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SYNTHESIS OF NOVEL PYRIMIDINE DERIVATIVE AND ITS BIOLOGICAL EVALUATION

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abstract: Nitrogen containing heterocyclic ring such as Pyrimidine is a promising structural moiety for drug designing. A series of 6-Bromo-3-(2-morpholino methyl amino)-6-substituted phenyl pyrimidine-4-yl-2H-chromone-2-one(6aM-6jM)&3-(2-((piperidine-1-yl)methylamino)-6-substituted phenylpyrimidin-4-yl)-7-bromo-2H-chromone-2-one (6aP-6jP) have been synthesized from 3-(2-amino-6-pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (5a-5j) which were synthesized from 3-acetyl-6-bromo-2H-chromen-2-one (3). The structures of the synthesized compounds were elucidated by I.R., ^{1}H NMR, ^{13}C NMR spectroscopic techniques. The synthesized compounds were screened for *in vivo* analgesic activity at a dose of 20 mg/kg body weight. Among them, compounds 6aP, 6aM, 6cM, 6iM and 6jM exhibited significant analgesic activity and compounds 6cM, 6iM and 6jM exhibited highly significant activity comparable with standard drug Diclofenac sodium using acetic acid-induced writhing model. Compounds 6aP, 6aM, 6cM, 6iM and 6jM were further evaluated for acute-ulcerogenesis activity. Among them, compound 6cM and 6iM were found to be most promising analgesic agent devoid of ulcerogenic effects.

key words: Analgesic activity; Knoevenagel reaction; Pyrimidines.

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1. Introduction

The investigation of compounds designed to treat both acute and chronic pain is challenging in pharmaceutical research [1], as pain is in fact a very important problem present in more than 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 leading 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

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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 heterocyclic ring such as pyrimidine is a promising structural moiety for drug design. Pyrimidine derivatives form a component in a number of useful drugs and are associated with many biological 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-tumour [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 starting from 3-acetyl-6-bromo-2H-chromen-2-one (Reaction Scheme). The compounds were screened for their *in vivo* analgesic and ulcerogenic activity. Thus, we have created new possibilities to explore the potent heterocyclic moieties for the pharmacological activities in medicinal chemistry.

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 $CDCl_3$ 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 (Schimadzu). The elemental analysis was carried out using Heraus CHN rapid analyzer. The homogeneity of the compounds was determined by thin layer chromatography (TLC) on alumina silica gel using as eluent toluene: ethyl acetate: formic acid" (5:4:1) and benzene: acetone (9:1); the migrated compounds were visualized 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 Table 1 and Table 2.

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 onto crushed ice. The solid separated was filtered, dried and recrystallized from ethanol (**3**). The formation of the compound can be explained on the basis of "Knoevenagel reaction". The purity of the compound was established by 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_6, δ, ppm): 2.58 (s, 3H, CH_3), 7.25-7.98 (m, 4H, 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; Anal. Calcd for $C_{11}H_7BrO_3$ (267.08): C, 70.21; H, 4.29.

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 benzaldehyde were refluxed in absolute ethanol using piperidine as a catalyst for 8-10 hours. The solution mixture was concentrated and poured onto crushed ice. The compound thus obtained was 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_6, 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_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;Anal. Calcd for $C_{18}H_{10}BrClO_3$ (389.63): C, 69.58; H, 3.57.

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_6, 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_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;Anal. Calcd for $C_{18}H_{10}BrClO_3$ (389.63): C, 69.58; H, 3.57.

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_6, 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_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;Anal. Calcd for $C_{18}H_{10}BrClO_3$ (389.63): C, 69.58; H, 3.57.

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_6,

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_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* 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.

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_6, 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_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; Anal. Calcd for $C_{18}H_{10}Br_2O_3$ (434.08): C, 60.87; H, 3.12.

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_6, 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_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; Anal. Calcd for $C_{18}H_{10}Br_2O_3$ (434.08): C, 60.87; H, 3.12.

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_6, 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_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; Anal. Calcd for $C_{19}H_{13}BrO_4$ (385.21): C, 74.50; H, 4.61.

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_6, 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_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* 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_6, 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_6, 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; Anal. Calcd for $C_{18}H_9BrCl_2O_3$ (424.27): C, 62.63; H, 2.92.

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_6, 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_6, 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; Anal. Calcd for $C_{18}H_9BrCl_2O_3$ (424.27): C, 62.63; H, 2.92.

2.1.3 Synthesis of compounds (5a-5j): general procedure

A mixture of compounds **(4a-4j)** (0.01 mole) and guanidine hydrochloride (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 hydrochloride. 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_6, δ, ppm): 4.256 (s, 2H, NH_2), 6.85-7.72 (m, 9H, 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; Anal. Calcd for $C_{19}H_{11}BrClN_3O_2$ (428.67): C, 65.24; H, 3.46; N, 12.01.

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 hydrochloride. 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_6, δ, ppm): 4.25 (s, 2H, NH_2), 6.92-7.36 (m, 9H, 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, 170.9; Anal. Calcd for $C_{19}H_{11}BrClN_3O_2$ (428.67): C, 65.24; H, 3.46; N, 12.01.

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 hydrochloride. 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_6, δ, ppm): 4.25 (s, 2H, NH_2), 7.02-7.50 (m, 9H, 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; Anal. Calcd for $C_{19}H_{11}BrClN_3O_2$ (428.67): C, 65.24; H, 3.46; N, 12.01.

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 hydrochloride. 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_6, δ, ppm): 4.96 (s, 2H, NH_2), 7.25-7.63 (m, 9H, 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 ;Anal. Calcd for $C_{19}H_{11}Br_2N_3O_2$ (473.12): C, 57.89; H, 3.07; N, 10.66.

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 hydrochloride. 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_6, δ, ppm): 4.27 (s, 2H, NH_2), 6.93-7.63 (m, 9H, 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; Anal. Calcd for $C_{19}H_{11}Br_2N_3O_2$ (473.12): C, 57.89; H, 3.07; N, 10.66.

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 hydrochloride**.** 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_6, δ, ppm): 4.16 (s, 2H, NH_2), 6.90-7.73 (m, 9H, 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; Anal. Calcd for $C_{19}H_{11}Br_2N_3O_2$ (473.12): C, 57.89; H, 3.07; N, 10.66.

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 hydrochloride. 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_6, 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_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; Anal. Calcd for $C_{20}H_{14}BrN_3O_3$ (424.25): C, 69.56; H, 4.38; N, 12.17.

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 hydrochloride. 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_6, 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_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; Anal. Calcd for $C_{20}H_{14}BrN_3O_3$ (424.25): C, 69.56; H, 4.38; N, 12.17.

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 hydrochloride. 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_6, 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_6, 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; Anal. Calcd for $C_{19}H_{10}BrCl_2N_3O_2$ (463.11): C, 59.39; H, 2.89; N, 10.94.

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 hydrochloride. 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_6, 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_6, 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; Anal.Calcd for $C_{19}H_{10}BrCl_2N_3O_2$ (463.11): C, 59.39; H, 2.89; N, 10.94.

2.1.4 Synthesis of compounds (6aM-6jM): general procedure

A mixture of compounds **(5a-5j)** (0.01 mole) and morpholine (0.01 mole) & formaldehyde (0.02) was refluxed in ethanol for 6-10 hours. The reaction mixture was reduced to half of its volume and poured onto crushed ice. The product so obtained was washed with water repeatedly, dried and recrystallized from ethanol. The formation of compounds (**6aM-6jM**) can be explained on the basis of "Mannich reaction".

2.1.4.1 Synthesis of 6-Bromo-3-(6-(2-Chlorophenyl)-2-(morpholinomethylamino) pyrimidin-4-yl)-2H-chromen-2-one (6aM): It was obtained from reacting (5a) with morpholine & formaldehyde. IR (KBr, cm^{-1}):3280.30 (N-H), 1706.90 (C=O),1605.16

(C=N),1542.35 (C=C),1130.80 (C-O-C);^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.08(s, 1H, NH),2.50 (t,4H,2 x CH_2), 3.73 (t,4H,2 x CH_2),6.87-7.73 (m, 9H, Ar-H); ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 51.3, 66.7, 72.6, 107.4, 121.4, 122.8, 124.9, 127.6, 128.6, 128.8, 129.2, 129.6, 130.2, 130.4, 132.5, 146.2, 152.6, 160.4, 161.8, 162.1, 165.9; Anal.Calcd for $C_{24}H_{20}BrClN_4O_3$ (526.04): C, 54.62; H, 3.82; N, 10.62.

2.1.4.2 Synthesis of 6-Bromo-3-(6-(3-Chlorophenyl)-2-(morpholinomethylamino) pyrimidin-4-yl)-2H-chromen-2-one (6bM): It was obtained from reacting **(5b)** with morpholine & formaldehyde. IR (KBr, cm^{-1}): 3285.12 (N-H), 1708.88 (C=O),16054.26 (C=N),1542.30 (C=C),1131.50 (C-O-C);^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.09(s, 1H, NH),2.38 (t,4H,2 x CH_2), 3.72 (t,4H,2 x CH_2),6.89-7.74 (m, 9H, Ar-H) ; ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 51.2,66.6,72.5,107.4, 121.5,122.8,125.7,127.6,128.8,129.3, 129.4,129.5,130.8, 134.9,146.3, 152.5,160.3,161.9,162.2,165.8; Anal. Calcd for $C_{24}H_{20}BrClN_4O_3$ (526.04): C, 54.62; H, 3.82; N, 10.62.

2.1.4.3 Synthesis of 6-Bromo-3-(6-(4-Chlorophenyl)-2-(morpholinomethylamino) pyrimidin-4-yl)-2H-chromen-2-one (6cM): It was obtained from reacting **(5c)** with morpholine & formaldehyde. IR (KBr, cm^{-1}): 3287.10 (N-H), 1710.06 (C=O),1604.16 (C=N),1541.45 (C=C),1132.45 (C-O-C);^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.04(s, 1H, NH,2.5 (t,4H,2 x CH_2), 3.72(t,4H,2 x CH_2),6.86-7.73 (m, 9H, Ar-H) ; ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 51.2, 66.7, 72.5, 107.4, 121.5, 122.8, 122.9, 124.9, 128.5, 128.7, 129.5, 131.4, 134.5, 146.3, 152.6, 160.4, 161.8, 162.2, 165.9;Anal.Calcd for $C_{24}H_{20}BrClN_4O_3$ (526.04): C, 54.62; H, 3.82; N, 10.62.

2.1.4.4 Synthesis of 6-Bromo-3-(6-(2-bromophenyl)-2-(morpholinomethylamino) pyrimidin-4-yl)-2H-chromen-2-one (6dM): It was obtained from reacting **(5d)** with morpholine & formaldehyde. IR (KBr, cm^{-1}): 3286.20 (N-H), 1707.80 (C=O),16053.12 (C=N),1540.38 (C=C),1129.70 (C-O-C);^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.02(s, 1H, NH),2.39 (t,4H,2 x CH_2), 3.74 (t,4H,2 x CH_2),6.85-7.73 (m, 9H, Ar-H) ; ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 51.3, 66.6, 72.5, 107.4, 120.5, 121.4, 122.8, 124.9, 128.4, 128.5, 129.2, 129.5, 131.2, 132.4, 139.7, 152.5, 161.8, 162.2, 165.9; Anal.Calcd for $C_{24}H_{20}Br_2N_4O_3$ (569.99): C, 50.37; H, 3.52; N, 9.79.

2.1.4.5 Synthesis of 6-bromo-3-(6-(3-bromophenyl)-2-(morpholinomethylamino) pyrimidin-4-yl)-2H-chromen-2-one (6eM): It was obtained from reacting **(5e)** with morpholine & formaldehyde. IR (KBr, cm^{-1}): 3284.40 (N-H), 1705.80 (C=O), 1606.10(C=N), 1543.30 (C=C), 1131.25 (C-O-C); ^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.03(s, 1H, NH), 2.36 (t, 4H, 2 x CH_2), 3.66(t, 4H, 2 x CH_2), 6.86-7.71 (m, 9H, Ar-H) ; ^{13}CNMR($CDCl_3$-d_6, δ, ppm):51.3, 66.4, 72.5, 107.4, 120.5, 121.5, 122.8, 123.7, 124.9, 126.7, 128.6, 128.6, 129.2, 129.5, 131.7, 133.2, 135.5, 146.2, 152.5, 160.2, 161.8, 165.9; Anal. Calcd for $C_{24}H_{20}Br_2N_4O_3$ (569.99): C, 50.37; H, 3.52; N, 9.79.

2.1.4.6 Synthesis of 6-bromo-3-(6-(4-bromophenyl)-2-(morpholinomethylamino) pyrimidin-4-yl)-2H-chromen-2-one (6fM): It was obtained from reacting **(5f)** with morpholine & formaldehyde. IR (KBr, cm^{-1}): 3282.15 (N-H), 1707.60 (C=O),1605.20 (C=N), 1544.40 (C=C),1128.90 (C-O-C);^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.05(s, 1H, NH), 2.34(t, 4H, 2 x CH_2), 3.63(t, 4H, 2 x CH_2), 6.84-7.71(m, 9H, Ar-H);^{13}CNMR($CDCl_3$-d_6, δ, ppm): 1.3, 66.6, 72.5, 107.4, 121.5, 122.9, 123.3, 124.9, 128.3, 128.5, 129.3, 129.5, 132.2, 132.4, 146.3, 152.5, 160.4, 161.8, 162.2, 165.9; Anal.Calcd for $C_{24}H_{20}Br_2N_4O_3$ (569.99):C, 50.37; H, 3.52; N, 9.79.

2.1.4.7 Synthesis of 6-bromo-3-(6-(2-methoxyphenyl)-2-(morpholinomethylamino) pyrimidin-4-yl)-2H-chromen-2-one (6gM): It was obtained from reacting **(5g)** with morpholine & formaldehyde. IR (KBr, cm^{-1}): 3282.50 (N-H), 1707.60 (C=O), 1604.25 (C=N), 1544.30 (C=C), 1132.50 (C-O-C);^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.10(s, 1H, NH), 2.49(t, 4H, 2 x CH_2), 3.69(t, 4H, 2 x CH_2), 6.94-7.25 (m, 9H, Ar-H) ;^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 51.3, 56.2, 66.7, 72.6, 107.4, 121.5, 121.7, 122.8, 124.9, 128.6, 129.3, 29.5, 146.3, 152.5, 157.6, 160.4, 161.8, 162.2, 165.9; Anal. Calcd for $C_{25}H_{23}BrN_4O_4$(522.09): C, 57.37; H, 4.43; N, 10.70.

2.1.4.8Synthesis of 6-bromo-3-(6-(3-methoxyphenyl)-2-(morpholinomethylamino) pyrimidin-4-yl)-2H-chromen-2-one (6hM): It was obtained from reacting **(5h)** with morpholine & formaldehyde. IR (KBr, cm^{-1}): 3285.20 (N-H), 1708.60 (C=O), 1603.30 (C=N), 1542.60 (C=C), 1129.60 (C-O-C);^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.09(s, 1H, NH), 2.35(t, 4H, 2 x CH_2), 3.73(t, 4H, 2 x CH_2), 6.77-7.25 (m, 9H, Ar-H);^{13}CNMR($CDCl_3$, d_6, δ, ppm):51.3, 55.8, 66.7, 72.5, 107.4, 111.5, 114.5, 119.9, 121.5, 122.9, 124.9, 128.6, 129.2, 129.5, 130.5, 134.3, 146.2, 152.5, 160.4, 161.8, 162.2, 165.9; Anal.Calcd for $C_{25}H_{23}BrN_4O_4$(522.09): C, 57.37; H, 4.43; N, 10.70.

2.1.4.9 Synthesis of 6-bromo-3-(6-(2,4-dichlorophenyl)-2-(morpholinomethylamino) pyrimidin-4-yl)-2H-chromen-2-one (6iM): It was obtained from reacting **(5i)** with morpholine & formaldehyde. IR (KBr, cm^{-1}): 3280.10 (N-H), 1705.50 (C=O), 1605.80 (C=N), 1540.95 (C=C), 1133.45 (C-O-C); ^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.08(s, 1H, NH), 2.49 (t, 4H, 2 x CH_2), 3.70(t, 4H, 2 x CH_2), 6.85-7.73 (m, 8H, Ar-H) ; ^{13}C NMR ($CDCl_3$-d_6, δ, ppm):51.2, 66.7, 72.5, 107.4, 121.5, 122.8, 124.9, 127.6, 128.3, 128.5, 129.2, 129.5, 130.5, 130.8, 133.7, 135.8, 146.3, 152.5, 157.6, 160.4, 161.8, 162.2, 165.9; Anal.Calcd for $C_{24}H_{19}BrCl_2N_4O_3$(560): C, 51.27; H, 4.21; N, 9.96.

2.1.4.10 Synthesis of 6-bromo-3-(6-(2, 6-dichlorophenyl)-2-(morpholinomethylamino) pyrimidin-4-yl)-2H-chromen-2-one (6jM): It was obtained from reacting **(5j)** with morpholine & formaldehyde. IR (KBr, cm^{-1}): 3287.20 (N-H), 1705.80 (C=O), 1610.16 (C=N), 1543.50 (C=C), 1132.40 (C-O-C); ^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.04(s, 1H, NH), 2.35 (t, 4H, 2 x CH_2), 3.65(t, 4H, 2 x CH_2), 6.84-7.73 (m, 8H, Ar-H); ^{13}C NMR($CDCl_3$-d_6, δ, ppm):51.2, 66.7, 72.5, 107.4, 121.5, 122.8, 124.9, 127.5, 127.6, 128.5, 129.3, 129.5, 131.7, 133.9146.3, 152.5, 157.6, 160.4, 162.2, 165.9 ;Anal.Calcd for $C_{24}H_{19}BrCl_2N_4O_3$ (560): C, 51.27; H, 4.21; N, 9.96.

2.1.5 Synthesis of compounds (6aP-6jP): general procedure

A mixture of compounds **(5a-5j)** (0.01 mole) and piperidine (0.01 mole) & formaldehyde (0.02) was refluxed in ethanol for 6-10 hours. The reaction mixture was reduced to half of its volume& Poured on crushed ice. The product so obtained was washed with water repeatedly, dried and recrystallized from ethanol. The formation of compounds (**6aP-6jP**) can be explained on the basis of "Mannich reaction".

2.1.5.1 Synthesis of 3-(2-((piperidin-1-yl)methylamino)-6-(2-chlorophenyl)pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (6aP): It was obtained from reacting **(5a)** with piperidine & formaldehyde. IR (KBr, cm^{-1}):3278.15 (N-H),1708.06 (C=O),1607.25 (C=N),1542.55 (C=C), 1132.40 (C-O-C); ^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.11 (s, 1H, NH), 4.16(s, 2H, CH_2), 1.61(m, 6H, 3 x CH_2), 2.39(t, 4H, 2 x CH_2), 6.82-7.51 (m, 9H, Ar-H) ; ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 25.6, 25.9, 52.11, 72.5, 108.1, 121.4, 122.5, 124.9 , 127.5,

128.6, 129.1, 129.3, 129.5, 129.6, 129.8, 130.1, 130.4, 132.1, 152.5, 160.4, 161.8, 162.3, 165.3; Anal. Calcd for $C_{25}H_{22}BrClN_4O_2$ (526.06): C, 57.10; H, 4.22; N, 10.66.

2.1.5.2 Synthesis of 3-(2-((piperidin-1-yl)methylamino)-6-(3-chlorophenyl)pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (6bP): It was obtained from reacting **(5b)** with piperidine & formaldehyde. IR (KBr, cm^{-1}): 3279.20 (N-H),1707.10 (C=O), 1608.30 (C=N), 1542.85(C=C), 1132.80 (C-O-C);^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.12 (s, 1H, NH), 1.53(m, 6H, 3 x CH_2), 2.27(t, 4H, 2 x CH_2), 6.85-7.73 (m, 9H, Ar-H) ; ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 25.7, 25.6, 26.1, 52.0, 52.1, 72.6, 107.5, 121.5, 122.6, 124.9, 125.9, 128.5, 129.2, 129.4, 130.8, 134.6, 134.9, 146.2, 152.3, 160.4, 161.1, 162.1, 165.9; Anal.Calcd for $C_{25}H_{22}BrClN_4O_2$ (526.06): C, 57.10; H, 4.22; N, 10.66.

2.1.5.3 Synthesis of 3-(2-((piperidin-1-yl)methylamino)-6-(4-chlorophenyl)pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (6cP): It was obtained from reacting **(5c)** with piperidine & formaldehyde. IR (KBr, cm^{-1}): 3276.80 (N-H), 1705.70(C=O), 1610.20(C=N), 1544.32 (C=C), 1131.50 (C-O-C);^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.10 (s, 1H, NH), 1.61(m, 6H, 3 x CH_2), 2.26(t, 4H, 2 x CH_2), 6.86-7.74 (m, 9H, Ar-H) ; ^{13}C NMR ($CDCl_3$-d_6, δ, ppm): 25.8, 25.9, 52.2, 72.7, 107.4, 122.8, 124.9, 128.6, 129.2, 129.5, 131.4, 134.5, 146.2, 152.6, 160.4, 161.9, 162.2, 165.9; Anal. Calcd for $C_{25}H_{22}BrClN_4O_2$ (525.82): C, 57.14; H, 4.24; N, 10.69.

2.1.5.4 Synthesis of 3-(2-((piperidin-1-yl)methylamino)-6-(2-bromophenyl)pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (6dP): It was obtained from reacting **(5d)** with piperidine & formaldehyde. IR (KBr, cm^{-1}): 3279.15 (N-H), 1707.26 (C=O), 1608.35 (C=N), 1541.50 (C=C), 1130.49 (C-O-C);^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.12 (s, 1H, NH), 1.56(m, 6H, 3 x CH_2), 2.38(t, 4H, 2 x CH_2), 6.90-7.25 (m, 9H, Ar-H) ; ^{13}C NMR ($CDCl_3$-d_6, δ, ppm):25.7, 25.1, 52.1, 72.6, 107.4, 120.4, 121.5, 122.8, 124.7, 128.2, 128.6, 129.1, 129.6, 131.2, 132.4, 139.9, 146.3, 152.5, 160.3, 161.8, 162.1, 165.7; Anal.Calcd for $C_{24}H_{22}Br_2N_4O_2$ (570.01): C, 52.65; H, 3.89; N, 9.82.

2.1.5.5 Synthesis of 3-(2-((piperidin-1-yl)methylamino)-6-(3-bromophenyl)pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (6eP): It was obtained from reacting **(5e)** with piperidine & formaldehyde. IR (KBr, cm^{-1}): 3278.85 (N-H), 1708.86(C=O), 1606.30(C=N), 1543.15 (C=C), 1130.90 (C-O-C);^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.11 (s, 1H, NH), 1.53(m, 6H, 3 x CH_2), 2.25(t, 4H, 2xCH_2), 6.85-7.74(m, 9H, Ar-H); ^{13}CNMR ($CDCl_3d_6$, δ, ppm): 25.7, 25.8, 52.2, 107.3, 121.4, 122.6, 124.9, 126.6, 128.5, 129.5, 131.6, 131.8, 133.2, 135.5, 146.2, 152.6, 160.4, 161.9, 162.3, 165.8; Anal.Calcd for $C_{25}H_{22}Br_2N_4O_2$ (570.01): C, 52.65; H, 3.89; N, 9.82.

2.1.5.6 Synthesis of 3-(2-((piperidin-1-yl)methylamino)-6-(4-bromophenyl)pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (6fP): It was obtained from reacting **(5f)** with piperidine & formaldehyde. IR (KBr, cm^{-1}): 3280.15 (N-H), 1708.60(C=O), 1606.85(C=N), 1542.35 (C=C), 1132.60 (C-O-C);^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.08(s, 1H, NH), 1.54(m, 6H, 3xCH_2), 2.40(t, 4H, 2xCH_2), 6.89-7.76 (m, 9H, Ar-H);^{13}CNMR($CDCl_3$-d_6, δ, ppm): 25.7, 25.8, 52.1, 72.6, 107.4, 121.5, 122.9, 123.3, 124.9, 128.6, 129.2, 129.5, 129.5, 129.8, 132.2, 132.4, 146.2, 152.5, 160.4, 161.8, 162.2, 165.7; Anal.Calcd for $C_{25}H_{22}Br_2N_4O_2$ (570.01): C, 52.65; H, 3.89; N, 9.82.

2.1.5.7 Synthesis of 3-(2-((piperidin-1-yl)methylamino)-6-(2-methoxyphenyl)pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (6gP): It was obtained from reacting **(5g)** with piperidine & formaldehyde. IR (KBr, cm^{-1}): 3276.95(N-), 1708.45(C=O), 1609.25(C=N),

1543.55(C=C), 1132.42(C-O-C);^{1}HNMR($CDCl_3$ d_6, δ, ppm): 4.09 (s, 1H, NH), 1.54(m, 6H, 3xCH_2), 2.38(t, 4H, 2xCH_2), 6.90-7.26(m, 9H, Ar-H);^{13}CNMR($CDCl_3$-d_6, δ, ppm): 25.7, 25.8, 52.2, 56.4, 72.5, 107.3, 114.9, 119.3, 121.4, 121.7, 122.8, 124.9, 128.6, 128.7, 129.2, 129.6, 129.9, 46.2, 152.6, 157.6, 160.4, 161.8, 165.9; Anal.Calcd for $C_{26}H_{25}BrN_4O_3$ (521.41): C, 59.89; H, 4.89; N, 10.75.

2.1.5.8 Synthesis of 3-(2-((piperidin-1-yl)methylamino)-6-(3-methoxyphenyl) pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (6hP): It was obtained from reacting **(5h)** with piperidine & formaldehyde. IR (KBr, cm^{-1}): 3278.90 (N-H), 1709.08(C=O), 1607.30(C=N), 1542.95(C=C), 1131.40 (C-O-C);^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.10(s, 1H, NH),1.53(m,6H,3xCH_2), 2.26(t, 4H, 2xCH_2), 6.88-7.7 (m, 9H, Ar-H);^{13}CNMR($CDCl_3$-d_6, δ, ppm): 25.7, 25.8, 52.8, 55.8, 72.5, 107.5, 114.5, 119.7, 121.5, 122.8, 124.9, 128.5, 129.2, 129.6, 130.4, 134.2, 146.2, 152.6, 160.4, 161.3, 162.2, 165.9; Anal.Calcd for $C_{26}H_{25}BrN_4O_3$ (521.41): C, 59.92; H, 4.85; N, 10.77.

2.1.4.9 Synthesis of 3-(2-((piperidin-1-yl)methylamino)-6-(2,4-dichlorophenyl) pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (6ip): It was obtained from reacting **(5i)** with piperidine & formaldehyde. IR (KBr, cm^{-1}): 3275.15 (N-H), 1705.06(C=O), 1607.55(C=N), 1542.25(C=C), 1134.60(C-O-C); ^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.13 (s, 1H, NH), 1.53(m, 6H, 3 x CH_2), 2.26(t, 4H, 2xCH_2), 6.82-7.74(m, 8H, Ar-H);^{13}CNMR($CDCl_3d_6$, δ, ppm): 25.6, 25.8, 52.8, 72.5, 107.4, 107.4, 121.3, 122.9, 124.9, 127.7, 128.3, 129.1, 129.5, 130.7, 133.6, 135.9, 152.5, 162.3, 161.7, 165.9;Anal.Calcd for $C_{25}H_{21}BrCl_2N_4O_2$ (521.41): C, 53.59; H, 3.78; N, 10.00.

2.1.4.10 Synthesis of 3-(2-((piperidin-1-yl)methylamino)-6-(2,6-dichlorophenyl) pyrimidin-4-yl)-6-bromo-2H-chromen-2-one (6jP): It was obtained from reacting **(5j)** with piperidine & formaldehyde. IR (KBr, cm^{-1}): 3277.95 (N-H), 1710.09 (C=O), 1610.25 (C=N), 1542.75 (C=C), 1132.24 (C-O-C);^{1}HNMR ($CDCl_3$-d_6, δ, ppm): 4.21 (s, 1H, NH), 1.51(m, 6H, 3 x CH_2), 2.21(t, 4H, 2 x CH_2), 6.85-7.81 (m, 8H, Ar-H);^{13}CNMR($CDCl_3$, d_6, δ, ppm): 25.5, 25.7, 52.3, 72.6, 107.1, 121.3, 122.9, 124.9, 127.5, 127.5, 127.9, 128.6, 129.3, 133.6, 146.9, 152.1, 162.8, 161.7, 160.7, 165.5; Anal.Calcd for $C_{25}H_{21}BrCl_2N_4O_2$ (521.41): C, 53.59; H, 3.78; N, 10.00.

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].

One hundred forty two mice were selected and divided into 22 groups (six in each group), starved for 16 h and pre-treated as follows. The first group which served as positive control orally received distilled water in appropriate volumes. The second to eleventh groups received the aqueous suspension of synthesized compounds (**6aM-6jM, 6aP-6jP**) orally in a dose of 20 mg/kg. The last group received orally Diclofenac sodium in a dose of 20 mg/kg. After 30 min, each mouse was administered 1% of an aqueous solution of acetic acid (10 mL/kg) and the mice were subsequently 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 5 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:

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

2.2.3 Acute-ulcerogenesis activity

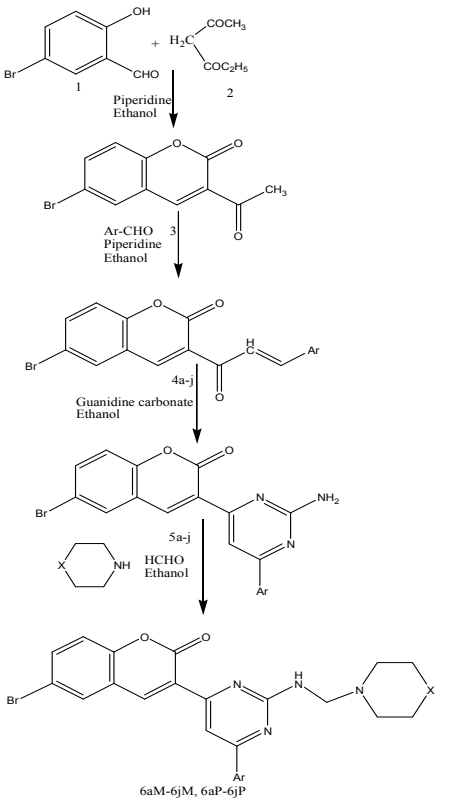
Acute ulcerogenesis test was done according to Cioli et al. [22]. Albino rats (150–200 g) were divided into different groups consisting of six animals in each group. Ulcerogenic activity was evaluated after p.o. administration of test compounds or standard drug at the dose of 60 mg/kg. Control rats received 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 6 and Fig. 2 and 3.

2.2.4 Determination of ulcerogenic activity by histological 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.



Scheme: *Schematic diagrams for the synthesis of pyrimidine derivatives (6aM-6jM, 6aP-6jP).*

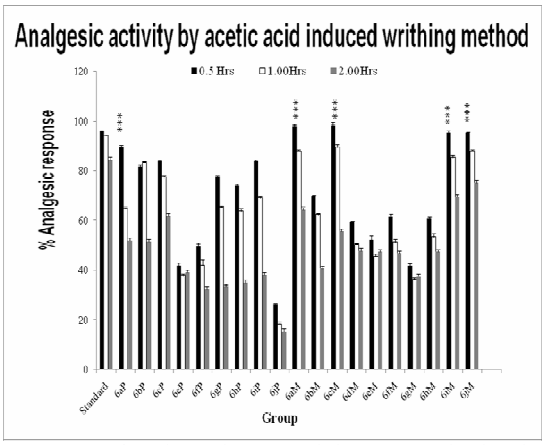


Fig. 1 *Analgesic responses of synthesized compounds by acetic acid induced writhing method. Values were expressed as Mean ± SEM and $p \leq 0.001$ indicates the level of statistical significance as compared with control.*

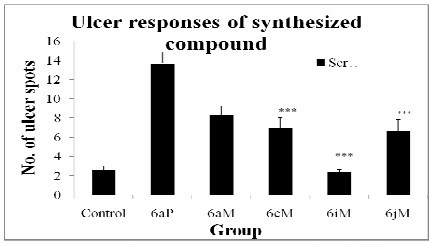


Fig. 2 *Values were expressed as mean ± SEM and and ***p ≤ 0.001 indicates the level of statistical significance as compared with control.*

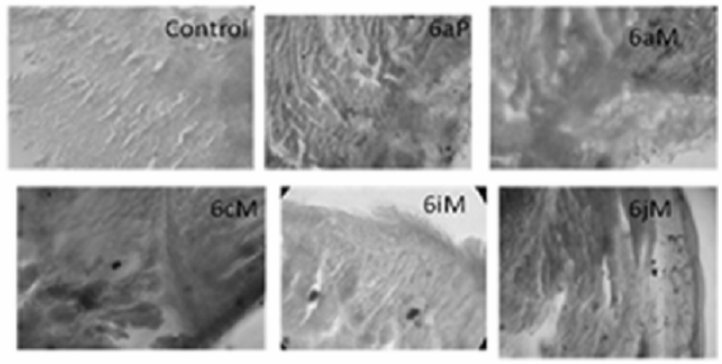
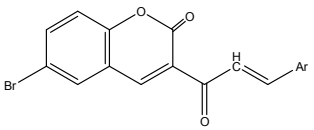


Fig. 3 *Picture showing histopathological examination of the transverse section of the greater curvature of stomach part of rats. The study was conducted on the six groups of rats i.e. control, compound 6aP, compound 6aM ,6cM, 6iM and compound 6jM*

Control Group (C): As it is clearly visible from the picture that there are very few or almost negligible ulcer spots in the transverse section of stomach of control group of rats. **Compound 6aP Group:** The picture highlights the ulcer spots. This group shows the significant difference from control group. **Compound 6aM Group:** The picture is showing the ulcer spots. This group shows the significant difference from control group. **Compound 6cM Group:** The picture is showing the ulcer spots. This group shows the significant difference from control group **Compound 6iM Group:** The picture is showing very less number of ulcer spots. This group does not show the significant difference from control group. **Compound 6jM Group:** The picture is showing the ulcer spots. This group shows the significant difference from control group

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

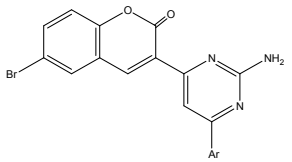


Compound [a]	-Ar	Yield (%) [b]	m.p. (°C) [c]	Rf value	Molecular Formula
4a		65	162-165	0.73	$C_{18}H_{10}BrClO_3$
4b		70	165-167	0.75	$C_{18}H_{10}BrClO_3$
4c		60	156-158	0.71	$C_{18}H_{10}BrClO_3$

4d		70	190-192	0.77	$C_{18}H_{10}Br_2O_3$
4e		75	185-187	0.76	$C_{18}H_{10}Br_2O_3$
4f		*75*	*185-188*	*0.69*	*$C_{18}H_{10}Br_2O_3$*
4g		65	180-182	0.64	$C_{19}H_{13}BrO_4$
4h		65	173-175	0.69	$C_{19}H_{13}BrO_4$
4i		70	175-177	0.71	$C_{18}H_9BrCl_2O_3$
4j		68	180-183	0.79	$C_{18}H_9BrCl_2O_3$

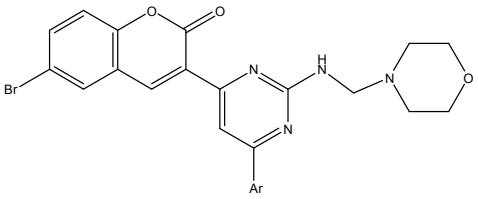
[a]Products were characterized by IR, NMR, MS and elemental analysis. [b]Synthesized yields. [c]M. p. are uncorrected.

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



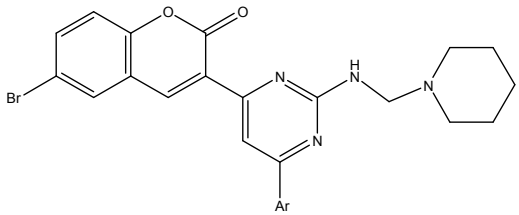
Compound[a]	-Ar	Yield (%)[b]	m.p. (°C)[c]	Rf value	Molecular Formula
5a		65	162-165	0.62	$C_{19}H_{11}BrClN_3O_2$
5b		60	165-167	0.74	$C_{19}H_{11}BrClN_3O_2$
5c		70	156-158	0.70	$C_{19}H_{11}BrClN_3O_2$
5d		65	190-192	0.75	$C_{19}H_{11}Br_2N_3O_2$
5e		50	185-187	0.72	$C_{19}H_{11}Br_2N_3O_2$
5f		60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

Table 3 Physical parameters of compounds (6aM-6jM).



Compound [a]	-Ar	Yield (%)[b]	m.p. (°C) [c]	Rf value	Molecular Formula
6aM		55.4	176-178 °C	0.76	$C_{24}H_{20}BrClN_4O_3$
6bM		60.9	175-177 °C	0.74	$C_{24}H_{20}BrClN_4O_3$
6cM		60.7	177-179 °C	0.66	$C_{24}H_{20}BrClN_4O_3$
6dM		65.1	180-182 °C	0.73	$C_{24}H_{20}Br_2N_4O_3$
6eM		50.9	175-178 °C	0.72	$C_{24}H_{20}Br_2N_4O_3$
6fM		60.5	178-180 °C	0.68	$C_{24}H_{20}Br_2N_4O_3$
6gM		65.3	177-179 °C	0.67	$C_{25}H_{23}BrN_4O_4$
6hM		65.5	173-175 °C	0.65	$C_{25}H_{23}BrN_4O_4$
6iM		60.3	175-177 °C	0.71	$C_{24}H_{19}BrCl_2N_4O_3$
6jM		55.1	171-173 °C	0.66	$C_{24}H_{19}BrCl_2N_4O_3$

Table 4 Physical parameters of compounds (6aP-6jP).



Compound [a]	-Ar	Yield (%)[b]	m.p. (°C) [c]	Rf value	Molecular Formula
6aP		50.4	168-170	0.72	$C_{25}H_{22}BrClN_4O_2$
6bP		60.7	170-172	0.73	$C_{25}H_{22}BrClN_4O_2$
6cP		60.5	176-178°C	0.66	$C_{25}H_{22}BrClN_4O_2$
6dP		65.3	172-174	0.73	$C_{25}H_{22}Br_2N_4O_2$
6eP		50.7	175-178	0.72	$C_{25}H_{22}Br_2N_4O_2$
6fP		60.5	178-180	0.68	$C_{25}H_{22}Br_2N_4O_2$
6gP		65.3	177-179	0.67	$C_{26}H_{25}BrN_4O_3$
6hP		65.8	173-175	0.65	$C_{26}H_{25}BrN_4O_3$
6iP		60.9	169-171°C	0.71	$C_{25}H_{21}BrCl_2N_4O_2$
6jP		55.6	187-189	0.66	$C_{25}H_{21}BrCl_2N_4O_2$

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

Table 5 Analgesic activity of compounds (6aP-6aj, 6aM-6jM) determined by acetic acid induced writhing response model.

Compounds Tested	Percent Protection		
	0.5 hrs	1 hrs	2 hrs
Diclofenac Sodium	95.87 ± 0.33	94.25 ± 0.31	84.53 ± 0.37
6aP	89.64 ± 0.61***	84.83 ± 0.47***	52.06 ± 0.76***
6bP	81.87 ± 0.48	83.25 ± 0.62	51.53 ± 0.21
6cP	83.94 ± 0.31	77.47 ± 0.95	61.86 ± 0.61
6dP	55.96 ± 0.98	40.31 ± 0.86	15.47 ± 1.23
6eP	41.97 ± 1.02**	37.70 ± 2.31	39.16 ± 1.96
6fP	49.74 ± 0.48	41.88 ± 0.67	32.47 ± 0.79
6gP	77.72 ± 0.48	65.44 ± 0.93	33.50 ± 0.61
6hP	74.09 ± 0.42	63.87 ± 0.85	35.05 ± 1.63
6iP	83.94 ± 0.48	69.12 ± 0.70	38.14 ± 1.69
6jP	26.43 ± 0.91	18.32 ± 0.58	15.47 ± 0.76
6aM	97.93 ± 0.21***	87.97 ± 0.60***	64.43 ± 1.28
6bM	69.95 ± 1.17	62.30 ± 0.93	40.71 ± 1.60
6cM	98.45 ± 0.22***	89.54 ± 0.61***	55.68 ± 1.33
6dM	59.59 ± 1.59	50.27 ± 1.25	47.94 ± 1.85
6eM	52.33 ± 0.88	45.55 ± 1.17	47.42 ± 2.00
6fM	61.66 ± 0.91	51.30 ± 1.02	46.89 ± 1.14
6gM	41.97 ± 1.02	36.13 ± 1.31	37.61 ± 0.95
6hM	60.62 ± 0.96	53.41 ± 0.95	47.42 ± 0.89
6iM	95.34 ± 0.43***	85.36 ± 0.56***	69.60 ± 1.30***
6jM	95.34 ± 0.43***	87.96 ± 0.48***	75.26 ± 0.37***

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.

Table 6 Ulcerogenic response of the various groups of compounds tested on the stomach of rats.

S. No.	Groups	No. of Ulcer spots
1.	Control Group	2.67 ± 0.33
2.	6aP	13.67 ± 1.20***
3.	6aM	8.33 ± 0.88***
4.	6cM	7.00 ± 1.00***
5.	6iM	2.33 ± 0.33
6.	6jM	6.67 ± 1.20

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

3. Results and discussion

From these data a preliminary SAR can be drawn for synthesized compounds. A novel series of compounds (**6aM-6jM,6aP-6jP**) 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, **6aP, 6aM, 6cM, 6iM,** and **6jM** exhibited significant analgesic activity, while compounds **6iM** and **6jM** 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, 1 hour and 2 hours observation period. The most potent effects were produced by derivative **6aP**, **6aM**, **6cM**, **6iM** and **6jM**. On the contrary, the weakest activity was displayed by **6eP**, **6jP** and **6gM**. From the data presented above, it can be concluded that the most active substance in the acetic acid induced writhing method is 6-bromo-3-(6-(4-chlorophenyl)-2-(morpholinomethylamino)pyrimidin-4-yl)-2H-chromen-2-one (**6cM**). Modification of chloro- from 2 position (as in compound 6aM) to position 2, 4 and 6 (as in compound **6iM, 6jM)** as well as some piperidine derivative such as 6aP also produced a potent analgesic compound (Table 5 and Fig. 1). The compounds which showed highly significant analgesic activity i.e. compounds **6aP, 6aM, 6cM, 6iM** and **6jM** were further evaluated for ulcerogenic activity (Table 6 and Figs. 2 and 3).

Conclusion

A new series of compounds (**6aP-6jP and 6aM-6jM**) i.e. pyrimidine analogues were synthesized and characterized. The synthesized compounds were screened for their *in vivo* analgesic activity. Some of the synthesized compounds viz., **6aP**, **6aM, 6cM, 6iM** and **6jM** exhibited significant analgesic activity and compounds **6cM, 6iM** and **6jM** 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 at 20 mg/kg body weights of the animals (Fig. 1). From all the tested compounds, five compounds i.e. **6aP**, **6aM, 6cM, 6iM** and **6jM** have been evaluated for ulcerogenic activity and compound **6iM** was found to be most promising analgesic agent which is devoid of ulcerogenic effects (Figs. 2 and 3).

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