Acta Poloniae Pharmaceutica – Drug Research, Vol. 68 No. 5 pp. 785–793, 2011 ISSN 0001-6837
Polish Pharmaceutical Society

SHORT COMMUNICATION

SYNTHESIS AND ANALGESIC ACTIVITY OF NOVEL PYRIMIDINE DERIVATIVES OF COUMARIN MOIETY

JITENDRA K. GUPTA[1]*, PRAMOD K. SHARMA[1], RUPESH DUDHE[1], SAMBHU C. MONDAL, ANSHU CHAUDHARY[2] and PRABHAKAR K. VERMA[3]

[1]Department of Pharmaceutical Technology, Meerut Institute of Engineering & Technology, NH-58, Baghpat Bypass Crossing, Meerut (U. P.) India-250005
[2]Vishveshwarya Institute of Medical Science Dadri, Gautambudh Nagar (U.P.) India-203207
[3]M.D. University, Rohtak, Haryana. India

Keywords: coumarin; pyrimidines; writhing test; analgesic activity

The investigation of compounds able to treat both acute and chronic pain is challenging in pharmaceutical research (1). Pain is in fact a very important problem present in 90% of diseases, from the simple back pain to pain associated with different forms of cancer. The classical therapies for pain treatment are mainly the non-steroidal anti-inflammatory drugs (NSAIDs) and opiates, whose lead compounds, acetylsalicylic acid and morphine, respectively, were isolated in 19th century. (2)

NSAIDs show side effects such as gastrointestinal irritation and lesions, renal toxicity and inhibition of platelet aggregation, while the use of opioids is limited to severe pain because of adverse secondary reactions as respiratory depression, dependence, sedation, and constipation. (3, 4) Hence, there is always a need for those drugs which have improved analgesic activity and less adverse effects. Nitrogen containing heterocycles, such as pyrimidine, are promising structural moieties for drug design. 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), antitubercular (9), anti-tumor (10), anti-neoplastic (11), antimalarial (12), diuretic (13) and cardiovascular agents(14). 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, 19).

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, here we have described the synthesis of new pyrimidine derivatives from 3-acetylcoumarins (Scheme 1). The compounds were screened for their *in vivo* analgesic activity. Thus, we have created new avenues to explore the potent heterocyclic moieties for the pharmacological activities in medicinal chemistry.

CHEMISTRY

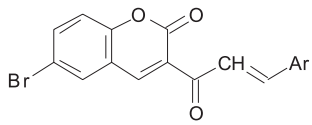
For the synthesis of target compounds, the reaction sequences outlined in Scheme 1, were followed. 5-Bromosalicyldehyde (**1**) was refluxed with ethyl acetoacetate (**2**) in the presence of piperidine yielding 3-acetyl-6-bromo-2H-chromen-2-one (**3**).

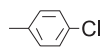
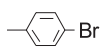
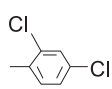
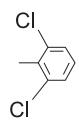
* Corresponding author: e- mail: jitendraeishwer@yahoo.co.in; jitendraeishwer@gmail.com; mobile: +91 9305109580

This compound condensed with different substituted benzaldehydes, using pipreridine as a catalyst, yielded 6-bromo-3-(3-substituted acryloyl)-2H-chromen -2-ones (**4a-4j**). 3-(2-Amino-6-substituted pyrimidine-4-yl)-6-bromo-2H-chromen-2-one (**5a-5j**) were prepared by treating compounds (**4a-4j**) with guanidine HCl. The structures of newly synthesized compounds were confirmed by their spectral analyses. The physical data of these compounds are summarized in Tables 1 and 2.

The structure of the precipitated product (**1**) was identified as 3-acetyl-6-bromo-2H-chromen-2-one (**3**) based on its spectral data. For example, IR spectrum of the isolated compound showed the character-

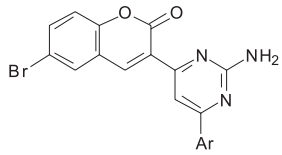
Table 1. Physical parameters of 6-bromo-3-(3-substituted acryloyl)-2H-chromen-2-ones [**4a**–**4j**].



Compound [a]	-Ar	Yield (%)[b]	M.p.[c] (°C)	Rf value
4a		65	162–165	0.73
4b		70	165–167	0.75
4c		60	156–158	0.71
4d		70	190–192	0.77
4e		75	185–187	0.76
4f		75	185–188	0.69
4g		75	177–179	0.72
4h		75	173–175	0.76
4i		80	175–177	0.71
4j		75	180–183	0.76

[a] Products were characterized by IR, NMR, MS and elemental analysis. [b] Synthesized yields. [c] uncorrected.

Table 2. Physical parameters of 3-(2-amino-6-(substituted)-pyrimidin-4-yl)-6-bromo-2H-chromen-2-ones (**5a–5j**).



Compound [a]	-Ar	Yield (%)[b]	M.p.[c](°C)	Rf value
5a		65	162–165	0.62
5b		60	165–167	0.74
5c		70	156–158	0.70
5d		65	190–192	0.75
5e		50	185–187	0.72
5f		60	185–188	0.68
5g		65	177–179	0.67
5h		65	173–175	0.65
5i		70	175–177	0.78
5j		68	180–183	0.70

[a] Products were characterized by IR, NMR, MS and elemental analysis. [b] Synthesized yields. [c] uncorrected

istic band at 1275 cm^{-1} for C-O-C group and ^{13}C-NMR spectrum revealed the presence of 11 carbon atoms in the form of 11 peak signals. Moreover the mass spectrum of the same product showed a peak at m/z 265 corresponding to its molecular ion peak. Formation of compound (**3**) can be explained on the basis of Knoevenagel reaction. The formation of compounds (**4a-4j**) can be explained on the basis of Claisen-Schmidt condensation. The structure of the final compounds (**5a-5j**) was assigned based on their elemental analyses and spectral data. For example, IR spectrum of compounds **5a-5j** revealed the broad band between 3300–3400 cm^{-1} due to the amino group. In addition to this, ^{1}H-NMR spectrum of the same compounds showed the singlet signal δ 4–5 ppm due to the same group. These signals were absent in the spectral data of compounds (**4a-4j**). Moreover, mass spectrum and elemental analysis data reported in experimental section also supported the structure assigned to the final compounds (**5a-5j**).

EXPERIMENTAL

Chemistry

All reagents and solvents were used as obtained from the supplier or recrystalized/redis-

tilled as necessary. The melting points of the products were determined by open capillaries method and are uncorrected. The IR spectra (KBr) were recorded on FTIR spectrophotometer (Shimadzu FTIR 84005, 4000–400 cm^{-1}). ^{1}H-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 ^{1}H 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 (Shimadzu) apparatus. The elemental analysis was carried out using Heraeus CHN rapid analyzer. The homogeneity of the compounds was checked by TLC on silica gel G with the solvent system toluene : ethyl acetate : formic acid (5: 4: 1, v/v/v) and detected by iodine vapors. The *in vivo* analgesic screening was done at Meerut Institute of Engineering and Technology, Meerut, India.

3-Acetyl-6-bromo-2H-chromen-2-one (3)

To a mixture of salicyldehyde (**1**) (0.02 mole) and ethyl acetoacetate (**2**) (0.03 mole) in ethanol, in round bottom flask, few drops of piperidine were added and refluxed for 2–3 hours. After completion of reaction, the content was poured on crushed ice. The solid separated was filtered, dried and recrystallized from ethanol. M.p. 115–117°C; IR (KBr, cm^{-1}): 1735.81 and 1674.10 (C=O), 1550.66 (C=C), 1230.50 (aryl ethers, C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 2.58 (s, 3H, CH_3), 7.25–7.98 (m, 4H, Ar-H). ^{13}C-NMR ($CDCl_3$, 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/z): 266 (100%) [M^+], 268 (15%) [M^++2], 270 (2%) [M^++4]. Analysis: for $C_{11}H_7BrO_3$ (267.08) calcd: C, 70.21; H, 4.29%; found: C, 70.15; H, 4.25%.

6-Bromo-3-(3-substituted acryloyl)-2H-chromen-2-ones (4a-4j)

Equimolar quantities of 3-acetyl-6-bromo-2H-chromen-2-one **(3)** and different substituted benzaldehydes were refluxed in absolute ethanol using piperidine as a catalyst for 8–10 h. The mixture was concentrated and poured onto crushed ice. The compounds so obtained were filtered, dried and recrystallized from ethanol to give pure crystalline solids.

6-Bromo-3-[(E)-3-(2-chlorophenyl)-acryloyl]-2H-chromen-2-one (4a)

Obtained from reaction of compound (**3**) with 2-chlorobenzaldehyde. IR (KBr, cm^{-1}): 1724.24 and 1662.52 (C=O), 1556.45 (C=C), 1184.21 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.02 (d, 1H, CH), 7.11–7.93 (m, 8H, Ar-H), 8.03 (d, 1H, CH). ^{13}C-NMR ($CDCl_3$, 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/z): 388 (100%) [M^+], 390 (35%) [M^++2], 392 (10%) [M^++4]. Analysis: for $C_{18}H_{10}BrClO_3$ (389.63) calcd.: C, 69.58; H, 3.57%; found: C, 69.52; H, 3.52%.

6-Bromo-3-[(E)-3-(3-chlorophenyl)-acryloyl]-2H-chromen-2-one (4b)

Obtained from reaction of compound (**3**) with 3-chlorobenzaldehyde. IR (KBr, cm^{-1}): 1728.10 and 1685.67 (C=O), 1558.38 (C=C), 1107.06 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.03 (d, 1H, CH), 7.15–8.02 (m, 8H, Ar-H), 8.66 (d, 1H, CH). ^{13}C-NMR ($CDCl_3$, 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/z): 388 (100%) [M^+], 390 (30%) [M^++2], 392 (5%) [M^++4]. Analysis: for $C_{18}H_{10}BrClO_3$ (389.63) calcd.: C, 69.58; H, 3.57%; found: C, 69.62; H, 3.52%.

6-Bromo-3-[(E)-3-(4-chlorophenyl)-acryloyl]-2H-chromen-2-one (4c)

Obtained from reaction of compound (**3**) with 4-chlorobenzaldehyde. IR (KBr, cm^{-1}): 1728.10 and 1685.67 (C=O), 1558.38 (C=C), 1107.06 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.36 (d, 1H, CH), 6.90 (d, 1H, CH), 7.02–8.48 (m, 8H, Ar-H). ^{13}C-NMR ($CDCl_3$, 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/z): 388 (100%) [M^+], 390 (33%) [M^++2], 392 (3%) [M^++4]. Analysis: for $C_{18}H_{10}BrClO_3$ (389.63) calcd.: C, 69.58; H, 3.57%; found: C, 69.55; H, 3.51%.

6-Bromo-3-[(E)-3-(2-bromophenyl)-acryloyl]-2H-chromen-2-one (4d)

Obtained from reaction of compound (**3**) with 2-bromobenzaldehyde. IR (KBr, cm^{-1}): 1724.24 and 1683.74 (C=O), 1556.43 (C=C), 1184.21 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.86 (d, 1H, CH), 7.02–7.93 (m, 8H, Ar-H), 8.00 (d, 1H, CH). ^{13}C-NMR ($CDCl_3$, 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/z): 433 (100%) [M^+], 435 (25%) [M^++2], 437 (2%) [M^++4]. Analysis: for $C_{18}H_{10}Br_2O_3$ (434.08) calcd.: C, 60.87; H, 3.12%; found: C, 60.81; H, 3.10%.

6-Bromo-3-[(E)-3-(3-bromophenyl)-acryloyl]-2H-chromen-2-one (4e)

Obtained from reaction of compound (**3**) with 3-bromobenzaldehyde. IR (KBr, cm^{-1}): 1728.10 and

1685.67 (C=O), 1558.38 (C=C), 1107.06 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.08 (d, 1H, CH), 7.11–7.99 (m, 8H, Ar-H), 8.05 (d, 1H, CH). ^{13}C-NMR ($CDCl_3$, 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/z): 433 (100%) [M^+], 435 (20%) [M^++2], 437 (1.6%) [M^++4]. Analysis: for $C_{18}H_{10}Br_2O_3$ (434.08) calcd.: C, 60.87; H, 3.12%; found: C, 60.81; H, 3.09%.

6-Bromo-3-[(E)-3-(4-bromophenyl)-acryloyl]-2H-chromen-2-one (**4f**)

Obtained from reaction of compound (**3**) with 4-bromobenzaldehyde. IR (KBr, cm^{-1}): 1739.67 and 1677.95 (C=O), 1558.38 (C=C), 1107.06 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.03 (d, 1H, CH), 7.11–7.94 (m, 8H, Ar-H), 8.23 (d, 1H, CH). ^{13}C-NMR ($CDCl_3$, 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/z): 433 (100%) [M^+], 435 (18%) [M^++2], 437 (2.5%) [M^++4]. Analysis: for $C_{18}H_{10}Br_2O_3$ (434.08) calcd.: C, 60.87; H, 3.12%; found: C, 60.90; H, 3.14%.

6-Bromo-3-[(E)-3-(2-methoxyphenyl)-acryloyl]-2H-chromen-2-one (**4g**)

Obtained from reaction of compound (**3**) with 2-methoxybenzaldehyde. IR (KBr, cm^{-1}): 1728.10 (C=O), 1685.67 (C=C), 1164.92 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.56 (s, 3H, CH_3), 6.86 (d, 1H, CH), 7.02–7.96 (m, 8H, Ar-H), 8.09 (d, 1H, CH). ^{13}C-NMR ($CDCl_3$, 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/z): 384 (100%) [M^+], 386 (25%) [M^++2], 388 (2%) [M^++4]. Analysis: for $C_{19}H_{13}BrO_4$ (385.21) calcd.: C, 74.50; H, 4.61%; found: C, 74.54; H, 4.57%.

6-Bromo-3-[(E)-3-(3-methoxyphenyl)-acryloyl]-2H-chromen-2-one (**4h**)

Obtained from reaction of compound (**3**) with 3-methoxybenzaldehyde. IR (KBr, cm^{-1}): 1735.81 (C=O), 1674.10 (C=C), 1137.92 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.90 (s, 3H, CH_3), 6.98 (d, 1H, Ar-H), 7.00-7.85 (m, 8H, Ar-H), 8.10 (d, 1H, CH). ^{13}C-NMR ($CDCl_3$, 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/z): 384 (100%) [M^+], 386 (20%) [M^++2], 388 (1.5%) [M^++4]. Analysis: for $C_{19}H_{13}BrO_4$ (385.21) calcd.: C, 74.50; H, 4.61%; found: C, 74.45; H, 4.56.

6-Bromo-3-[(E)-3-(2,4-dichlorophenyl)-acryloyl]-2H-chromen-2-one (**4i**)

Obtained from reaction of compound (**3**) with 2,4-dichlorobenzaldehyde. IR (KBr, cm^{-1}): 1739.67 (C=O), 1677.95 (C=C), 1103.21 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.98 (s, 1H, CH), 7.00–7.85 (m, 6H, Ar-H), 7.93 (s, 1H, CH), 8.43 (s, 1H, CH). ^{13}C-NMR ($CDCl_3$, δ, ppm): 121.9, 122.9, 123.2, 125.9, 126.5, 127.9, 128, 128.6, 129.3, 129.9, 132.5, 136.5,

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

Compounds Tested	Percent protection		
	0.5 h	1 h	2 h
Diclofenac sodium	94.25 ± 0.33	92.85 ± 0.47	84.0 ± 0.57
5a	42.52 ± 2.77**	69.04 ± 2.67**	56.0 ± 2.62***
5b	54.02 ± 4.20**	47.61 ± 3.95	32.0 ± 3.90
5c	39.08 ± 2.38	4.76 ± 2.33	20.0 ± 2.49
5d	51.72 ± 3.13	4.76 ± 3.40	0.00 ± 3.39
5e	25.94 ± 1.65**	59.52 ± 1.62	40.0 ± 1.77
5f	40.22 ± 3.84	0.00 ± 3.77	0.00 ± 3.54
5g	48.27 ± 3.05	14.28 ± 3.03	20.0 ± 2.99
5h	36.78 ± 2.42	47.61 ± 2.48	40.0 ± 2.07
5i	52.87 ± 1.81**	95.23 ± 0.76***	92.0 ± 1.05***
5j	88.50 ± 2.90***	85.71 ± 1.32 ***	92.0 ± 0.66***

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); **$p \leq 0.01$ and ***$p \leq 0.001$ when compared to control. Statistical analysis performed by one-way ANOVA followed by Dunnet's test.

136.9, 150.3, 152.6, 165.9, 166.3, 182.3. MS (m/z): 423 (100%) [M+], 425 (25%) [M+ +2], 427 (2%) [M+ +4]. Analysis: for $C_{18}H_9BrCl_2O_3$ (424.27) calcd.: C, 62.63; H, 2.92%; found: C, 62.56; H, 2.96%.

6-Bromo-3-[(E)-3-(2,6-dichlorophenyl)-acryloyl]-2H-chromen-2-one (4j)

Obtained from reaction of compound (**3**) with 2,6-dichlorobenzaldehyde. IR (KBr, cm^{-1}): 1739.67 (C=O), 1677.95 (C=C), 1161.07 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.87 (s, 1H, CH), 7.00–7.95 (m, 6H, Ar-H), 8.0 (s, 1H, CH), 8.43 (s, 1H, CH). ^{13}C-NMR ($CDCl_3$, δ, ppm): 121.1, 122.2, 123.9, 125, 126.9, 127.5, 128, 128.9, 129.3, 130.9, 132.4, 136.9, 138.9, 151.9, 155.5, 167.9, 169.5, 185.8. MS (m/z): 423 (100%) [M+], 425 (23%) [M+ +2], 427 (2.2%) [M+ +4]. Analysis: for $C_{18}H_9BrCl_2O_3$ (424.27) calcd.: C, 62.63; H, 2.92%; found: C, 62.66; H, 2.90%.

2-Amino-6-(substituted)-pyrimidin-4-yl)-6-bromo-2H-chromen-2-ones (5a–5j)

A mixture of 6-bromo-3-(3-Substituted acryloyl)-2H-chromen-2-one (**4a-4j**) (0.01 mole) and guanidine HCl (0.02 mole) was refluxed in ethanol for 8–10 h. The mixture was evaporated to dryness and the product was washed repeatedly with water and recrystallized from ethanol.

3-[2-Amino-6-(2-chlorophenyl)-pyrimidin-4-yl]-6-bromo-2H-chromen-2-one (5a)

Obtained by reacting (**4a**) with guanidine HCl. IR (KBr, cm^{-1}): 3431.55 (N-H), 1709.55 (C=O), 1612.04 (C=N), 1535.90 (C=C), 1129.17 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.256 (s, 2H, NH_2), 6.85–7.72 (m, 9H, Ar-H). ^{13}C-NMR ($CDCl_3$, 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/z): 427 (100%) [M+], 429 (40%) [M+ +2], 431 (10%) [M+ +4]. Analysis: for $C_{19}H_{11}BrClN_3O_2$ (428.67) calcd.: C, 65.24; H, 3.46; N, 12.01%; found: C, 65.30; H, 3.48; N, 12.06%.

3-[2-Amino-6-(3-chlorophenyl)-pyrimidin-4-yl]-6-bromo-2H-chromen-2-one (5b)

Obtained by reacting (**4b**) with guanidine HCl. IR (KBr, cm^{-1}): 3174.61 (N-H), 1654.81 (C=O), 1596.95 (C=N), 1546.80 (C=C), 1234.36 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.25 (s, 2H, NH_2), 6.92–7.36 (m, 9H, Ar-H). ^{13}C-NMR ($CDCl_3$, 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, 170.9. MS (m/z): 427 (100%) [M+], 429 (45%) [M+ +2], 431 (15%) [M+ +4]. Analysis: for $C_{19}H_{11}BrClN_3O_2$ (428.67) calcd.: C, 65.24; H, 3.46; N, 12.01%; found: C, 65.20; H, 3.40; N, 12.0%.

3-[2-Amino-6-(4-chlorophenyl)-pyrimidin-4-yl]-6-bromo-2H-chromen-2-one (5c)

Obtained by reacting (**4c**) with guanidine HCl. IR (KBr, cm^{-1}): 3340.48 (N-H), 1685.67 (C=O), 1593.09 (C=C), 1542.95 (C=N), 1238.61 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.25 (s, 2H, NH_2), 7.02–7.50 (m, 9H, Ar-H). ^{13}C-NMR ($CDCl_3$, 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/z): 427 (100%) [M+], 429 (47%) [M+ +2], 431 (17%) [M+ +4]. Analysis: for $C_{19}H_{11}BrClN_3O_2$ (428.67) calcd.: C, 65.24; H, 3.46; N, 12.01%. found: C, 65.19; H, 3.50; N, 12.02%.

3-[2-Amino-6-(2-bromophenyl)-pyrimidin-4-yl]-6-bromo-2H-chromen-2-one (5d)

Obtained by reacting (**4d**) with guanidine HCl. IR (KBr, cm^{-1}): 3355.91 (N-H), 1654.81 (C=O), 1600.81 (C=N), 1542.95 (C=C), 1238.21 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.96 (s, 2H, NH_2), 7.25–7.63 (m, 9H, Ar-H). ^{13}C-NMR ($CDCl_3$, 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/z): 472 (100%) [M+], 474 (50%) [M+ +2], 476 (20%) [M+ +4]. Analysis: for $C_{19}H_{11}Br_2N_3O_2$ (473.12) calcd.: C, 57.89; H, 3.07; N, 10.66%; found: C, 57.85; H, 3.02; N, 10.60%.

3-(2-Amino-6-(3-bromophenyl)-pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (5e)

Obtained by reacting (**4e**) with guanidine HCl. IR (KBr, cm^{-1}): 3355.91 (N-H), 1654.81 (C=O), 1542.95 (C=N), 1477.37 (C=C), 1269.07 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.27 (s, 2H, NH_2), 6.93–7.63 (m, 9H, Ar-H). ^{13}C-NMR ($CDCl_3$, 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/z): 472 (100%) [M+], 474 (45%) [M+ +2], 476 (15%) [M+ +4]. Analysis:. for $C_{19}H_{11}Br_2N_3O_2$ (473.12) calcd.: C, 57.89; H, 3.07; N, 10.66%; found: C, 57.92; H, 3.05; N, 10.60%.

3-(2-Amino-6-(4-bromophenyl)-pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (5f)

Obtained by reacting (**4f**) with guanidine HCl**.** IR (KBr, cm^{-1}): 3417.63 (N-H), 1666.38 (C=O), 1604.66 (C=N), 1477.37 (C=C), 1234.36 (C-O-C).

^{1}H-NMR ($CDCl_3$, δ, ppm): 4.16 (s, 2H, NH_2), 6.90–7.73 (m, 9H, Ar-H). ^{13}C-NMR ($CDCl_3$, 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/z): 472 (100%) [M^+], 474 (55%) [M^++2], 476 (15%) [M^++4]. Analysis: for $C_{19}H_{11}Br_2N_3O_2$ (473.12) calcd.: C, 57.89; H, 3.07; N, 10.66%; found: C, 57.85; H, 3.01; N, 10.60%.

3-[2-Amino-6-(2-methoxyphenyl)-pyrimidin-4-yl]-6-bromo-2H-chromen-2-one (5g)

Obtained by reacting (**4g**) with guanidine HCl. IR (KBr, cm^{-1}): 3382.91 (N-H), 1670.24 (C=O), 1600.81 (C=N), 1477.37 (C=C), 1245.93 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.87 (s, 3H, CH_3), 4.25 (s, 2H, NH_2), 6.92–8.00 (m, 9H, Ar-H). ^{13}C-NMR ($CDCl_3$, 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/z): 423 (100%) [M^+], 425 (25%) [M^++2], 427 (5%) [M^++4]. Analysis: for $C_{20}H_{14}BrN_3O_3$ (424.25) calcd.: C, 69.56; H, 4.38; N, 12.17%; found: C, 69.62; H, 4.35; N, 12.11%.

3-[2-Amino-6-(3-methoxyphenyl)-pyrimidin-4-yl]-6-bromo-2H-chromen-2-one (5h)

Obtained by reacting (**4h**) with guanidine HCl. IR (KBr, cm^{-1}): 3367.48 (N-H), 1666.38 (C=O), 1600.81 (C=N), 1577.66 (C=C), 1265.22 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.81 (s, 3H, CH_3), 4.04 (s, 2H, NH_2), 6.86–7.25 (m, 9H, Ar-H). ^{13}C-NMR ($CDCl_3$, 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/z): 423 (100%) [M^+], 425 (20%) [M^++2], 427 (8%) [M^++4]. Analysis: for $C_{20}H_{14}BrN_3O_3$ (424.25) calcd.: C, 69.56; H, 4.38; N, 12.17%; found: C, 69.50; H, 4.34; N, 12.15%.

3-(2-Amino-6-(2,4-dichlorophenyl)-pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (5i)

Obtained by reacting (**4i**) with guanidine HCl. IR (KBr, cm^{-1}): 3417.63 (N-H), 1677.95 (C=O), 1589.23 (C=N), 1473.51 (C=C), 1234.36 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.06 (s, 2H, NH_2), 7.0–7.40 (m, 7H, Ar-H), 7.95 (s, 1H, CH). ^{13}C-NMR ($CDCl_3$, δ, ppm): 105.4, 120.5, 121.9, 123.5, 124.6, 127.9, 128.5, 128.9, 129.9, 130, 132.6, 133.6, 135.6, 138.7 145.6, 150.3, 154.9, 160.8, 165.9. MS (m/z) 462 (100%) [M^+], 464 (22%) [M^++2], 466 (5%) [M^++4]. Analysis: for $C_{19}H_{10}BrCl_2N_3O_2$ (463.11) calcd.: C, 59.39; H, 2.89; N, 10.94%; found: C, 59.44; H, 2.85; N, 10.90%.

3-[2-Amino-6-(2,6-dichlorophenyl)-pyrimidin-4-yl]-6-bromo-2H-chromen-2-one (5j)

Obtained by reacting (**4j**) with guanidine HCl. IR (KBr, cm^{-1}): 3425.34 (N-H), 1604.66 (C=O), 1600.81 (C=N), 1577.66 (C=C), 1265.22 (C-O-C). ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.03 (s, 2H, NH_2),

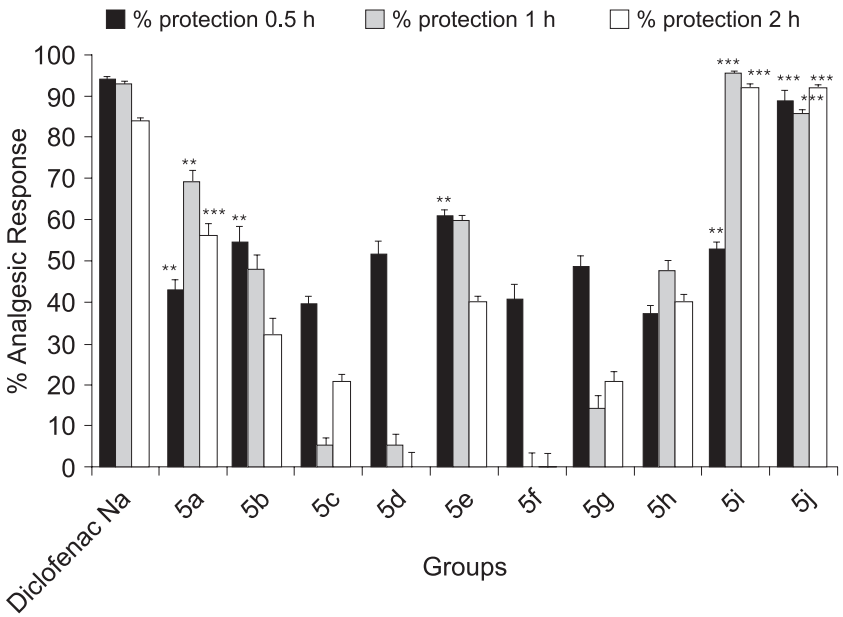
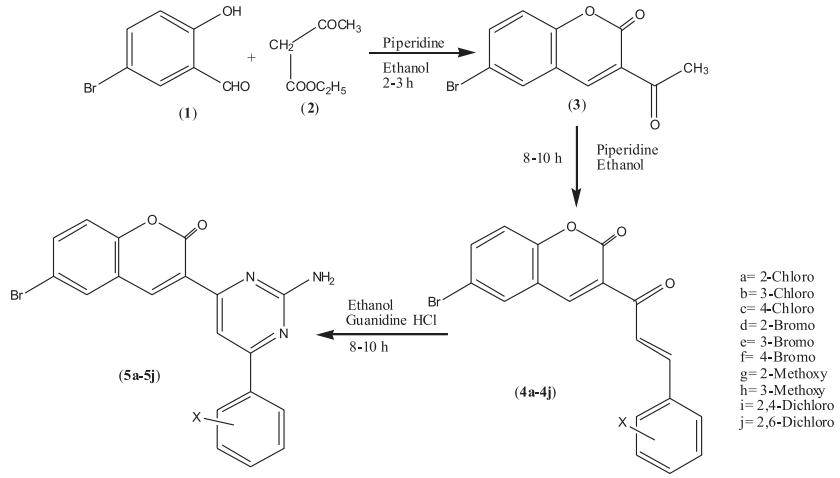


Figure 1. Analgesic responses of synthesized compounds were evaluated by acetic acid induced writhing method. Values were expressed as the mean ± SEM; **p ≤ 0.01 and ***p ≤ 0.001 indicates the level of statistical significance as compared with control.



Scheme 1. Schematic diagrams for the synthesis of pyrimidine derivatives (**5a**–**5j**).

7.10–7.60 (m, 7H, Ar-H), 7.95 (s, 1H, CH). ^{13}C-NMR ($CDCl_3$, δ, ppm): 104.5, 120.9, 121.9, 123.9, 124.8, 126.7, 127.5, 129.1, 129.9, 130.2, 132.9, 133.7, 135, 140.7, 150.6, 150.9, 154.9, 157.03, 165.9. MS (m/z): 462 (100%) [M^+], 464 (19%) [M^+ +2], 466 (7%) [M^+ +4]. Analysis: for $C_{19}H_{10}BrCl_2N_3O_2$ (463.11) calcd.: C, 59.39; H, 2.89; N, 10.94%; found: C, 59.36; H, 2.90; N, 10.90%.

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 rations 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 investigating their analgesic activity in acetic acid induced writhing response in Swiss albino mice following the method of Turner (20) and Collier et al. (21). Seventy two mice were selected and divided into 12 groups (six in each group), starved for 16 h and pre-treated as follows: the first group which served as a positive control was orally received distilled water in appropriate volumes. The second to eleventh groups were receiving the aqueous suspension of synthesized compounds (**5a-5j**) orally in a dose of 20 mg/kg. The last group was orally receiving 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 15 min after acetic acid injection at 0.5, 1 and 2 h. 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:

$$\% \text{ protection} = (\text{control mean} - \text{treated mean}/\text{control mean}) \times 100$$

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons of all com-

pounds in various pharmacological assays. Data are expressed as the mean ± SEM.

RESULTS AND DISCUSSION

Among all the synthesized compounds, some compounds viz., **5a**, **5b** and **5e** exhibited significant analgesic activity and compounds **5i** and **5j** have shown highly significant activity. The remaining compounds have shown less analgesic activity comparable to that of standard drug diclofenac sodium in the acetic acid induced writhing response model (Fig. 1).

CONCLUSION

All derivatives tested significantly suppressed the spontaneous locomotor activity of mice during a 30 min observation period. The most potent effects were produced by derivatives **5i** and **5j**. On the contrary, the weakest activity in this test was displayed by compounds **5c**, **5d** and **5f**. From the data obtained, it follows that the most active substance in the acetic acid induced writhing method is 3-(2-amino-6-(2,4-dichlorophenyl)-pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (**5i**). Modification of position of chlorine from 2 and 4 position as in compound **5i**, to position 2 and 6 as in compound **5j** also produced the potent analgesic compound (Table 3 and Fig 1).

Acknowledgment

This research work was financially supported by the Meerut Institute of Engineering & Technology, Meerut, India-250005.

REFERENCES

1. Williams M., Kowaluk E.A., Arneric S.P.: J. Med. Chem. 9, 1481 (1999).
2. Dardonville C., Rozas I., Goya P., Giron R., Goicoechea C., Martýn M. I.: Bioorg. Med. Chem. 11, 1283 (2003).
3. Giovannoni M.P., Vergelli C., Ghelardini C., Galeotti N., Bartolini A. DalPiaz V.: J. Med. Chem. 46, 1055 (2003).
4. Walsh T. D.: J. Pain Symptom Manage. 5, 362 (1990).
5. Patel R., Desai K., Chikhalia K.: J. Ind. Chem. Soc. 80, 138 (2003).
6. Desai K., Patel R., Chikhalia K.: J. Ind. Chem. 45 (B), 773 (2006).
7. Amr A.E., Nermien M.S., Abdulla M.M.: Monatsh. Chem. 138, 699 (2007).
8. Fujiwara N., Nakajima T., Ueda Y., Fujita H., Kawakami H.: Bioorg. Med. Chem. 16, 9804 (2008).
9. Ballell L., Field R.A., Chung G.A.C., Young R.J.: Bioorg. Med. Chem. Lett. 17, 1736 (2007).
10. Wagner E., Al-Kadasi K., Zimecki M., Sawka-Dobrowolska W.: Eur. J. Med. Chem. 43, 2498 (2008).
11. Cordeu L., Cubedo E., Bandres E., Rebollo A., Saenz X., Chozas H. et al.: Bioorg. Med. Chem. 15, 1659 (2007).
12. Gorlitzer K., Herbig S., Walter R.D.: Pharmazie 52, 670 (1997).
13. Ukrainets I.V., Tugaibei I.A., Bereznykova N.L., Karvechenko V.N., Turov A.V.: Khim. Geterotsiklich. Soed. 5, 718 (2008).
14. Kurono M., Hayashi M., Miura K., Isogawa Y., Sawai K: Sanwa Kagaku Kenkyusho Co., Japan, Kokai Tokkyo Koho JP 62, 267, 272, (1987); Chem. Abstr. 109, 37832t (1988).
15. Wang S.Q., Fang L., Liu X.J., Zhao K.: Chinese Chem. Lett. 15, 885 (2004).
16. Yang W., Ruan Z., Wang Y., Van Kirk K., Ma Z., Arey B. J. et al.: J. Med. Chem. 52, 1204 (2009).
17. Gillespie R.J., Bamford S.J., Botting R., Comer M., Denny, S., Gaur S. et al.: J. Med. Chem. 52, 33 (2009).
18. Kulkarni M.V., Kulkarni G.M., Lin C.H., Sun C.M.: Curr. Med. Chem. 13, 2795 (2006).
19. Keri R.S., Hosamani K.M., Shingalapur R.V., Hugar M.H.: Eur. J. Med. Chem. 45, 2597 (2010).
20. Turner R.A.: In Analgesics: Screening Methods in Pharmacology. Turner, R.A., Ed.; p. 100, Academic Press, London 1965.
21. Collier H.D.J., Dinnin L.C., Johnson C.A., Schneider C.: Br. J. Pharmacol. 32, 295 (1968).

Received: 21. 08. 2010