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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Analgesic study of novel pyrimidine derivatives linked with coumarin moiety

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Abstract A novel series of 2-amino-4-(coumarin-3-yl)-6-substituted phenyl pyrimidines (**5a**–**h**) were synthesized from 3-acetylcoumarin (**3**). The structures of the synthesized compounds were elucidated by I.R., ^{1}H NMR, ^{13}C NMR, and Mass spectroscopic techniques. The synthesized compounds were screened for in vivo analgesic activities at a dose of 20 mg/kg body weight (b.w). Among them, compounds **5b** and **5h** exhibited significant analgesic activity comparable with control as well as standard drug diclofenac sodium using acetic acid-induced writhing model.

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Introduction

Inflammation is a complex defensive mechanism of the body to any noxious stimulus; this process may vary from a localized to a generalized response characterized by the accumulation of fluids and leukocytes leading to edema and pain (Black, 2005). This inflammatory response seems to be mediated by different physiological and immunological mediators that play a role in acute and chronic inflammation. The acute inflammation occurs as the initial response to tissue injury, being mediated by the release of autocoids, for example, histamine, bradykinin, prostaglandins, and leukotrienes (Black, 2005; Sherwood and Toliver-Kinsky, 2004). On the other hand, the chronic inflammatory process involves the release of diverse mediators, as interleukins, interferon and tumor necrosis factor a (TNF-α), a cytokine that plays a major role in this kind of inflammatory process and whose production is associated with some inflammatory diseases such as rheumatoid arthritis (Black, 2005; Sherwood and Toliver-Kinsky, 2004). Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used for the treatment of pain and inflammation associated with different diseases particularly rheumatoid arthritis (Sorbera *et al*., 2001).

Coumarins have a variety of bioactivities including analgesic, antimicrobial (Radulovic *et al*., 2006), anticoagulant, vasodilator, anthelmintic, sedative and hypnotics, and antipyretic activity (Soine, 1964; O'Kennedy and Thornes, 1997). In addition to this, studies are performed on coumarin derivatives for their therapeutic role in the

treatment of cancer (Lacy and O'Kennedy, 2004). Various pyrimidine derivatives have been reported as anti-microbial (El-Sayed Ali, 2009), analgesic and anti-inflammatory (Amr *et al*., 2007; Abu-Hashem *et al*., 2010; El-Gazzar *et al*., 2009; Chikhale *et al*., 2009), anticonvulsants (Gupta *et al*., 2009), anti-HIV (Fujiwara *et al*., 2008), antiviral (Hockov *et al*., 2004), anti-tubercular (Ballell *et al*., 2007), anti-tumor (Wagner *et al*., 2008), anti-neoplastic (Xie *et al*., 2009), antioxidant, and radioprotective agents (Ghorab *et al*., 2010).

Both the pyrimidines and coumarins exhibit diverse biological properties (Kulkarni *et al*., 2006; Shafiee *et al*., 2010). It was observed that linking of these two active pharmacophores would generate novel molecular templates which are likely to exhibit interesting biological properties in animal models (Keri *et al*., 2010). Owing to the importance, we wish to describe the synthesis of new pyrimidine derivatives linked with coumarin moiety from 3-acetylcoumarins (Reaction Scheme 1). The compounds were screened for their in vivo analgesic activity.

Chemistry

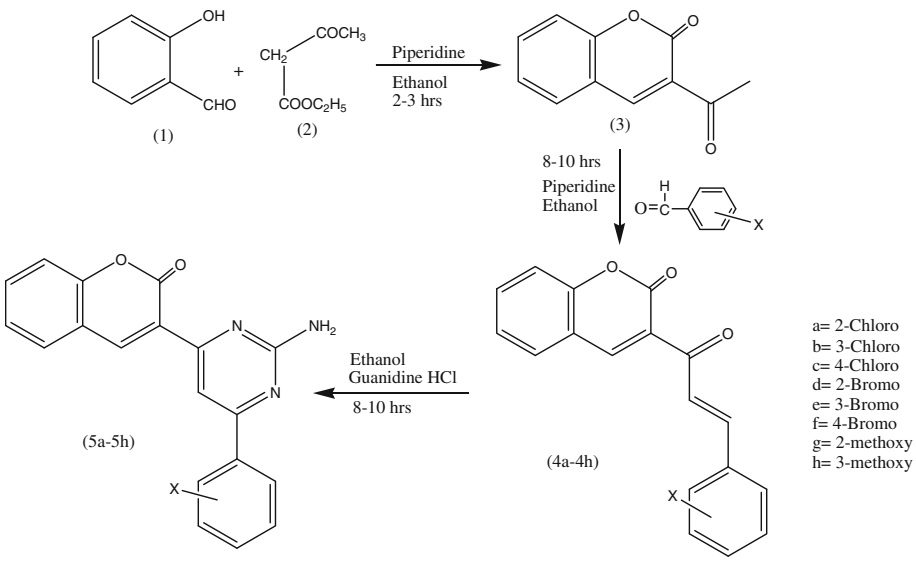
For the synthesis of target compounds, the reaction sequence outline in Scheme 1, were followed. Salicylaldehyde (**1**) is reflux with ethyl acetoacetate (**2**) in the presence of piperidine which gave 3-acetyl coumarin (**3**). This synthesized compound condensed with different substituted benzaldehydes using piperidine as a catalyst yielded 3-substituted phenyl-1-(coumarin-3-yl) prop-2-ene-1-ones (**4a–h**). 2-amino-4-(coumarin-3-yl)-6-substituted phenyl pyrimidines (**5a–h**) were prepared by treating compounds (**4a–h**) with guanidine HCl. The structures of newly synthesized compounds were confirmed by their spectral analysis. The physical data of these compounds are summarized in Tables 1 and 2.

When salicylaldehyde (**1**) is treated with ethyl acetoacetate (**2**) in refluxing ethanol in the presence of piperidine, it afforded a single precipitated product that was analyzed correctly for $C_{11}H_8O_3$. The reaction takes 2–3 h for completion. The structure of the precipitated product was identified as 3-acetyl coumarin (**3**) based on its spectral data. For example, IR spectrum of the isolated compound showed the characteristic band at 1,275 cm^{-1}, ^{13}C NMR spectrum revealed the presence of 11 carbon atoms in the form of 11 peak signal.

In the second step, treatment of 3-acetyl coumarin (**3**) with different types of benzaldehydes in the presence of piperidine for 8–10 h using ethanol as a solvent yielded one precipitated product (**4a–h**) respective of their benzaldehydes. The isolated product was identified as 3-substituted phenyl-1-(coumarin-3-yl) prop-2-ene-1-ones (**4a–h**). The IR spectrum of the isolated product revealed in each case bands due to carbonyl function and aryl ethers functions. Moreover, the ^1H NMR spectrum of compound (**4a–h**), taken as an example, revealed two doublet signals between δ 6–8 ppm, and one multiplet at δ 7–8 ppm due to methylene protons and aromatic protons, respectively.

The formation of compounds (**4a–h**) can be explained on the basis of "Claisen–Schmidt condensation".

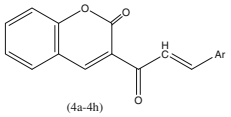
In the third step, compound (**4a–h**) is refluxed with guanidine HCl, in equimolar quantity in ethanol as a solvent for 8–10 h, which afforded the final compounds (**5a–h**). The structure of the final compounds was assigned based on their elemental analysis and spectral data. For example IR spectrum of the compounds (**5a–h**) revealed the broad band between 3,300 and 3,400 cm^{-1} due to



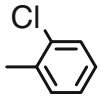
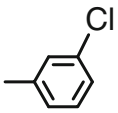
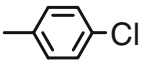
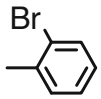
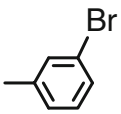
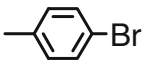
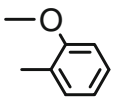
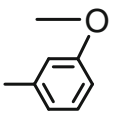
Scheme 1 Schematic representation for the synthesis of pyrimidine derivatives

Table 1 Physical data of the synthesized compounds (**4a–h**)

Physical data of 3-substituted phenyl-1-(coumarin-3-yl) prop-2-ene-1-one (**4a–h**)



(4a-4h)

Compound[a]	-Ar	Yield (%)[b]	m.p. (°C)[c]	R_f value	Molecular formula
Compound **4a**		65	156–158	0.73	$C_{18}H_{11}O_3Cl$
Compound **4b**		70	165–167	0.75	$C_{18}H_{11}O_3Cl$
Compound **4c**		60	162–165	0.71	$C_{18}H_{11}O_3Cl$
Compound **4d**		70	185–188	0.77	$C_{18}H_{11}O_3Br$
Compound **4e**		75	185–187	0.76	$C_{18}H_{11}O_3Br$
Compound 4f		75	190–192	0.69	$C_{18}H_{11}O_3Br$
Compound **4g**		75	173–175	0.72	$C_{19}H_{14}O_4$
Compound **4h**		75	177–179	0.76	$C_{19}H_{14}O_4$

[a] Products were characterized by IR, NMR, MS, and elemental analysis

[b] Isolated yields

[c] Melting points are uncorrected

amino group. In addition to this 1H NMR spectrum showed the singlet signal δ 4–5 ppm due to the amino group. These signals were absent in the spectral data of compounds (**4a–h**). Moreover, mass spectrum and elemental analysis data also supported the structure assigned to the final compounds (**5a–h**).

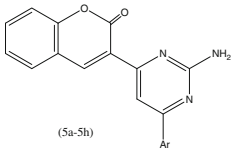
Result and discussion

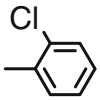
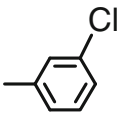
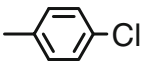
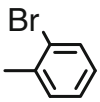
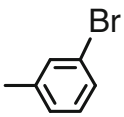
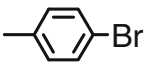
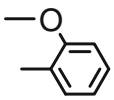
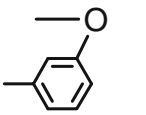
Analgesic activity

Analgesic activity was performed according to Turner (1965) technique using Swiss albino mice. The results of

Table 2 Physical data of the synthesized compounds (**5a–h**)

Physical data of 2-amino-4-(coumarin-3-yl)-6-substituted phenyl pyrimidines (**5a–h**)



Compound[a]	-Ar	Yield (%)[b]	m.p. (°C)[c]	R_f value	Molecular formula
Compound **5a**		65	145–147	0.62	$C_{19}H_{12}N_3O_2Cl$
Compound **5b**		60	170–172	0.74	$C_{19}H_{12}N_3O_2Cl$
Compound **5c**		70	175–177	0.70	$C_{19}H_{12}N_3O_2Cl$
Compound **5d**		65	142–145	0.75	$C_{19}H_{12}N_3O_2Br$
Compound **5e**		50	133–135	0.72	$C_{19}H_{12}N_3O_2Br$
Compound **5f**		60	170–173	0.68	$C_{19}H_{12}N_3O_2Br$
Compound **5g**		65	190–191	0.67	$C_{20}H_{15}N_3O_3$
Compound **5h**		65	173–175	0.65	$C_{20}H_{15}N_3O_3$

[a] Products were characterized by IR, NMR, MS, and elemental analysis

[b] Isolated yields

[c] Melting points are uncorrected

analgesic activity indicated that all test compounds, except **5a** and **5c**, exhibited significant analgesic activity. Compounds **5b** and **5h** have approximately half of the analgesic activity as that of reference drug, and the remaining compounds had moderate analgesic activity (Table 3).

Conclusion

A novel series of 2-amino-4-(coumarin-3-yl)-6-(substituted phenyl) pyrimidines analogues were synthesized and characterized. The synthesized compounds screened for

Table 3 Analgesic activity of synthesized compounds (**5a–h**) by acetic acid-induced writhing response model

Compounds tested	Percent protection		
	0.5 h	1 h	2 h
Diclofenac sodium	91.13 ± 0.33	96.76 ± 0.47	93.35 ± 0.57
Compound **5a**	8.22 ± 2.77	12.05 ± 2.67	11.63 ± 2.62
Compound **5b**	52.21 ± 4.20*[a,b]	60.58 ± 3.95*[a,b]	56.50 ± 3.90*[a,b]
Compound **5c**	9.49 ± 2.38	12.35 ± 2.33	10.80 ± 2.49
Compound **5d**	23.41 ± 3.13	30.58 ± 3.40	27.70 ± 3.39
Compound **5e**	25.94 ± 1.65	33.52 ± 1.62	31.57 ± 1.77
Compound **5f**	28.16 ± 3.84	29.41 ± 3.77	26.59 ± 3.54
Compound **5g**	35.44 ± 3.05	35.58 ± 3.03	33.79 ± 2.99
Compound **5h**	46.51 ± 2.42*[a,b]	53.52 ± 2.48*[a,b]	49.30 ± 2.07*[a,b]

Method: acetic acid-induced writhing response model; test animals: albino mice; number of animals per group: 6; route of administration: oral; standard: Diclofenac sodium (20 mg/kg)

Statistical analysis: the statistical analysis was performed by one-way ANOVA followed Dunnet's test

* $P \leq 0.05$, [*a]when compared to control, and [*b]standard drug

Values are expressed as mean ± SEM and *$P \geq 0.05$, indicates the level of statistical significance as compared with control (*a) as well as standard drug (*b)

their in vivo analgesic activity. Some of the synthesized compounds viz., **5b** and **5h** exhibited good analgesic activity in comparison to that of standard drug diclofenac sodium in the acetic acid-induced writhing response model at 20 mg/kg body weights of the animals.

Experimental section

Chemistry

All reagents and solvents were used as obtained from the supplier or recrystallized/redistilled as necessary. The melting points of the products were determined by open capillary method and are uncorrected. I.R. Spectra (KBr) were recorded on FTIR Spectrophotometer (Shimadzu FTIR 84005, 4,000–400 cm^{-1}). ^1H NMR and ^{13}C NMR spectra were recorded on a JEOL AL300 FTNMR 300 MHz spectrometer in $CDCl_3$ using TMS as an internal standard, with ^1H resonance frequency of 300 MHz and ^{13}C resonance frequency of 75 MHz. Chemical shift values are expressed in δ ppm. Mass spectra were recorded on a 70 eV EI-MS-QP 1000 EX (Schimadzu). The elemental analysis was carried out using Heraus CHN rapid analyzer. The homogeneity of the compounds was determined by TLC on alumina silica gel 60 F254 (Merck) detected by UV light (254 nm) and iodine vapors. The in vivo analgesic activity was performed at Meerut Institute of Engineering and Technology, Meerut, India.

General procedures for the preparation of compounds

*Synthesis of 3-acetyl coumarin (**3**): general procedure*

A mixture of salicylaldehyde (**1**) (0.02 mol) and ethyl acetoacetate (**2**) (0.03 mol) in ethanol were taken in round bottom flask. To this mixture, few drops of piperidine were added and refluxed for 2–3 h. After completion of reaction, the content was poured on crushed ice. The solid separated was filtered, dried, and recrystallized from ethanol. The purity of compound was established on the basis of TLC. M. P. 111–113°C, I. R. (KBr, cm^{-1}): 1741.60 and 1677.95 (C=O), 1275 (aryl ethers, C–O–C); ^1H NMR ($CDCl_3$-d_6, δ, ppm): 2.58 (s, 3H, CH_3), 7.25–7.98 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 35.50, 120.9, 123.8, 126.6, 127.3, 130.5, 132.5, 139.8, 155.7, 163, 200.6; MS, [M^+], *m/z* 188 (100%), [M^+ +2], *m/z* 190 (15%), [M^+ +4], *m/z* 192 (2%); Anal. Calcd for $C_{11}H_8O_3$ (188.18): C, 70.21; H, 4.29. Found: C, 70.15; H, 4.25.

*Synthesis of 3-substituted phenyl-1-(coumarin-3-yl) prop-2-ene-1-ones (**4a–h**): general procedure*

Equimolar quantities of 3-acetyl coumarin (**3**) and different substituted benzaldehydes were refluxed in absolute ethanol using piperidine as a catalyst for 8–10 h. The solution mixture was concentrated and poured on to crushed ice. The precipitate was filtered, dried, and recrystallized from ethanol to give pure crystalline solid.

*Synthesis of 3-(2-chlorophenyl)-1-(coumarin-3-yl) prop-2-ene-1-one (**4a**)* It was obtained from reaction of compound (**3**) with 2-chlorobenzaldehyde. IR (KBr, cm^{-1}): 1724.24 and 1662.52 (C=O), 1184.21 (C–O–C); ^1HNMR ($CDCl_3$-d_6, δ, ppm): 6.02 (d, 1H, CH), 7.11–7.93 (m, 9H, Ar-H), 8.03 (d, 1H, CH); ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 120.3, 124.2, 125.3, 125.9, 129.1, 129.9, 130, 131.9, 132.5, 133, 138.9, 142.6, 143.9, 145.2, 147.6, 157.8, 159.6, 180.5; MS, [M^+], *m/z* 310 (100%), [M^+ +2], *m/z* 312 (35%), [M^+ +4], *m/z* 314 (10%); Anal. Calcd for $C_{18}H_{11}ClO_3$ (310.73): C, 69.58; H, 3.57. Found: C, 69.52; H, 3.52.

*Synthesis of 3-(3-chlorophenyl)-1-(coumarin-3-yl) prop-2-ene-1-one (**4b**)* It was obtained from reaction of compound (**3**) with 3-chlorobenzaldehyde. IR (KBr, cm^{-1}): 1728.10 and 1685.67 (C=O), 1107.06 (C–O–C); ^1HNMR ($CDCl_3$-d_6, δ, ppm): 7.03 (d, 1H, CH), 7.15–8.02 (m, 9H, Ar-H), 8.66 (d, 1H, CH); ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 120.9, 122.9, 124.6, 125.9, 127.6, 128.9, 130.2, 130.9 131.5, 132.7, 133, 135.7,

138.9, 144.9, 148.2, 158.3, 160.5, 178.6; MS, [M^+], *m/z* 309 (100%), [$M^+ +2$], *m/z* 311 (30%), [$M^+ +4$], *m/z* 313 (5%); Anal. Calcd for $C_{18}H_{11}ClO_3$ (310.73): C, 69.58; H, 3.57. Found: C, 69.62; H, 3.52.

*Synthesis of 3-(4-chlorophenyl)-1-(coumarin-3-yl) prop-2-ene-1-one (**4c**)* It was obtained from reaction of compound (**3**) with 4-chlorobenzaldehyde. IR (KBr, cm^{-1}): 1728.10 and 1685.67 (C=O), 1107.06 (C–O–C). ^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 6.36 (d, 1H, CH), 6.90 (d, 1H, CH), 7.02–8.48 (m, 9H, Ar-H); ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 120.5, 123.4, 124.6, 127.5, 128.4, 128.6, 128.9, 130.5, 130.9, 131.5, 131.7, 132.6, 132.9, 144.4, 145.6, 157.2, 158.6, 182.9; MS, [M^+], *m/z* 309 (100%), [$M^+ +2$], *m/z* 311 (33%), [$M^+ +4$], *m/z* 313 (3%); Anal. Calcd for $C_{18}H_{11}ClO_3$ (310.73): C, 69.58; H, 3.57. Found: C, 69.55; H, 3.51.

*Synthesis of 3-(2-bromophenyl)-1-(coumarin-3-yl) prop-2-ene-1-one (**4d**)* It was obtained from reaction of compound (**3**) with 2-bromobenzaldehyde. IR (KBr, cm^{-1}): 1724.24 and 1683.74 (C=O), 1184.21 (C–O–C); ^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 6.86 (d, 1H, CH), 7.02–7.93 (m, 9H, Ar-H), 8.00 (d, 1H, CH); ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 120.1, 120.9, 121.5, 121.9, 124.6, 125.6, 127.6, 127.9, 128.6, 128.9, 129.4, 129.9, 130.9, 145.6, 149.3, 159.6, 161.9, 178.5; MS, [M^+], *m/z* 354 (100%), [$M^+ +2$], *m/z* 356 (25%), [$M^+ +4$], *m/z* 358 (2%); Anal. Calcd for $C_{18}H_{11}BrO_3$ (355.18): C, 60.87; H, 3.12. Found: C, 60.81; H, 3.10.

*Synthesis of 3-(3-bromophenyl)-1-(coumarin-3-yl) prop-2-ene-1-one (**4e**)* It was obtained from reaction of compound (**3**) with 3-bromobenzaldehyde. IR (KBr, cm^{-1}): 1728.10 and 1685.67 (C=O), 1107.06 (C–O–C). ^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 7.08 (d, 1H, CH), 7.11–7.99 (m, 9H, Ar-H), 8.05 (d, 1H, CH); ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 1209, 123.5, 124.6, 125.9, 126.9, 127.8, 128.7, 129, 129.4, 130, 131.5, 131.6, 134.6, 140, 147.3, 150.6, 158.3, 179.2; MS, [M^+], *m/z* 354 (100%), [$M^+ +2$], *m/z* 356 (20%), [$M^+ +4$], *m/z* 358 (1.6%); Anal. Calcd for $C_{18}H_{11}BrO_3$ (355.18): C, 60.87; H, 3.12. Found: C, 60.81; H, 3.09.

*Synthesis of 3-(4-bromophenyl)-1-(coumarin-3-yl) prop-2-ene-1-one (**4f**)* It was obtained from reaction of compound (**3**) with 4-bromobenzaldehyde. IR (KBr, cm^{-1}): 1739.67 and 1677.95 (C=O), 1107.06 (C–O–C). ^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 7.03 (d, 1H, CH), 7.11–7.94 (m, 9H, Ar-H), 8.23 (d, 1H, CH); ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 121.9, 122.3, 123.6, 124.6, 125.3, 125.9, 128.6, 128.9, 129.5, 129.9, 130.5, 132.3, 135, 145.6, 150, 160.3, 164.2, 165.1, 180; MS, [M^+], *m/z* 354 (100%), [$M^+ +2$], *m/z* 356 (18%), [$M^+ +4$], *m/z* 358 (2.5%); Anal. Calcd for $C_{18}H_{11}BrO_3$ (355.18): C, 60.87; H, 3.12. Found: C, 60.90; H, 3.14.

*Synthesis of 3-(2-methoxyphenyl)-1-(coumarin-3-yl) prop-2-ene-1-one (**4g**)* It was obtained from reaction of compound (**3**) with 2-methoxybenzaldehyde. IR (KBr, cm^{-1}): 1728.10 (C=O), 1164.92 (C–O–C). ^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 3.56 (s, 3H, CH_3), 6.86 (d, 1H, CH), 7.02–7.96 (m, 9H, Ar-H), 8.09 (d, 1H, CH); ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 62.7, 113.5, 118.6, 120.3, 121.6, 123.6, 125.9, 127.6, 128, 128.9, 129, 129.9, 143.9, 150, 155.6, 160.3, 163.5, 163.9, 179; MS, [M^+], *m/z* 305 (100%), [$M^+ +2$], *m/z* 307 (25%), [$M^+ +4$], *m/z* 309 (2%); Anal. Calcd for $C_{19}H_{14}O_4$ (306.31): C, 74.50; H, 4.61. Found: C, 74.54; H, 4.57.

*Synthesis of 3-(3-methoxyphenyl)-1-(coumarin-3-yl) prop-2-ene-1-one (**4h**)* It was obtained from reaction of compound (**3**) with 3-methoxybenzaldehyde. IR (KBr, cm^{-1}): 1735.81 (C=O), 1137.92 (C–O–C). ^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 3.90 (s, 3H, CH_3), 6.98 (d, 1H, Ar-H), 7.00–7.85 (m, 9H, Ar-H), 8.10 (d, 1H, CH); ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 63.2, 112.5, 118.2, 120.9, 122.9, 122.5, 126.9, 127.9, 128, 128.6, 129.3, 129.9, 142.6, 150.3, 154.6, 160.8, 163.6, 165.9, 182.3; MS, [M^+], *m/z* 305 (100%), [$M^+ +2$], *m/z* 307 (20%), [$M^+ +4$], *m/z* 309 (1.5%); Anal. Calcd for $C_{19}H_{14}O_4$ (306.31): C, 74.50; H, 4.61. Found: C, 74.45; H, 4.56.

*Synthesis of 2-amino-4-(7-substituted coumarin-3-yl)-6-substituted phenyl pyrimidines-(**5a–h**): general procedure*

A mixture of 3-substituted phenyl-1-(coumarin-3-yl) prop-2-ene-1-ones (0.01 mol) and guanidine HCl (0.02 mol) was refluxed in ethanol for 8–10 h. The content was evaporated to dryness and the product so obtained was washed with water repeatedly and recrystallized from ethanol.

*Synthesis of 2-amino-4-(coumarin-3yl)-6-(2-chlorophenyl) pyrimidines (**5a**)* It was obtained from reaction of guanidine HCl with compound (**4a**). IR (KBr, cm^{-1}): 3151.47 (br, NH), 1758.96 (C=O), 1666.38 (C=N), 1114.78 (C–O–C); ^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.256 (s, 2H, NH_2), 6.85–7.72 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$-d_6, δ, ppm):110.1, 124.2, 125.3, 128.6, 129.1, 129.9, 130, 131.9, 132.5, 135.5, 138.9, 142.6, 143.9, 145.2, 147.6, 157.8, 165.6, 168.5, 170.5; MS, [M^+], *m/z* 348 (100%), [$M^+ +2$], *m/z* 350 (40%), [$M^+ +4$], *m/z* 352 (10%); Anal. Calcd for $C_{19}H_{12}ClN_3O_2$ (349.77): C, 65.24; H, 3.46; N, 12.01. Found: C, 65.30; H, 3.48; N, 12.06.

*Synthesis of 2-amino-4-(coumarin-3yl)-6-(3-chlorophenyl) pyrimidines (**5b**)* It was obtained from reaction of guanidine HCl with compound (**4b**). IR (KBr, cm^{-1}): 3352.05 (br, NH), 1758.96 (C=O), 1635.52 (C=N), 1242.07 (C–O–C); ^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.25 (s, 2H, NH_2), 6.92–7.36 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$-d_6, δ, ppm):

109.2, 122.9, 124.6, 125.9, 127.6, 128.9, 130.2, 131.5, 132.7, 133, 135.7, 138.9, 144.9, 148.2, 158.3, 160.5, 161.4, 163.4; MS, [M^+], *m/z* 348 (100%), [M^+ +2], *m/z* 350 (45%), [M^+ +4], *m/z* 352 (15%); Anal. Calcd for $C_{19}H_{12}ClN_3O_2$ (349.77): C, 65.24; H, 3.46; N, 12.01. Found: C, 65.20; H, 3.40; N, 12.0.

*Synthesis of 2-amino-4-(coumarin-3yl)-6-(4-chlorophenyl) pyrimidines (**5c**)* It was obtained from reaction of guanidine HCl with compound (**4c**). IR (KBr, cm^{-1}): 3367.48 (br, NH), 1662.52 (C=O), 1585.38 (C=N), 1226.64 (C–O–C); ^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.25 (s, 2H, NH_2), 7.02–7.50 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 110.5, 123.4, 124.6, 127.5, 128.4, 128.6, 128.9, 130.5, 130.9, 131.5, 131.7, 132.6, 132.9, 144.4, 145.6, 157.2, 158.6, 160.9, 163.7; MS, [M^+], *m/z* 348 (100%), [M^+ +2], *m/z* 350 (47%), [M^+ +4], *m/z* 352 (17%); Anal. Calcd for $C_{19}H_{12}ClN_3O_2$ (349.77): C, 65.24; H, 3.46; N, 12.01. Found: C, 65.19; H, 3.50; N, 12.02.

*Synthesis of 2-amino-4-(coumarin-3yl)-6-(2-bromophenyl) pyrimidines (**5d**)* It was obtained from reaction of guanidine HCl with compound (**4d**). IR (KBr, cm^{-1}): 3402.20 (N–H), 1674.10 (C=O), 1546.80 (C=N), 1110.92 (C–O–C); ^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.96 (s, 2H, NH_2), 7.25–7.63 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 107.9, 120.5, 121.5, 121.9, 124.6, 125.6, 127.6, 127.9, 128.6, 128.9, 129.4, 129.9, 130.9, 145.6, 149.3, 159.6, 161.9, 162.8, 164.9; MS, [M^+], *m/z* 393 (100%), [M^+ +2], *m/z* 395 (50%), [M^+ +4], *m/z* 397 (20%); Anal. Calcd for $C_{19}H_{12}BrN_3O_2$ (394.22): C, 57.89; H, 3.07; N, 10.66. Found: C, 57.85; H, 3.02; N, 10.60.

*Synthesis of 2-amino-4-(coumarin-3yl)-6-(3-bromophenyl) pyrimidines (**5e**)* It was obtained from reaction of guanidine HCl with compound (**4e**). IR (KBr, cm^{-1}): 3421.48 (N–H), 1654.81 (C=O), 1488.94 (C=N), 1110.92 (C–O–C); ^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.27 (s, 2H, NH_2), 6.93–7.63 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$-d_6, δ, ppm):109.9, 123.5, 124.6, 125.9, 126.9, 127.8, 128.7, 129, 129.4, 130, 131.5, 131.6, 134.6, 140, 147.3, 150.6, 158.3, 160, 165.8; MS, [M^+], *m/z* 393 (100%), [M^+ +2], *m/z* 395 (45%), [M^+ +4], *m/z* 397 (15%); Anal. Calcd for $C_{19}H_{12}BrN_3O_2$ (394.22): C, 57.89; H, 3.07; N, 10.66. Found: C, 57.92; H, 3.05; N, 10.60.

*Synthesis of 2-amino-4-(coumarin-3yl)-6-(4-bromophenyl) pyrimidines (**5f**)* It was obtained from reaction of guanidine HCl with compound (**4f**). IR (KBr, cm^{-1}): 3348.19 (N–H), 1674.10 (C=O), 1542.95 (C=N), 1107.06 (C–O–C); ^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.16 (s, 2H, NH_2), 6.90–7.73 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 109.3, 122.3, 123.6, 124.6, 125.3, 125.9, 128.6, 128.9, 129.5, 129.9, 130.5, 132.3, 135, 145.6, 150, 160.3, 164.2, 165.1, 167; MS, [M^+], *m/z* 393 (100%), [M^+ +2], *m/z* 395 (55%), [M^+ +4], *m/z* 397 (15%); Anal. Calcd for $C_{19}H_{12}BrN_3O_2$ (394.22): C, 57.89; H, 3.07; N, 10.66. Found: C, 57.85; H, 3.01; N, 10.60.

*Synthesis of 2-amino-4-(coumarin-3yl)-6-(2-methoxyphenyl) pyrimidines (**5g**)* It was obtained from reaction of guanidine HCl with compound (**4g**). IR (KBr, cm^{-1}): 3421.48 (N–H), 1635.52 (C=O), 1600.81 (C=N), 1164.92 (C–O–C); ^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 3.87 (s, 3H, CH_3), 4.25 (s, 2H, NH_2), 6.92–8.00 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 63.7, 106.3, 113.5, 118.6, 120.3, 121.6, 123.6, 125.9, 127.6, 128, 128.9, 129, 129.9, 143.9, 150, 155.6, 160.3, 163.5, 163.9, 166.3; MS, [M^+], *m/z* 344 (100%), [M^+ +2], *m/z* 346 (25%), [M^+ +4], *m/z* 348 (5%); Anal. Calcd for $C_{20}H_{15}N_3O_3$ (345.35): C, 69.56; H, 4.38; N, 12.17. Found: C, 69.62; H, 4.35; N, 12.11.

*Synthesis of 2-amino-4-(coumarin-3yl)-6-(3-methoxyphenyl) pyrimidines (**5h**)* It was obtained from reaction of guanidine HCl with compound (**4h**). IR (KBr, cm^{-1}): 3413.77 (N–H), 1639.38 (C=O), 1585.38 (C=N), 1157.21 (C–O–C); ^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 3.81 (s, 3H, CH_3), 4.04(s, 2H, NH_2), 6.86–7.25 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 63.2, 106.6, 112.5, 118.2, 120.9, 122.9, 122.5, 126.9, 127.9, 128, 128.6, 129.3, 129.9, 142.6, 150.3, 154.6, 160.8, 163.6, 165.9, 167.5; MS, [M^+], *m/z* 344 (100%), [M^+ +2], *m/z* 346 (20%), [M^+ +4], *m/z* 348 (8%); Anal. Calcd for $C_{20}H_{15}N_3O_3$ (345.35): C, 69.56; H, 4.38; N, 12.17. Found: C, 69.50; H, 4.34; N, 12.15.

Pharmacological screening

Animals

Albino-Swiss mice weighing (20–25 g) were used for studying in vivo analgesic activity. Animals were maintained under standard laboratory conditions (24 ± 2°C relative humidity 60–70%). Study protocol was approved by the institutional Animal Ethics Committee for the Purpose of Control and Supervision on Experiments on Animals (IAEC, Approval No. 711/02/a/CPCSEA) before experiment. Albino-Swiss mice from Laboratory Animal House Section, Department of Pharmaceutical Technology, Meerut Institute of Engineering & Technology, Meerut 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.

Analgesic activity (acetic acid-induced writhing response model)

The compounds were selected for screening of their analgesic activity in acetic acid-induced writhing response in Swiss albino mice following the method of Turner (Turner, 1965). Sixty mice were selected and divided into 10 groups (six in each group), starved for 16 h and pretreated as follows, the first group which served as control positive was orally received distilled water in appropriate volumes. The second to ninth groups were received the aqueous suspension of synthesized compounds orally in a dose of 20 mg/kg. The last group was orally received diclofenac sodium in a dose of 20 mg/kg. After 30 min, each mice was administered 1% of an aqueous solution of acetic acid (10 ml/kg) and the mice were then placed in transparent boxes for observation. The number of writhes was counted for 30 min after acetic acid injection. The number of writhes in each treated group was compared to that of a control group. The number of writhing was recorded and the percentage protection was calculated using the following ratio:

$$\% \text{ Protection} = (\text{Control mean} - \text{Treated mean}/\text{Control mean}) * 100$$

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.

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