139.8, 145.6, 149.0, 162.3, 165.1, 175.7; ESI-MS: 396 (M^+); Anal. Calcd for $C_{26}H_{20}O_4$: C, 78.77; H, 5.09; Found C, 78.80; H, 5.10%.

*6.2.3.3. 6-Chloro-4-[4-(3-Oxo-3-phenyl-propenyl)-phenoxymethyl]-chromen-2-one **4**(**c**).* Shiny pale yellow crystals, Yield 63%, m.p.145–147 °C, IR (KBr, υ cm^{-1}): 3088 (= CH-), 1715 (C=O of coumarin), 1647 (α, β-unsaturated keto group), 1532 (C=C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 5.38 (s, 2H, CH_2O), 6.53 (s, 1H, C_3H), 7.32 (d, $J = 15.9$ Hz, 1H, –CH=CH), 15.6 (d, $J = 15.8$ Hz, 1H,–CH=CH), 6.76–7.98 (m, 12H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 75.8, 104.6, 112.6, 113.6, 117.5, 119.4, 122.0, 123.6, 125.3, 127.2, 129.6, 131.3, 132.5, 132.9, 134.3, 135.2, 136.3, 141.2, 148.3, 156.8, 159.1, 161.5, 184.4; ESI-MS: 418 $[M+1]^+$; Anal. Calcd for $C_{26}H_{17}ClO_4$: C, 72.03; H, 4.11; Found C, 72.00; H, 4.14%.

*6.2.3.4. 1-[4-(3-Oxo-3-phenyl-propenyl)-phenoxymethyl]-benzo[f] chromen-3-one **4**(**d**).* Yellow crystals, Yield 73%, m.p.242–243 °C, IR (KBr, υ cm^{-1}): 3103 (= CH-), 1728 (C=O of coumarin), 1664 (α, β-unsaturated keto group), 1542 (C=C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 5.13 (s, 2H, CH_2O), 6.37 (s, 1H, C_3H), 7.54 (d, $J = 15.4$ Hz, 1H, –CH=CH), 7.83 (d, $J = 15.5$ Hz 1H,–CH=CH), 7.02- 8.41 (m, 15H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 83.5, 106.3, 112.6, 113.3, 115.2, 116.7, 121.6, 123.3, 124.7, 126.5, 127.6, 128.3, 129.3, 130.2, 131.3, 133.1, 136.5, 140.8, 150.2, 158.3, 160.4, 163.3, 177.2; ESI-MS: 433$[M+1]^+$; Anal. Calcd for $C_{29}H_{20}O_4$: C, 80.54; H, 4.66; Found C, 80.53; H, 4.69%.

*6.2.3.5. 4-[4-(3-Oxo-3-phenyl-propenyl)-phenoxymethyl]-benzo[h] chromen-2-one **4**(**e**).* Reddish crystals, Yield 58%, m.p.221–222 °C, IR (KBr, υ cm^{-1}): 3093 (= CH-), 1725 (C=O of coumarin), 1655 (α, β-unsaturated keto group), 1526 (C=C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 5.36 (s, 2H, CH_2O), 6.42 (s, 1H, C_3H), 7.36 (d, $J = 16.2$ Hz, 1H, –CH=CH), 7.92 (d, $J = 16.3$ Hz 1H, –CH=CH), 6.89–8.23 (m, 15H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 81.6, 104.8, 111.6, 114.5, 115.6, 118.3, 120.4, 122.3, 123.6, 125.9, 128.1, 128.9, 129.5, 130.5, 131.0, 132.3, 135.3, 138.7, 144.9, 148.7, 160.0, 161.3, 162.8, 180.0; ESI-MS: 433 $[M+1]^+$; Anal. Calcd for $C_{29}H_{20}O_4$: C, 80.54; H, 4.66; Found C, 80.57; H, 4.69%.

*6.2.4. Preparation of 4-[4-(6-phenyl-pyrimidin-4-yl)-phenoxymethyl]-chromen-2-one [(**5**–**7**)**a**–**e**]*

Chalcone **4**(**a**–**e**) (0.01 mol) and urea, thiourea or guanidine hydrochloride (0.01 mol) were dissolved in DMF (20 ml). Few drops of concentrated HCl were added and the reaction mixture was refluxed and the reaction was monitored by TLC. After completion of reaction, the reaction mixture was poured onto 250 ml of ice-cold water and kept aside for some time. The crude solid was filtered and subjected to column chromatography. Elution with solvent system ethyl acetate/petroleum ether (60–80 °C) gave pure compound.

*6.2.4.1. 4-[4-(2-Hydroxy-6-phenyl-pyrimidin-4-yl)-phenoxymethyl]-6-methyl- chromen-2-one **5**(**a**).* Colourless shiny crystals, IR (KBr, υ cm^{-1}): 3498 (–OH), 3055 (Ar–H), 1718 (C=O of coumarin), 1603

(C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 2.16 (s, 3H, C_6-CH_3), 5.47 (s, 2H, CH_2O), 6.46 (s, 1H, C_3H), 6.61 (s, 1H, pyrimidine proton), 6.88–8.15 (m, 12H, Ar–H), 10.7 (br s, OH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 21.5, 79.3, 101.0, 107.4, 113.2, 115.2, 117.2, 120.2, 124.0, 125.4, 126.2, 127.0, 127.8, 128.3, 128.6, 129.0, 130.8, 133.4, 134.6, 136.3, 145.0, 147.8, 155.3, 161.3, 164.6 171.3; ESI-MS: 437 $[M+1]^+$.

*6.2.4.2. 4-[4-(2-Hydroxy-6-phenyl-pyrimidin-4-yl)-phenoxymethyl]-7-methyl-chromen-2- one **5(b)**.* Colourless shiny crystals, IR (KBr, υ cm^{-1}): 3486 (–OH), 3073 (Ar–H), 1721 (C=O of coumarin), 1589 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 2.25 (s, 3H, C_7-CH_3), 5.53 (s, 2H, CH_2O), 6.33 (s, 1H, C_3H), 6.67 (s, 1H, pyrimidine proton), 7.05–8.44 (m, 12H, Ar–H), 11.6 (br s, OH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 19.4, 80.3, 104.6, 108.5, 112.6, 115.8, 117.8, 121.7, 125.3, 126.6, 127.7, 128.9, 129.3, 130.4, 131.6, 132.9, 136.8, 138.7, 147.6, 150.8, 157.3, 159.2, 161.7, 176.8; ESI-MS: 437 $[M+1]^+$.

*6.2.4.3. 6-Chloro-4-[4-(2-hydroxy-6-phenyl-pyrimidin-4-yl)-phenoxymethyl]-chromen-2- one **5(c)**.* Colourless shiny crystals, IR (KBr, υ cm^{-1}): 3508 (–OH), 3067 (Ar–H), 1725 (C=O of coumarin), 1612 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 5.53 (s, 2H, CH_2O), 6.41 (s, 1H, C_3H), 6.68 (s, 1H, pyrimidine proton), 6.91–8.33 (m, 12H, Ar–H), 10.87 (br s, OH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 77.7, 103.6, 109.3, 111.5, 113.1, 115.9, 119.3, 125.7, 126.8, 127.2, 127.6, 128.4, 128.9, 130.5, 134.2, 135.5, 146.3, 147.9, 155.8,160.1, 163.8 176.2; ESI-MS: 458 $[M+1]^+$.

*6.2.4.4. 1-[4-(2-Hydroxy-6-phenyl-pyrimidin-4-yl)-phenoxymethyl]-benzo[f]chromen-3- one **5(d)**.* Yellow shiny crystals, IR (KBr, υ cm^{-1}): 3517 (-OH), 3088 (Ar–H), 1731 (C=O of coumarin), 1606 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 5.43 (s, 2H, CH_2O), 6.35 (s, 1H, C_3H), 6.71 (s, 1H, pyrimidine proton), 7.03–8.42 (m, 15H, Ar–H), 11.04 (br s, OH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 80.8, 102.3, 108.0, 114.8, 115.8, 116.0, 117.3, 120.1, 122.3, 124.5, 126.3, 127.1, 127.7, 128.3, 128.9, 129.2, 130.6, 131.8, 133.8, 135.3, 136.9, 144.3, 151.3, 156.9, 158.6, 162.3, 164.2 172.1; ESI-MS: 473 $[M+1]^+$.

*6.2.4.5. 4-[4-(2-Hydroxy-6-phenyl-pyrimidin-4-yl)-phenoxymethyl]-benzo [h]chromen-2-one **5(e)**.* Reddish shiny crystals, IR (KBr, υ cm^{-1}): 3503 (-OH), 3065 (Ar–H), 1726 (C=O of coumarin), 1589 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 5.53 (s, 2H, CH_2O), 6.35 (s, 1H,C_3–H), 6.88 (s, 1H, pyrimidine proton), 6.97–8.13 (m, 15H, Ar–H), 10.65 (br s, OH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 77.5, 99.4, , 111.0, 113.4, 115.2, 115.9, 116.7, 119.6, 122.0, 124.1, 126.3, 127.3, 127.9, 128.1, 128.8, 129.3, 129.9, 130.5, 131.6, 134.6, 138.5, 147.7, 154.3, 159.5, 160.4, 161.6, 175.5; ESI-MS: 473 $[M+1]^+$.

*6.2.4.6. 4-[4-(2-Mercapto-6-phenyl-pyrimidin-4-yl)-phenoxymethyl]-6-methyl-chromen-2-one **6(a)**.* Colourless shiny crystals, IR (KBr, υ cm^{-1}): 3048 (Ar–H), 2592 (SH), 1719 (C=O of coumarin), 1609 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 2.22 (s, 3H, C_6-CH_3), 5.52 (s, 2H, CH_2O), 6.38 (s, 1H, C_3–H), 6.70 (s, 1H, pyrimidine proton), 6.76–8.04 (m, 12H, Ar–H), 12.02 (s, SH, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 20.1, 79.7, 103.5, 109.3, 112.7, 113.3, 115.3, 122.3, 125.8, 126.3, 127.5, 128.1, 128.8, 129.6, 129.9, 130.5, 132.7, 135.7, 136.8, 146.0, 148.7, 155.8, 158.8, 159.8, 162.3, 166.2, 187.5; ESI-MS: 453 $[M+1]^+$.

*6.2.4.7. 4-[4-(2-Mercapto-6-phenyl-pyrimidin-4-yl)-phenoxymethyl]-7-methyl-chromen-2-one **6(b)**.* Colourless shiny crystals, IR (KBr, υ cm^{-1}): 3053 (Ar–H), 2603 (SH), 1722 (C=O of coumarin), 1613 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 2.13 (s, 3H, C_6-CH_3), 5.46 (s, 2H, CH_2O), 6.43 (s, 1H, C_3–H), 6.65 (s, 1H, pyrimidine proton), 7.06–8.34 (m, 12H, Ar–H), 11.87 (s, SH, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 17.9, 81.2, 104.6, 110.6, 112.9, 114.6, 115.8, 121.7, 126.5, 126.9, 127.9, 128.2, 128.7, 129.2, 129.8, 130.7, 133.7, 138.3, 145.8, 149.7, 157.8, 158.9, 161.7, 163.9, 184.3; ESI-MS: 453 $[M+1]^+$.

*6.2.4.8. 6-Chloro-4-[4-(2-Mercapto-6-phenyl-pyrimidin-4-yl)-phenoxymethyl]-chromen-2-one **6(c)**.* Colourless shiny crystals, IR (KBr, υ cm^{-1}): 3078 (Ar–H), 2587 (SH), 1719 (C=O of coumarin), 1609 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 5.29 (s, 2H, CH_2O), 6.36 (s, 1H,C_3H), 6.89 (s, 1H, pyrimidine proton), 7.04–8.43 (m, 12H, Ar–H), 11.78 (s, SH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 82.1, 104.6, 107.1, 111.9, 115.6, 118.2, 121.5, 123.6, 125.8, 126.8, 127.5, 127.9, 128.2, 128.6, 129.9, 132.8, 136.9, 137.3, 144.9, 146.7, 157.8, 159.7, 161.8, 162.4, 179.6; ESI-MS: 474 $[M+1]^+$.

*6.2.4.9. 1-[4-(2-Mercapto-6-phenyl-pyrimidin-4-yl)-phenoxymethyl]-benzo[f]chromen-3- one **6(d)**.* Yellow shiny crystals, IR (KBr, ν cm^{-1}): 3093 (Ar–H), 2599 (SH), 1726 (C=O of coumarin), 1603 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 4.88 (s, 2H, CH_2O), 6.27 (s, 1H, C_3-H), 6.88 (s, 1H, pyrimidine proton), 7.12–8.51 (m, 15H, Ar–H), 12.13 (s, SH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 79.3, 106.7, 110.5, 113.2, 115.3, 117.2, 121.6, 123.6, 126.2, 126.7, 127.4, 128.0, 128.3, 128.9, 129.8, 130.2, 132.8, 134.9, 136.9, 149.4, 153.2, 157.4, 161.7, 163.0, 165.1, 184.6; ESI-MS: 489 $[M+1]^+$.

*6.2.4.10. 4-[4-(2-Mercapto-6-phenyl-pyrimidin-4-yl)-phenoxymethyl]-benzo[h]chromen-2-one **6(e)**.* Red shiny crystals, IR (KBr, υ cm^{-1}): 3087 (Ar–H), 2607 (SH), 1732 (C=O of coumarin), 1596 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 5.12 (s, 2H, CH_2O), 6.42 (s, 1H,C_3–H), 6.79 (s, 1H, pyrimidine proton), 7.07–8.36 (m, 15H, Ar–H), 11.76 (s, SH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 80.0, 107.5, 112.8, 113.3, 116.1, 117.5, 119.9, 122.9, 125.9, 125.9, 126.4, 127.8, 128.1, 128.7, 129.0, 129.3, 130.6, 131.5, 135.2, 138.7, 150.1, 159.2, 160.6, 164.2, 166.6, 182.2; ESI-MS: 489 $[M+1]^+$.

*6.2.4.11. 4-[4-(2-Amino-6-phenyl-pyrimidin-4-yl)-phenoxymethyl]-6-methyl-chromen-2-one **7(a)**.* Colourless crystals, IR (KBr, ν cm^{-1}): 3433 and 3316 (NH_2), 3065 (Ar–H), 1722 (C=O of coumarin), 1601 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 2.31 (s, 3H, C_6-CH_3), 5.07 (s, 2H, CH_2O), 5.75 (s, 2H,NH_2, D_2O exchangeable), 6.88 (s, 1H, pyrimidine proton), 7.09–8.32 (m, 12H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 17.9, 80.3, 107.3, 112.8, 113.2, 114.5, 117.0, 120.3, 125.7, 126.8, 127.3, 128.0, 128.4, 128.9, 129.3, 129.9, 133.2, 134.8, 135.3, 147.6, 152.2, 158.0, 160.3, 161.3, 167.7, 170.3; ESI-MS: 436 $[M+1]^+$.

*6.2.4.12. 4-[4-(2-Amino-6-phenyl-pyrimidin-4-yl)-phenoxymethyl]-7-methyl-chromen-2-one **7(b)**.* Colourless crystals, IR (KBr, v cm^{-1}): 3427 and 3299 (NH_2), 3042 (Ar–H), 1719 (C=O of coumarin), 1597 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 2.25 (s, 3H, C_7-CH_3), 5.56 (s, 2H, CH_2O), 5.85 (s, 2H,NH_2, D_2O exchangeable), 6.76 (s, 1H, pyrimidine proton), 7.01–8.42 (m, 12H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 18.6, 78.5, 106.8, 110.2, 113.7, 114.3, 116.7, 119.6, 124.3, 127.0, 127.5, 128.1, 128.4, 128.9, 129.1, 129.7, 133.7, 134.0, 135.8, 137.7, 145.3,149.8, 157.5, 160.1, 161.7, 164.3, 168.1, 169.2; ESI-MS: 436 $[M+1]^+$.

*6.2.4.13. 4-[4-(2-Amino-6-phenyl-pyrimidin-4-yl)-phenoxymethyl]-6-chloro-chromen-2-one **7(c)**.* Colourless crystals, IR (KBr, υ cm^{-1}): 3408 and 3319 (NH_2), 3032 (Ar–H), 1726 (C=O of coumarin), 1614 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 5.21 (s, 2H,

CH_2O), 5.47 (s, 2H,NH_2, D_2O exchangeable), 6.57 (s, 1H, pyrimidine proton), 6.97–8.24 (m, 12H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 82.6, 102.5, 107.2, 113.4, 115.8, 116.7, 121.8, 127.1, 127.8, 128.0, 128.6, 129.1, 129.8, 131.5, 135.9, 147.3, 151.2, 157.8, 162.6, 167.4, 168.1; ESI-MS: 457 $[M+1]^+$.

6.2.4.14. 1-[4-(2-Amino-6-phenyl-pyrimidin-4-yl)-phenoxymethyl]-benzo[f]chromen-3- one ***7(d)***. Yellow shiny crystals, IR (KBr, υ cm^{-1}): 3423 and 3335 (NH_2), 3025 (Ar–H), 1731 (C=O of coumarin), 1607 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 5.43 (s, 2H, CH_2O), 5.68(s, 2H,NH_2, D_2O exchangeable), 6.75 (s, 1H, pyrimidine proton), 7.07–8.36 (m, 15H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 81.7, 107.2, 112.5, 114.8, 115.6, 116.0, 117.7, 121.0, 123.9, 126.0, 127.2, 127.6, 127.9, 128.5, 128.9, 129.4, 129.9, 131.6, 135.3, 145.9, 150.2, 153.8, 159.2, 162.1, 163.1, 166.6, 168.1; ESI-MS: 472 $[M+1]^+$.

6.2.4.15. 4-[4-(2-Amino-6-phenyl-pyrimidin-4-yl)-phenoxymethyl]-benzo[h]chromen-2-one ***7(e)***. Red shiny crystals, IR (KBr, υ cm^{-1}): 3417 and 3326 (NH_2), 3020 (Ar–H), 1726 (C=O of coumarin), 1600 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 5.31 (s, 2H, CH_2O), 5.54 (s, 2H,NH_2, D_2O exchangeable), 6.69 (s, 1H, pyrimidine proton), 6.92–8.26 (m, 15H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 78.9, 106.7, 110.4, 113.2, 114.7, 115.7, 117.0, 118.4, 120.7, 122.4, 125.7, 126.8, 127.0, 127.9, 128.1, 128.7, 129.3, 129.9, 130.5, 131.6, 134.6, 148.7, 157.9, 160.3, 162.4, 165.1, 166.3; ESI-MS: 472 $[M+1]^+$.

6.3. Pharmacology

6.3.1. Animals

Albino-Swiss mice weighing (20–25 g) and albino Wistar rats weighing (150–200 g) were used for studying *in-vivo* analgesic and anti-pyretic activities. Animals were maintained under standard laboratory conditions (24 ± 2 °C; relative humidity 60–70%). Study protocol was approved by the institutional Animal Ethics Committee (IAEC, Reg. No. 346/ CPCSEA: Dated. 03-01-2001) before experiment. Albino-Swiss mice and albino Wistar rats from Laboratory Animal Resource Section, Department of Pharmacy, Luqman College of pharmacy, Gulbarga were used in the study. The animals were kept in polypropylene cages and maintained on balanced ration with free access to clean drinking water. All experimental procedures were conducted in accordance with the guide for Care and use of laboratory animals and in accordance with the Local animal care and use committee.

6.3.2. Acute toxicity studies

For testing the acute toxicity potential of the test compounds, albino rats of either sex weighing 25–300 g were selected, separated into groups each containing six rats. The dosage was varied from 100 up to 3000 mg kg^{-1} body weight. The rats were continuously observed for 8 h for any signs of acute toxicity such as increased–decreased motor activity, ataxia, tremors, convulsions, sedation, lacrimation, etc. After 24 h the rats were sacrificed, stomach, intestine, and liver were inspected under the magnifying lenses for any ulcer-haemorrhagic spots.

6.3.3. Analgesic activity

The animals were divided into groups as shown in Table 2. The reaction time was measured at the end of 0, 30, 60 and 90 min after the administration of the compound and the standard employed was analgin. The drugs were administered orally. The tail-flick latency was assessed by the time taken by the rat to withdraw its tail from the organ bath containing hot water (temperature 55 ± 0.5 °C). The tail-flick latency of treated animals was compared with control and standard.

6.3.4. Anti-pyretic activity

The anti-pyretic activity was evaluated using brewer's yeast-induced pyrexia in rats [24]. Fever was induced by injecting 20 ml/kg (s.c.) of 20% aqueous suspension of brewer's yeast in normal saline below the nape of the neck and rectal temperature was recorded by clinical thermometer immediately before (andminus;18 h) and 18 h after (0 h) brewer's yeast injection. Prior to the experiment, the rats were maintained in separate cages for 7 days and the animals with approximately constant rectal temperature were selected for the study. Aspirin (300 mg/kg, p.o.) was used as standard drug for comparing the anti-pyretic action of compounds. The experimental rats showed a mean increase of about 0.86 °C in rectal temperature, 18 h after brewer's yeast injection. Compounds at 100 mg/kg produced significant ($P < 0.05$ and $P < 0.01$, respectively) anti-pyretic activity at 1 h and 3 h after drug administration.

6.3.5. Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons of all compounds in various pharmacological assays. Data are expressed as mean ± SEM.

6.3.6. DNA cleavage experiment

6.3.6.1. Preparation of culture media. DNA cleavage experiments were done according to the literature [25]. Nutrient broth [peptone, 10; yeast extract, 5; NaCl, 10; ins (g/l)] was used for culturing of Escherichia coli. 50 ml media was prepared, autoclaved for 15 min at 121 °C under 15 lb pressure. The autoclaved media were inoculated for 24 h at 37 °C.

6.3.6.2. Isolation of DNA. The fresh bacterial culture (1.5 ml) is centrifuged to obtain the pellet which is then dissolved in 0.5 ml of lysis buffer (100 mM tris pH 8.0, 50 mM EDTA, 10% SDS). To this 0.5 mL of saturated phenol was added and incubated at 55 °C for 10 min, then centrifuged at 10,000 rpm for 10 min and to the supernatant, equal volume of chloroform: isoamyl alcohol (24:1) and 1/20th volume of 3 M sodium acetate (pH 4.8) was added. Again centrifuging at 10,000 rpm for 10 min and to the supernatant, 3 volumes of chilled absolute alcohol were added. The precipitated DNA was separated by centrifugation and the pellet was dried and dissolved in TAE buffer (10 mM Tris pH 8.0, 1 mM EDTA) and stored in cold condition.

6.3.6.3. Agarose gel electrophoresis. Cleavage products were analyzed by agarose gel electrophoresis method [25]. Test samples (1 mg/ml) were prepared in DMF. The samples (25 mg) were added to the isolated DNA of *E. coli*. The samples were incubated for 2 h at 37 °C and then 20 ml of DNA sample (mixed with bromophenol blue dye at 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 L) and finally loaded on agarose gel and passed the constant 50 V of electricity for around 30 min. Removing the gel and being stained with 10.0 mg/ml ethidium bromide for 10–15 min, the bands were observed under Vilberlourmate Gel documentation system and then photographed to determine the extent of DNA cleavage. Henceforth the results were compared with standard DNA marker.

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