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SYNTHESIS, ANALGESIC AND ULCEROGENIC ACTIVITY OF NOVEL PYRIMIDINE DERIVATIVE OF COUMARIN MOIETY

J. K. Gupta *, P.K. Sharma *, R. Dudhe **, Anshu Chaudhary * and P.K. Verma ******

abstract: A novel series of 3-(2-amino-6-pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (**5a-5j**) were synthesized from 3-acetyl-6-bromo-2H-chromen-2-one (**3**). The structures of the synthesized compounds were elucidated by IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopic techniques. The synthesized compounds were screened for in-vivo analgesic activity at a dose of 20 mg/kg body weight. Among them, compounds **5a**, **5b** and **5e** exhibited significant analgesic activity and compounds **5i** and **5j** exhibited highly significant activity comparable with standard drug Diclofenac sodium using Acetic acid induced writhing model. Compounds **5a**, **5i** and **5j** were further evaluated for acute-ulcerogenesis activity. Among them, compound **5j** was found to be most promising analgesic agent devoid of ulcerogenic effects.

key words: Coumarin; Pyrimidines; Knoevenagel reaction; Claisen-schmidt condensation; Writhing Test; Analgesic activity; Ulcerogenic activity.

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1. Introduction

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

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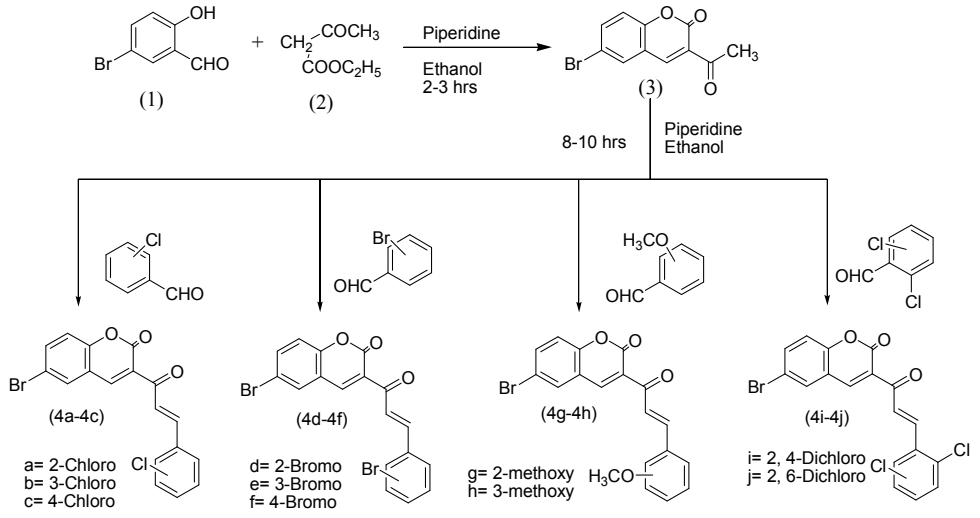
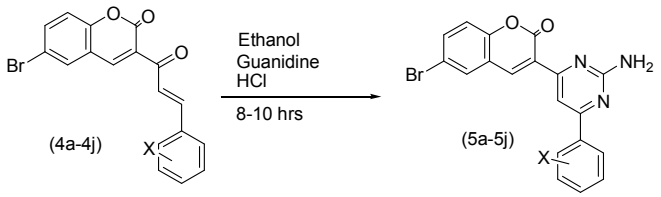
constipation [3,4]. Hence there is always a need for those drugs which have improved analgesic activity and less adverse effects.



Scheme 1 *Stepwise schematic diagrams for the synthesis of pyrimidine derivatives (4a-4j).*



X=2-Cl, 3-Cl, 4-Cl, 2-Br, 3-Br, 4-Br, 2-OCH_3, 3-OCH_3, 2,4-Dichloro, 2,6-Dichloro

Scheme 2 *Stepwise schematic diagrams for the synthesis of pyrimidine derivatives (5a-5j).*

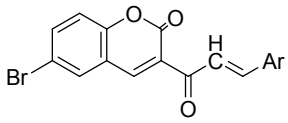
Nitrogen containing heterocycles such as Pyrimidine is a promising structural moiety for drug designing. Pyrimidine derivatives form a component in a number of useful drugs and are associated with many biological, pharmaceutical and therapeutical activities [5]. Condensed pyrimidine derivatives have 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 [18,19].

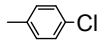
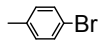
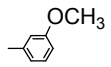
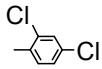
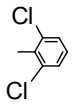
It was envisaged that these two active pharmacophores, if linked together would generate novel molecular templates which are likely to exhibit interesting biological properties in animal models. The above-cited applications prompted us to synthesize a series of new compounds reported in this article.

Owing to the importance, here we have described the synthesis of new pyrimidine derivatives from 3-acetyl-6-bromo-2H-chromen-2-one (Reaction Scheme 1 & 2). The

compounds were screened for their in-vivo analgesic and ulcerogenic activity. Thus, we have created new avenues to explore the potent heterocyclic moieties for the pharmacological activities in medicinal chemistry.

Table 1 *Physical parameters of Compounds (**4a-4j**).*



Compound [a]	-Ar	Yield (%) [b]	m.p.(°C) [c]	Rf value	Molecular Formula
4a		65	162-165 ºC	0.73	$C_{18}H_{10}BrClO_3$
4b		70	165-167 ºC	0.75	$C_{18}H_{10}BrClO_3$
4c		60	156-158 ºC	0.71	$C_{18}H_{10}BrClO_3$
4d		70	190-192 ºC	0.77	$C_{18}H_{10}Br_2O_3$
4e		75	185-187 ºC	0.76	$C_{18}H_{10}Br_2O_3$
4f		75	185-188 ºC	0.69	$C_{18}H_{10}Br_2O_3$
4g		75	177-179 ºC	0.72	$C_{19}H_{13}BrO_4$
4h		75	173-175 ºC	0.76	$C_{19}H_{13}BrO_4$
4i		80	175-177 ºC	0.71	$C_{18}H_9BrCl_2O_3$
4j		75	180-183 ºC	0.76	$C_{18}H_9BrCl_2O_3$

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

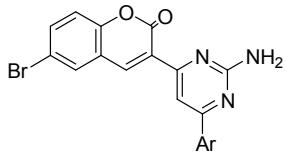
2. Experimental

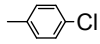
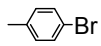
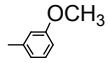
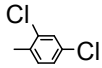
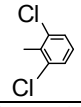
2.1 Chemistry

All reagents and solvents were used as obtained from the supplier. The melting points of the products were determined by open capillaries method and are uncorrected. I.R. Spectra (KBr) were recorded on FTIR Spectrophotometer (Shimadzu FTIR 84005, 4000-400cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL AL300 FTNMR 300 MHz spectrometer in $CDCl_3$-d 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 an 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 described by TLC on alumina silica gel using solvent system "Toluene:Ethylacetate:Formic acid" (5:4:1) detected by iodine vapours. The in-vivo analgesic activity and acute ulcerogenesis activity was performed at Meerut Institute of Engineering and Technology, Meerut, India. The physical data of all these compounds are summarized in Tables 1 and 2.

Table 2 *Physical parameters of Compounds (**5a-5j**).*



Compound [a]	-Ar	Yield (%)[b]	m.p.(°C)[c]	Rf value	Molecular Formula
5a		65	162-165 ºC	0.62	$C_{19}H_{11}BrClN_3O_2$
5b		60	165-167 ºC	0.74	$C_{19}H_{11}BrClN_3O_2$
5c		70	156-158 ºC	0.70	$C_{19}H_{11}BrClN_3O_2$
5d		65	190-192 ºC	0.75	$C_{19}H_{11}Br_2N_3O_2$
5e		50	185-187 ºC	0.72	$C_{19}H_{11}Br_2N_3O_2$
5f		60	185-188 ºC	0.68	$C_{19}H_{11}Br_2N_3O_2$
5g		65	177-179 ºC	0.67	$C_{20}H_{14}BrN_3O_3$
5h		65	173-175 ºC	0.65	$C_{20}H_{14}BrN_3O_3$
5i		70	175-177 ºC	0.78	$C_{19}H_{10}BrCl_2N_3O_2$
5j		68	180-183 ºC	0.70	$C_{19}H_{10}BrCl_2N_3O_2$

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

General Procedures for the Preparation of Compounds

2.1.1 Synthesis of 3-acetyl-6-bromo-2H-chromen-2-one (3): General Procedure

A mixture of salicyldehyde **(1)** (0.02 moles) and ethyl acetoacetate **(2)** (0.03 moles) in ethanol were taken in round bottom flask. To this mixture 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. Formation of compound (**3**) can be explained on the basis of "Knoevenagel reaction". The purity of compound was established on the basis of TLC. M. P. 115-117ºC; 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 ($CDCl_3$-*d*, δ, ppm): 2.58 (s, 3H, CH_3), 7.25-7.98 (m, 4H, Ar-H); ^{13}C NMR ($CDCl_3$-*d*, 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* 266 (100%), [M^+ +2], *m/z* 268 (15%), [M^+ +4], *m/z* 270 (2%); Anal. Calcd for $C_{11}H_7BrO_3$ (267.08): C, 70.21; H, 4.29. Found: C, 70.15; H, 4.25.

2.1.2 Synthesis of Compounds (4a-4j): General Procedure

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 hours. The solution mixture was concentrated and poured on to crushed ice. The compound so obtained were filtered at pump, dried and recrystallized from ethanol to get pure crystalline solid. The formation of compounds (**4a-4j**) can be explained on the basis of "Claisen-schmidt condensation".

2.1.2.1 Synthesis of 6-bromo-3-((E)-3-(2-chlorophenyl)-acryloyl)-2H-chromen-2-one (4a): It was 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}HNMR ($CDCl_3$-*d*, δ, ppm): 6.02 (d, 1H, CH), 7.11-7.93 (m, 8H, Ar-H), 8.03 (d, 1H, CH); ^{13}C NMR ($CDCl_3$-*d*, 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* 388 (100%), [M^+ +2], *m/z* 390 (35%), [M^+ +4], *m/z* 392 (10%); Anal. Calcd for $C_{18}H_{10}BrClO_3$ (389.63): C, 69.58; H, 3.57. Found: C, 69.52; H, 3.52.

2.1.2.2 Synthesis of 6-bromo-3-((E)-3-(3-chlorophenyl)-acryloyl)-2H-chromen-2-one (4b): It was 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}HNMR ($CDCl_3$-*d*, δ, ppm): 7.03 (d, 1H, CH), 7.15-8.02 (m, 8H, Ar-H), 8.66 (d, 1H, CH); ^{13}C NMR ($CDCl_3$-*d*, 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* 388 (100%), [M^+ +2], *m/z* 390 (30%), [M^+ +4], *m/z* 392 (5%); Anal. Calcd for $C_{18}H_{10}BrClO_3$ (389.63): C, 69.58; H, 3.57. Found: C, 69.62; H, 3.52.

2.1.2.3 Synthesis of 6-bromo-3-((E)-3-(4-chlorophenyl)-acryloyl)-2H-chromen-2-one (4c): It was 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}HNMR ($CDCl_3$-*d*, δ, ppm): 6.36 (d, 1H, CH), 6.90 (d, 1H, CH), 7.02-8.48 (m, 8H, Ar-H); ^{13}C NMR ($CDCl_3$-*d*, 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* 388 (100%), [M^+ +2], *m/z* 390 (33%), [M^+ +4], *m/z* 392 (3%); Anal. Calcd for $C_{18}H_{10}BrClO_3$ (389.63): C, 69.58; H, 3.57. Found: C, 69.55; H, 3.51.

2.1.2.4 Synthesis of 6-bromo-3-((E)-3-(2-bromophenyl)-acryloyl)-2H-chromen-2-one (4d): It was 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}HNMR ($CDCl_3$-*d*, δ, ppm): 6.86 (d, 1H, CH), 7.02-7.93 (m, 8H, Ar-H), 8.00 (d, 1H, CH); ^{13}C NMR ($CDCl_3$-*d*,

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* 433 (100%), [M^+ +2], *m/z* 435 (25%), [M^+ +4], *m/z* 437 (2%); Anal. Calcd for $C_{18}H_{10}Br_2O_3$ (434.08): C, 60.87; H, 3.12. Found: C, 60.81; H, 3.10.

2.1.2.5 Synthesis of 6-bromo-3-((E)-3-(3-bromophenyl)-acryloyl)-2H-chromen-2-one (4e): It was 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}HNMR ($CDCl_3$-*d*, δ, ppm): 7.08 (d, 1H, CH), 7.11-7.99 (m, 8H, Ar-H), 8.05 (d, 1H, CH); ^{13}C NMR ($CDCl_3$-*d*, 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* 433 (100%), [M^+ +2], *m/z* 435 (20%), [M^+ +4], *m/z* 437 (1.6%); Anal. Calcd for $C_{18}H_{10}Br_2O_3$ (434.08): C, 60.87; H, 3.12. Found: C, 60.81; H, 3.09.

2.1.2.6 Synthesis of 6-bromo-3-((E)-3-(4-bromophenyl)-acryloyl)-2H-chromen-2-one (4f): It was 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}HNMR ($CDCl_3$-*d*, δ, ppm): 7.03 (d, 1H, CH), 7.11-7.94 (m, 8H, Ar-H), 8.23 (d, 1H, CH); ^{13}C NMR ($CDCl_3$-*d*, 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* 433 (100%), [M^+ +2], *m/z* 435 (18%), [M^+ +4], *m/z* 437 (2.5%); Anal. Calcd for $C_{18}H_{10}Br_2O_3$ (434.08): C, 60.87; H, 3.12. Found: C, 60.90; H, 3.14.

2.1.2.7 Synthesis of 6-bromo-3-((E)-3-(2-methoxyphenyl)-acryloyl)-2H-chromen-2-one (4g): It was obtained from reaction of compound **(3)** with 2-Methoxybenzaldehyde. IR (KBr, cm^{-1}): 1728.10 (C=O), 16085.67 (C=C), 1164.92 (C-O-C). ^{1}HNMR ($CDCl_3$-*d*, 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 ($CDCl_3$-*d*, 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* 384 (100%), [M^+ +2], *m/z* 386 (25%), [M^+ +4], *m/z* 388 (2%); Anal. Calcd for $C_{19}H_{13}BrO_4$ (385.21): C, 74.50; H, 4.61. Found: C, 74.54; H, 4.57.

2.1.2.8 Synthesis of 6-bromo-3-((E)-3-(3-methoxyphenyl)-acryloyl)-2H-chromen-2-one (4h): It was 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}HNMR ($CDCl_3$-*d*, 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 ($CDCl_3$-*d*, 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* 384 (100%), [M^+ +2], *m/z* 386 (20%), [M^+ +4], *m/z* 388 (1.5%); Anal. Calcd for $C_{19}H_{13}BrO_4$ (385.21): C, 74.50; H, 4.61. Found: C, 74.45; H, 4.56.

2.1.2.9 Synthesis of 6-bromo-3-((E)-3-(2, 4-dichlorophenyl)-acryloyl)-2H-chromen-2-one (4i): It was 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}HNMR ($CDCl_3$-*d*, 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 ($CDCl_3$-*d*, 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, 136.9, 150.3, 152.6, 165.9, 166.3, 182.3; MS, [M^+], *m/z* 423 (100%), [M^+ +2], *m/z* 425 (25%), [M^+ +4], *m/z* 427 (2%); Anal. Calcd for $C_{18}H_9BrCl_2O_3$ (424.27): C, 62.63; H, 2.92. Found: C, 62.56; H, 2.96.

2.1.2.10 Synthesis of 6-bromo-3-((E)-3-(2, 6-dichlorophenyl)-acryloyl)-2H-chromen-2-one (4j): It was 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}HNMR ($CDCl_3$-*d*, 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 ($CDCl_3$-*d*, 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^+], *m/z* 423 (100%), [M^++2], *m/z* 425 (23%), [M^++4], *m/z* 427 (2.2%); Anal. Calcd for $C_{18}H_9BrCl_2O_3$ (424.27): C, 62.63; H, 2.92. Found: C, 62.66; H, 2.90.

2.1.3 Synthesis of Compounds (5a-5j): General Procedure

A mixture of Compounds **(4a-4j)** (0.01 mole) and guanidine HCl (0.02 mole) was refluxed in ethanol for 8-10 hours. The content was evaporated to dryness and the product so obtained was washed with water repeatedly and recrystallized from ethanol.

2.1.3.1 Synthesis of 3-(2-amino-6-(2-chlorophenyl)-pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (5a): It was obtained from 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}HNMR ($CDCl_3$-*d*, δ, ppm): 4.256 (s, 2H, NH_2), 6.85-7.72 (m, 9H, Ar-H); ^{13}C NMR ($CDCl_3$-*d*, 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* 427 (100%), [M^+ +2], *m/z* 429 (40%), [M^+ +4], *m/z* 431 (10%); Anal. Calcd for $C_{19}H_{11}BrClN_3O_2$ (428.67): C, 65.24; H, 3.46; N, 12.01. Found: C, 65.30; H, 3.48; N, 12.06.

2.1.3.2 Synthesis of 3-(2-amino-6-(3-chlorophenyl)-pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (5b): It was obtained from 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}HNMR ($CDCl_3$-*d*, δ, ppm): 4.25 (s, 2H, NH_2), 6.92-7.36 (m, 9H, Ar-H); ^{13}C NMR ($CDCl_3$-*d*, 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^+], *m/z* 427 (100%), [M^+ +2], *m/z* 429 (45%), [M^+ +4], *m/z* 431 (15%); Anal. Calcd for $C_{19}H_{11}BrClN_3O_2$ (428.67): C, 65.24; H, 3.46; N, 12.01. Found: C, 65.20; H, 3.40; N, 12.0.

2.1.3.3 Synthesis of 3-(2-amino-6-(4-chlorophenyl)-pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (5c): It was obtained from reacting **(4c)** with guanidine HCl. IR (KBr, cm^{-1}): 3340.48 (N-H), 1685.67 (C=O), 1593.09 (C=N), 1542.95 (C=N), 1238.61(C-O-C); ^{1}HNMR ($CDCl_3$-*d*, δ, ppm): 4.25 (s, 2H, NH_2), 7.02-7.50 (m, 9H, Ar-H); ^{13}C NMR ($CDCl_3$-*d*, 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* 427 (100%), [M^++2], *m/z* 429 (47%), [M^+ +4], *m/z* 431 (17%); Anal. Calcd for $C_{19}H_{11}BrClN_3O_2$ (428.67): C, 65.24; H, 3.46; N, 12.01. Found: C, 65.19; H, 3.50; N, 12.02.

2.1.3.4 Synthesis of 3-(2-amino-6-(2-bromophenyl)-pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (5d): It was obtained from reacting **(4d)** with guanidine HCl. IR (KBr, cm^{-1}): 3355.91 (N-H), 1654.81 (C=O), 1600.81 (C=N), 1542.95 (C=N), 1238.21 (C-O- C); ^{1}HNMR ($CDCl_3$-*d*, δ, ppm): 4.96 (s, 2H, NH_2), 7.25-7.63 (m, 9H, Ar-H); ^{13}C NMR ($CDCl_3$-*d*, 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* 472 (100%), [M^++2], *m/z* 474 (50%), [M^+ +4], *m/z* 476 (20%); Anal. Calcd for $C_{19}H_{11}Br_2N_3O_2$ (473.12): C, 57.89; H, 3.07; N, 10.66. Found: C, 57.85; H, 3.02; N, 10.60.

2.1.3.5 Synthesis of 3-(2-amino-6-(3-bromophenyl)-pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (5e): It was obtained from reacting **(4e)** with guanidine HCl. IR (KBr, cm^{-1}): 3355.91 (N-H), 1654.81 (C=O), 1542.95 (C=N), 1477.37 (C=N), 1269.07 (C-O-C); ^{1}HNMR ($CDCl_3$-*d*, δ, ppm): 4.27 (s, 2H, NH_2), 6.93-7.63 (m, 9H, Ar-H); ^{13}C NMR ($CDCl_3$-*d*, 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* 472 (100%), [M^+ +2], *m/z* 474 (45%), [M^+ +4], *m/z* 476 (15%); Anal. Calcd for $C_{19}H_{11}Br_2N_3O_2$ (473.12): C, 57.89; H, 3.07; N, 10.66. Found: C, 57.92; H, 3.05; N, 10.60.

2.1.3.6 Synthesis of 3-(2-amino-6-(4-bromophenyl)-pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (5f): It was obtained from reacting **(4f)** with guanidine HCl**.** IR (KBr, cm^{-1}): 3417.63 (N-H), 1666.38 (C=O), 1604.66 (C=N), 1477.37 (C=N), 1234.36 (C-O-C); ^{1}HNMR ($CDCl_3$-*d*, δ, ppm): 4.16 (s, 2H, NH_2), 6.90-7.73 (m, 9H, Ar-H); ^{13}C NMR ($CDCl_3$-*d*, 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* 472 (100%), [M^+ +2], *m/z* 474 (55%), [M^+ +4], *m/z* 476 (15%); Anal. Calcd for $C_{19}H_{11}Br_2N_3O_2$ (473.12): C, 57.89; H, 3.07; N, 10.66. Found: C, 57.85; H, 3.01; N, 10.60.

2.1.3.7 Synthesis of 3-(2-amino-6-(2-methoxyphenyl)-pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (5g): It was obtained from reacting **(4g)** with guanidine HCl. IR (KBr, cm^{-1}) : 3382.91 (N-H), 1670.24 (C=O), 1600.81 (C=N), 1477.37 (C=N), 1245.93 (C-O-C); ^{1}HNMR ($CDCl_3$-*d*, 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 ($CDCl_3$-*d*, 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* 423 (100%), [M^+ +2], *m/z* 425 (25%), [M^+ +4], *m/z* 427 (5%); Anal. Calcd for $C_{20}H_{14}BrN_3O_3$ (424.25): C, 69.56; H, 4.38; N, 12.17. Found: C, 69.62; H, 4.35; N, 12.11.

2.1.3.8 Synthesis of 3-(2-amino-6-(3-methoxyphenyl)-pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (5h): It was obtained from reacting **(4h)** with guanidine HCl. IR (KBr, cm^{-1}): 3367.48 (N-H), 1666.38 (C=O), 1600.81 (C=N), 1577.66 (C=N), 1265.22 (C-O-C); ^{1}HNMR ($CDCl_3$-*d*, 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 ($CDCl_3$-*d*, 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* 423 (100%), [M^+ +2], *m/z* 425 (20%), [M^+ +4], *m/z* 427 (8%); Anal. Calcd for $C_{20}H_{14}BrN_3O_3$ (424.25): C, 69.56; H, 4.38; N, 12.17. Found: C, 69.50; H, 4.34; N, 12.15.

2.1.3.9 Synthesis of 3-(2-amino-6-(2, 4-dichlorophenyl)-pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (5i): It was obtained from reacting **(4i)** with guanidine HCl. IR (KBr, cm^{-1}): 3417.63 (N-H), 1677.95 (C=O), 1589.23 (C=N), 1473.51 (C=N), 1234.36 (C-O-C); ^{1}HNMR ($CDCl_3$-*d*, δ, ppm): 4.06 (s, 2H, NH_2), 7.0-7.40 (m, 7H, Ar-H), 7.95 (s, 1H, CH); ^{13}C NMR ($CDCl_3$-*d*, 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^+], *m/z* 462 (100%), [M^+ +2], *m/z* 464 (22%), [M^+ +4], *m/z* 466 (5%); Anal. Calcd for $C_{19}H_{10}BrCl_2N_3O_2$ (463.11): C, 59.39; H, 2.89; N, 10.94. Found: C, 59.44; H, 2.85; N, 10.90.

2.1.3.10 Synthesis of 3-(2-amino-6-(2, 6-dichlorophenyl)-pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (5j): It was obtained from reacting **(4j)** with guanidine HCl. IR (KBr, cm^{-1}): 3425.34 (N-H), 1604.66 (C=O), 1600.81 (C=N), 1577.66 (C=N), 1265.22 (C-O-C); ^{1}HNMR ($CDCl_3$-*d*, δ, ppm): 4.03 (s, 2H, NH_2), 7.10-7.60 (m, 7H, Ar-H), 7.95 (s, 1H, CH);

^{13}C NMR ($CDCl_3$-*d*, 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^+], *m/z* 462 (100%), [M^+ +2], *m/z* 464 (19%), [M^+ +4], *m/z* 466 (7%); Anal.Calcd for $C_{19}H_{10}BrCl_2N_3O_2$ (463.11): C, 59.39; H, 2.89; N, 10.94. Found: C, 59.36; H, 2.90; N, 10.90.

Table 3 *Analgesic activity of compounds (5a-5j) by Acetic acid induced writhing response model.*

Compounds Tested	Percent Protection		
	0.5 hrs	1 hrs	2 hrs
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); $p \leq 0.001$ when compared to control. Statistical analysis: the statistical analysis was performed by one-way ANOVA followed by Dunnet's test.

2.2 Pharmacological Screening

2.2.1 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.

2.2.2 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 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 control positive was orally received distilled water in appropriate volumes. The second to eleventh groups were received the aqueous suspension of synthesized compounds (**5a-5j**) 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 were 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 hr, 1 hr and 2 hr (Table 3 and Fig. 1). 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:

$$\% \text{ Protection} = \frac{(\text{ControlMean-TreatedMean})}{\text{ControlMean}} \times 100$$

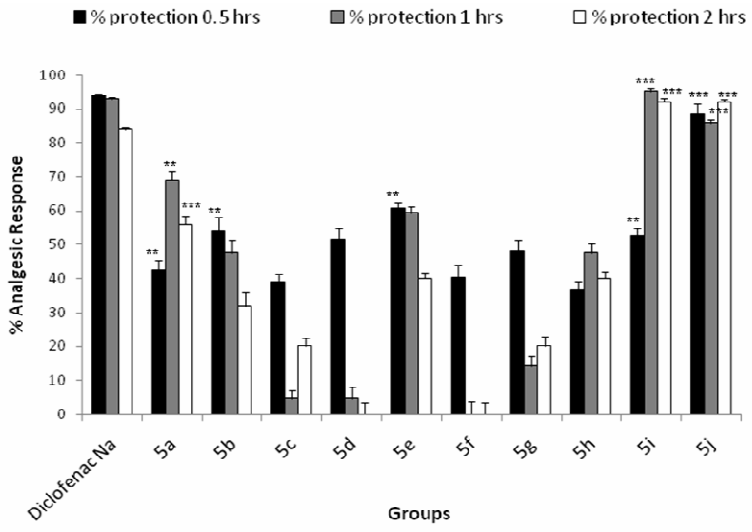


Fig. 1 *Analgesic responses of synthesized compounds by acetic acid induced writhing method.*
Values were expressed as Mean ± SEM and $p \leq 0.001$ indicates
the level of statistical significance as compared with control.

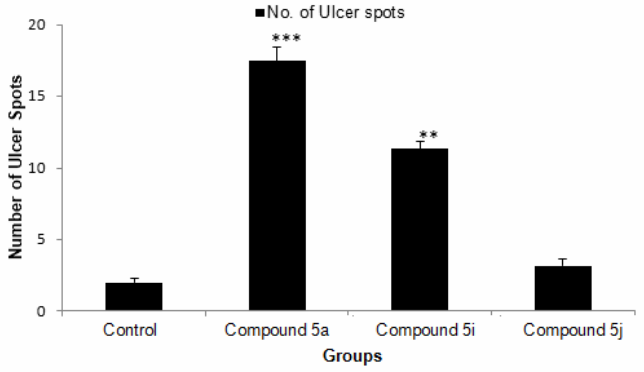


Fig. 2 *Ulcerogenic responses of synthesized compounds.*
Values were expressed as Mean ± SEM and and ***$p \leq 0.001$, **$p \leq 0.05$ indicates
the level of statistical significance as compared with control.

2.2.3 Acute-Ulcerogenesis Activity

Acute ulcerogenesis test was done according to Cioli et al.[22]. Albino rats (150–200 g) were divided into different groups consisting of six animals in each group. Ulcerogenic activity was evaluated after p.o. administration of test compounds or standard drug at the

dose of 60 mg/kg. Control rats received p.o. administration of vehicle (suspension of 1% methyl cellulose). Food but not water was removed 24 h before administration of the test compounds. After the drug treatment, the rats were fed normal diet for 17 h and then sacrificed. The stomach was removed and opened along the greater curvature, washed with distilled water and opened along the greater curvature, washed with distilled water and cleaned gently by dipping in saline. The gastric mucosa of the rats was examined by means of a 4x binocular magnifier. The lesions were counted and reported in Table 4 and Fig. 2.

Table 4 *Ulcerogenic response of the various groups of compounds tested on the stomach of rats.*

S. No.	Groups	No. of Ulcer spots
1.	Control Group	2.00 ± 0.25
2.	Compound 5a	17.50 ± 0.91***
3.	Compound 5i	11.33 ± 0.49**
4.	Compound 5j	3.16 ± 0.47

Values are expressed as Mean ± SEM and ***$p \leq 0.001$, **$p \leq 0.05$ indicates the level of statistical significance as compared with control.

2.2.4 Determination of ulcerogenic activity by Histopathological examination

A transverse section of the greater curvature of stomach was collected from formalin fixed stomach. Paraffin-embedded tissue sections were prepared at a thickness of 5 µm and stained with hematoxylin and eosin (H & E) for evaluation of cellular structure (Fig. 3). All histological examinations were performed by evaluating one stomach section per animal, using an Olympus microscope (Model BX 04).

2.2.5 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.

3. Result & Discussion

From these data a preliminary SAR can be drawn for synthesized compounds.

A novel series of compounds (**5a-5j**) were synthesized and characterized. The synthesized compounds screened for their in vivo analgesic activity according to the method by Turner [20] and Collier [21] using Swiss albino mice. Some of the synthesized 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**). All derivatives tested significantly suppressed the spontaneous locomotor activity of mice during a 30 min observation period. The most potent effects were produced by derivative **5i** and **5j**. On the contrary, the weakest activity in this test was displayed by **5c**, **5d** & **5f**. The data for compounds, tested for analgesic activity, are presented in **Fig. 1**. From the data presented above, 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). The compounds which showed highly significant analgesic activity i.e. compounds **5a**, **5i** and **5j** are further evaluated for ulcerogenic activity (Table 4 and Figs. 2 and 3).

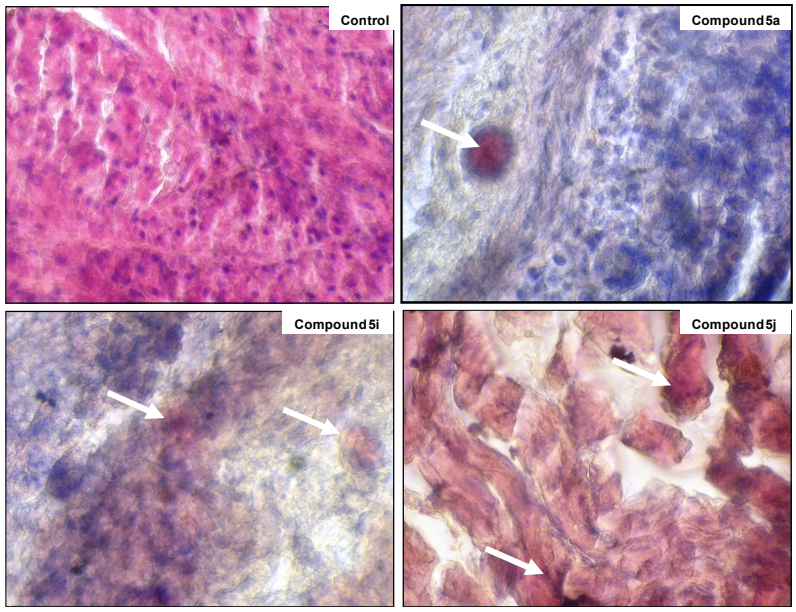


Fig. 3 *Picture showing histopathological examination of the transverse section of the greater curvature of stomach part of rats. The study was conducted on the four groups of rats i.e. Control, Compound 5a, Compound 5i and Compound 5j. Arrow marks are showing the ulcer spots in the compound group 5a, 5i and 5j.*

Control Group (C): As it is clearly visible from the picture that there are very less or almost negligible ulcer spots in the transverse section of stomach of control group of rats. **Compound 5a Group:** The picture is showing the ulcer spots. This group shows the significant difference from control group. **Compound 5i Group:** The picture is showing the ulcer spots. This group shows the significant difference from control group. **Compound 5j Group:** The picture is showing very less number of ulcer spots. This group does not show the significant difference from control group.

4. Conclusion

A new series of Compounds (**5a-5j**) i.e. pyrimidine analogues were synthesized and characterized. The synthesized compounds screened for their *in vivo* analgesic activity. Some of the synthesized compounds viz., **5i** and **5j** have shown highly significant activity and compounds **5a**, **5b** and **5e** exhibited significant analgesic 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 at 20 mg/kg body weights of the animals (Fig. 1). From all the tested compounds, three compounds i.e. **5a**, **5i** and **5j** have been evaluated for ulcerogenic activity and compound **5j** was found to be most promising analgesic agent which is devoid of ulcerogenic effects (Figs. 2 and 3).

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