Synthesis and Characterization of Some Heteroaromatic Derivatives of 3-But-2-enoyl-chromen-2-one and Their Potential as Anti-inflammatory Agents

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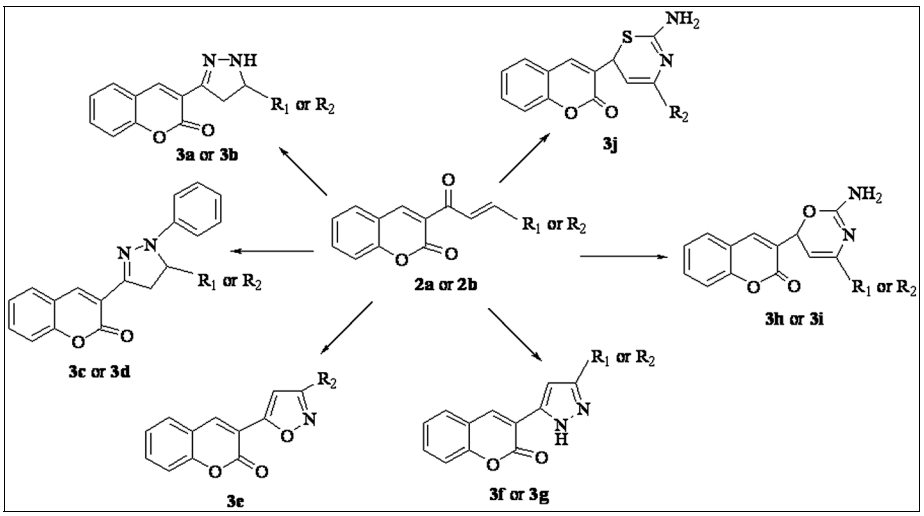

A novel series of chromen-2-ones containing pyrazole, isoxazole, oxazine, and thiazine substitutions have been synthesized by reacting 3-[3-(4-chloro-phenyl)-acryloyl]-chromen-2-one and 3-[3-(3-methoxy-phenyl)-acryloyl]-chromen-2-one with various cyclizing agents such as hydrazine, phenylhydrazine, urea, and thiourea. The structures of all the synthesized compounds were confirmed by the use of IR, ^{1}H-NMR, mass spectroscopy, and elemental analysis data. All the newly synthesized compounds were evaluated for their anti-inflammatory activity at a dose of 100 mg/kg body weight in carrageenan-induced rat paw edema model. The entire series of the compounds exhibited moderate to good anti-inflammatory activity, with the percentage inhibition of edema formation ranging from 39.99 to 63.15 against the reference drug ibuprofen (100 mg/kg) that showed 78.96% inhibition at the third hour. Compounds **3a**, **3c**, and **3d** showed good inhibitory activity, whereas compounds **3b**, **3e**, **3f**, and **3j** showed moderate inhibitory activity at the third hour.

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INTRODUCTION

Inflammation is a complex phenomenon involving humoral and cellular reactions through a number of inflammatory mediators [1]. The classical nonsteroidal anti-inflammatory drugs (NSAIDs) are important therapeutic agents widely used in the treatment of pain and inflammation [2,3]. The existence of the enzyme cyclooxygenase (COX) in its two distinct isoforms, and thus nonselective action of classical NSAIDs, results in certain mechanism-based side effects including dyspepsia, gastrointestinal ulcerations, bleeding, and nephrotoxicity [4,5]. Therefore, development of novel compounds having anti-inflammatory and analgesic activities with an improved safety profile is still a necessity. Thus, the discovery of novel anti-inflammatory agents has been attracting a lot of interest [6,7]. Chromen-2-ones (Coumarins) have been reported to possess anti-inflammatory, anticancer, antimicrobial activities, and antioxidant properties [8–14]. Coumarin and its hydroxy-derivative can reduce tissue edema and inflammation by inhibiting prostaglandin biosynthesis, which involves fatty acid hydroperoxy intermediates. Natural products such as esculetin, fraxetin, daphnetin, and other related coumarin derivatives are recognized as inhibitors not only of the lipoxygenase and cycloxygenase enzymic systems, but also of the neutrophil-dependent superoxide anion generation [15]. Pyrazole derivatives such as phenylbutazone, oxyphenbutazone, and celecoxib exhibit anti-inflammatory, antipyretic, and analgesic properties [16]. 3,4-Diarylisoxazole scaffold is one of the frequently found pharmacophore in a wide variety of NSAIDs (such as Valdecoxib), protein kinase

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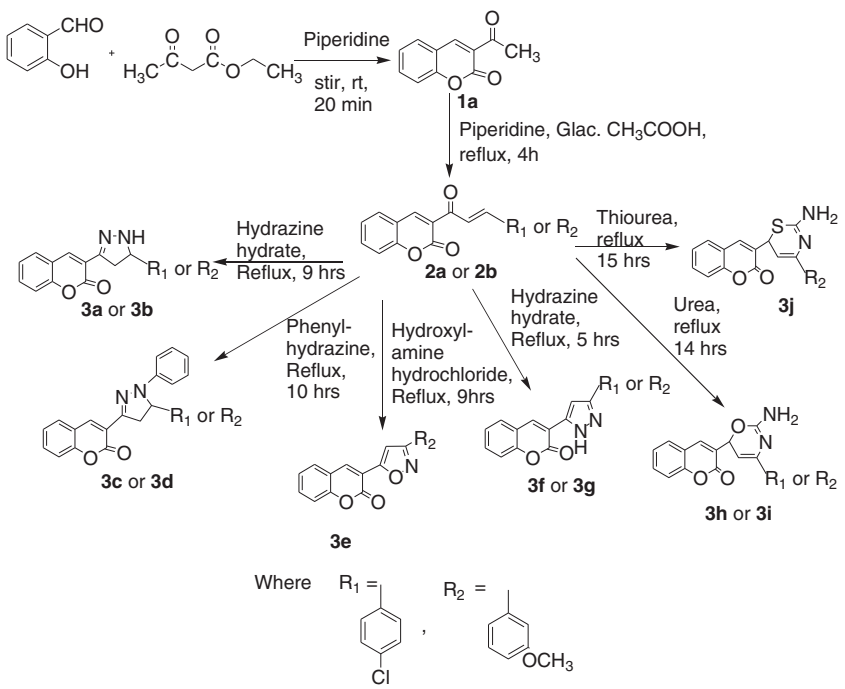
inhibitors, and antihypertensive agents. The phenysulphonamide group located at the *para*-position is known to interact effectively with the COX-II side pocket through slow tight-binding kinetics [5]. 1,3-Oxazine ring with a wide range of substituents, such as 1,3-oxazinanes, are being explored as anti-inflammatory agents and agents for treating ulcers, allergies, asthma, arthritis, and diabetes. 1,3-Oxazine ring system also occur in some natural antibiotics [17]. In view of these observations, it was considered appropriate to synthesize some new chemical entities, incorporating the two active pharmacophores in a single molecular framework and to evaluate their anti-inflammatory activity [18].

RESULTS AND DISCUSSION

In the first step, 3-acetylcoumarin (**1a**) was synthesized by Knoevenagel reaction by reacting salicylaldehyde with ethylacetoacetate in the presence of catalytic quantity of piperidine at room temperature [6]. Acryloyl-chromen-2-ones (**2a–b**) were synthesized by the reaction of 3-acetylcoumarin with aromatic aldehydes (4-chloro and 3-methoxybenzaldehyde), in the second step of the reaction, which is an example of Claisen–Schmidt condensation reaction. Refluxing of acryloyl-chromen-2-ones such as 3-[3-(4-chloro-phenyl)-acryloyl]-chromen-2-one (**2a**) and 3-[3-(3-methoxy-phenyl)-acryloyl]-chromen-2-one (**2b**), with hydrazine hydrate, phenylhydrazine, urea, and thiourea resulted in the synthesis of pyrazole, isoxazole, oxazine, and thiazine substituted chromen-2-ones in the third step of the reaction [18–20]. A total of 10 compounds (**3a–j**) were synthesized as analogs of chromen-2-one. The synthesis of the new compounds was carried out as outlined in Scheme 1.

All the newly synthesized compounds were evaluated for their anti-inflammatory activity, at a dose of 100 mg/kg body weight (b.w.), using carrageenan-induced rat paw edema model by Winter *et al*. [21]. This model reliably predicts the anti-inflammatory efficacy of the NSAIDs, and during the second phase, it detects the compounds that act as anti-inflammatory agents as a result of inhibition of prostaglandin amplification [22]. The entire series of investigated compounds exhibited moderate to good anti-inflammatory activity, with the percentage inhibition of edema formation ranging from 39.99 to 63.15, against the reference drug ibuprofen (100 mg/kg) that showed a 78.96% inhibition at third hour (Figure 1). Compounds **3c** and **3d** were found to be the most active compounds in the series and showed good inhibitory activity, whereas compounds **3a**, **3b**, **3e**, **3f**, and **3j** showed moderate inhibitory activity. The anti-inflammatory activity of these compounds was comparable with that of the standard drug ibuprofen, although not equal (Tables 1 and 2). The synthesized derivatives of chromen-2-one may be free from the gastrointestinal side effects, as they do not possess any free carboxylic group.

Scheme 1. Synthesis of chromen-2-one derivatives.



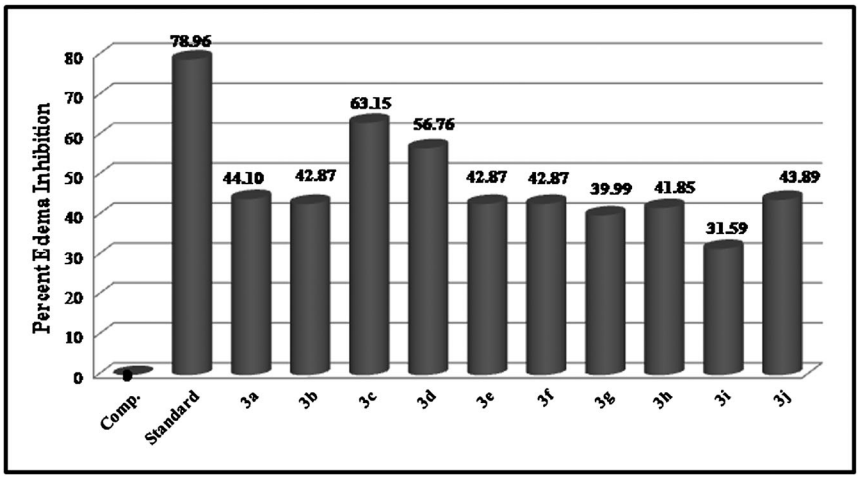


Figure 1. Percent edema inhibition at third hour after treatment with chromen-2-one analogs.

Table 1

Mean paw volume measured after treatment with chromen-2-one analogs (**3a–j**).

Compounds	Dose (mg/kg b.w.)	Mean paw volume ± SEM			
		1 h	3 h	5 h	7 h
Control	0.5% CMC	0.486 ± 0.009	0.522 ± 0.012	0.522 ± 0.015	0.558 ± 0.012
Ibuprofen	100	0.380 ± 0.009	0.282 ± 0.009	0.254 ± 0.009	0.240 ± 0.009
3a	100	0.344 ± 0.005	0.290 ± 0.005	0.254 ± 0.005	0.242 ± 0.007
3b	100	0.420 ± 0.008	0.369 ± 0.012	0.342 ± 0.009	0.334 ± 0.007
3c	100	0.368 ± 0.007	0.302 ± 0.006	0.266 ± 0.007	0.244 ± 0.008
3d	100	0.382 ± 0.004	0.298 ± 0.005	0.264 ± 0.008	0.252 ± 0.008
3e	100	0.348 ± 0.004	0.300 ± 0.005	0.276 ± 0.005	0.268 ± 0.006
3f	100	0.354 ± 0.10	0.302 ± 0.008	0.278 ± 0.009	0.258 ± 0.010
3g	100	0.448 ± 0.009	0.386 ± 0.010	0.360 ± 0.010	0.344 ± 0.008
3h	100	0.428 ± 0.008	0.370 ± 0.008	0.342 ± 0.010	0.344 ± 0.008
3i	100	0.338 ± 0.014	0.288 ± 0.015	0.270 ± 0.017	0.202 ± 0.018
3j	100	0.444 ± 0.017	0.384 ± 0.017	0.354 ± 0.019	0.336 ± 0.017

Values are expressed as mean ± SEM, $n = 5$, $P < 0.01$ compared with vehicle treated group using one-way analysis of variance followed by Dunnett's test. b.w., body weight; CMC, carboxymethyl cellulose.

Table 2

Percent edema inhibition after treatment with chromen-2-one analogs (**3a–j**).

Compounds	Dose (mg/kg b.w.)	Anti-inflammatory activity (% edema inhibition)			
		1 h	3 h	5 h	7 h
Control	0.5% CMC				
Ibuprofen	100	−45.45	78.96	32.13	12.00
3a	100	−18.75	44.10	41.37	12.00
3b	100	−40.53	42.87	5.55	3.03
3c	100	−56.25	63.15	39.99	11.52
3d	100	−36.36	56.76	30.00	22.23
3e	100	−38.70	42.87	20.01	11.10
3f	100	−28.14	42.87	20.01	21.42
3g	100	−29.28	39.99	23.07	16.68
3h	100	−39.48	41.85	24.33	8.82
3i	100	−80.01	31.59	17.64	9.39
3j	100	−21.93	43.89	23.67	8.55

Values are expressed as mean ± SEM, $n = 5$, $P < 0.01$ compared with vehicle treated group using one-way analysis of variance followed by Dunnett's test. b.w., body weight; CMC, carboxymethyl cellulose.

CONCLUSIONS

From the results, it has been concluded that

- Although considering all the newly synthesized compounds of the series together, the presence of chlorine and methoxy groups in the aromatic ring at the fifth-position of the pyrazoline nucleus gave rise to an increase in the anti-inflammatory activity.
- The bulkier substituents while increasing lipophilicity gave better anti-inflammatory activity.
- The results obtained expound that the compounds substituted with pyrazoline nucleus **3a**, **3b**, **3c**, and **3d** exhibited the maximum per cent inhibition of edema. This also confirmed that the compound **3c** and **3d**, substituted with *N*-phenyl pyrazoline at third position of chromen-2-one, gave rise to increased anti-inflammatory activity.
- Compound **3e** substituted with 3-methoxy-phenyl and isoxazole showed more activity than the compound **3g** substituted with 3-methoxy-phenyl and pyrazole.
- The synthesized derivatives of chromen-2-one may be free from the gastrointestinal side effects, as they do not possess any free carboxylic group.
- The synthesized derivatives of chromen-2-one may act as selective COX-2 inhibitors, as carrageenan-induced rat paw edema model predicts the anti-inflammatory activity of the NSAIDs during the second phase by detecting the result of inhibition of prostaglandins.

EXPERIMENTAL

The chemicals and reagents were procured from S. D. Fine Chemicals, Mumbai, India and were used as such. Melting points were determined by open capillary method and are uncorrected. Progress of the reactions was monitored by TLC on precoated silica gel G plates, using iodine vapors and UV chamber as visualizing agents. IR spectra were recorded on Shimadzu 8400S and Perkin Elmer RX1 FTIR spectrophotometers, and values are expressed in cm^{-1}. ^{1}H-NMR spectra were recorded on Bruker DRX-300 (at 300 MHz) spectrometer, and chemical shifts are reported in parts per million (δ value), taking TMS (δ 0 ppm for ^{1}H-NMR) as an internal standard. Mass spectra were recorded on a JEOL-AccuTOF JMS-T100LC mass spectrometer instrument using ESI technique. Elemental analysis was performed on an Elemental Vario EL III analyzer.

General procedure for the synthesis of 3-acetylcoumarin (1a). Salicyladehyde (0.2 M, 20.94 mL), ethylacetoacetate (0.2 M, 25.32 mL), and a few drops (2 mL) of piperidine were mixed at room temperature. A small magnetic stirrer bead was introduced, and the contents were stirred vigorously until a viscous solution was formed (usually in 30 min). The flask was removed from the stirrer and the yellowish solid filtered off. The filter cake (precipitate) was washed with distilled water. The product was recrystallized from absolute ethanol to obtain a yellow crystalline solid. The progress of the reaction was monitored by TLC, using chloroform and *n*-hexane (1:9) as the solvent system [6].

State: Solid; Color: Light yellow; Yield: 85.10%; Melting Range: 70–72°C; IR (KBr, cm^{-1}): 1739.67 (Cyclic C═O stretching), 1677.95 (C═O stretching), 1614.31 (C═C stretching); MS *m/z* (M + 1): 189.03.

General procedure for the synthesis of acryloyl-chromen-2-ones (2a–b). 3-Acetylcoumarin (0.01 M, 1.88 g) and 4-chlorobenzaldehyde (0.012 M, 1.69 g) or 3-methoxybenzaldehyde (0.012 M, 1.63 mL) were dissolved in 10 mL of *n*-butanol with heating, and glacial acetic acid (0.3 mL) and piperidine (0.3 mL) were added. The reaction mixture was refluxed for 7–8 h, and then the solvent was removed under vacuum. The residue was triturated with 10 mL of ethanol until a precipitate was formed. The precipitate was filtered off, recrystallized from acetone, and re-precipitated with ethanol. The progress of the reaction was monitored by TLC using chloroform and *n*-hexane (2:9) as the solvent system.

3-[3-(4-Chloro-phenyl)-acryloyl]-chromen-2-one (2a). State: Solid; Color: Dark yellow; Yield: 65.55%; Melting Range: 110–112°C; IR (KBr, cm^{-1}): 1718.46 (Cyclic C═O stretching), 1608.52 (C═O stretching), 1560.30 (C═C stretching); MS *m/z* (M + 1): 311.06.

3-[3-(3-Methoxy-phenyl)-acryloyl]-chromen-2-one (2b). State: Solid; Color: Dark yellow; Yield: 60.35%; Melting Range: 92–94°C; IR (KBr, cm^{-1}): 1722.31 (Cyclic C═O stretching), 1604.66 (C═O stretching), 1487.01(C═C stretching); MS *m/z* (M + 1): 307.12.

General procedure for the synthesis of chromen-2-one analogs of pyrazoline (3a–d). To a solution of **2a** (0.01 M, 3.10 g)/**2b** (0.01 M, 3.06 g) with hydrazine hydrate (0.01 M, 0.49 mL) or phenylhydrazine (0.01 M, 0.99 mL) in 20 mL of ethanol, alcoholic NaOH (0.025 M, 5 mL) was added, and the reaction mixture was refluxed for 8–10 h. The excess solvent was removed, and the reaction mixture was poured onto crushed ice. The precipitated solid was filtered, dried, and recrystallised from acetone. The progress of the reaction was monitored by TLC, using methanol and chloroform as the solvent system.

***3-[5-(4-Chloro-phenyl)-4,5-dihydro-1H*-pyrazol-3-yl]-chromen-2-one (3a).** State: Solid; Color: Dark yellow; Yield: 82.20%; Melting Range: 172–174°C; IR (KBr, cm^{-1}): 1598.88 (C═N stretching), 1490.87 (C═C stretching), 1234.36 (C—O); ^{1}H-NMR ($CDCl_3$, δ ppm): 1.257–1.283 (d, 2H of pyrazoline), 2.076–2.173 (t, 1H of pyrazoline), 7.141–7.115 (d, 1H, Ar—H), 7.261–7.232 (d, 1H of chromen-2-one), 7.321–7.352 (t, 1H of chromen-2-one), 8.722 (s, 1H, 4-H of chromen-2-one); MS *m/z* (M + 1): 325.10; *Anal.* Calcd for $C_{18}H_{13}ClN_2O_2$: C, 68.24; H, 5.03; N, 8.24. Found: C, 68.47; H, 5.05; N, 8.46.

3-[5-(3-Methoxy-phenyl)-4,5-dihydro-1H-pyrazol-3-yl]-chromen-2-one (3b). State: Solid; Color: Dark yellow; Yield: 82.81%; Melting Range: 188–190°C; IR (KBr, cm^{-1}): 1598.88 (C═N stretching), 1487.01 (C═C stretching), 1261.36 (C—O); ^{1}H-NMR ($CDCl_3$, δ ppm): 2.171 (s, 3H, —OCH_3), 2.341–2.386 (d, 2H of pyrazoline), 7.341–7.360 (d, 1H of chromen-2-one), 7.125–7.148 (d, 1H, Ar—H), 6.933–6.940 (d, 1H of chromen-2-one), 6.851–6.879 (d, 1H, Ar—H), 8.013 (s, 1H, 4-H of chromen-2-one); MS *m/z* (M + 1): 321.14; *Anal.* Calcd for $C_{19}H_{16}N_2O_3$: C, 75.54; H, 5.08; N, 7.07. Found: C, 75.56; H, 5.29; N, 7.08.

3-[5-(4-Chloro-phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-chromen-2-one (3c). State: Solid; Color: Reddish brown; Yield: 64.30%; Melting Range: 170–172 °C; IR (KBr, cm^{-1}): 1598.88 (C═N stretching), 1487.01 (C═C stretching), 1257.50 (C—O), 756.04 (C—Cl); ^{1}H-NMR ($CDCl_3$, δ ppm):

2.043–2.057 (t, 1H of pyrazoline), 2.743–2.829 (d, 2H of pyrazoline), 7.375–7.400 (d, 1H of chromen-2-one), 7.283–7.301 (d, 1H, Ar—H), 7.740–7.794 (t, 1H of chromen-2-one), 7.939 (s, 1H, 4-H of chromen-2-one); MS *m/z* (M + 1): 401.15; *Anal*. Calcd for $C_{24}H_{17}ClN_2O_2$: C, 68.19; H, 4.27; N, 6.19. Found: C, 68.28; H, 4.38; N, 6.31.

3-[5-(3-Methoxy-phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-chromen-2-one (3d). State: Solid; Color: Reddish brown; Yield: 75.31%; Melting Range: 177–179°C; IR (KBr, cm^{-1}): 1598.88 (C═N stretching), 1485.09 (C═C stretching), 1257.50 (C—O), 756.04 (C—Cl); ^{1}H-NMR ($CDCl_3$, δ ppm): 1.297–1.317 (d, 2H of pyrazoline), 3.740–3.769 (t, 1H of pyrazoline), 3.930 (s, 3H, —OCH_3), 6.133–6.165 (d, 1H, Ar—H), 6.754–6.820 (t, 1H, Ar—H), 7.170–7.224 (t, 1H of chromen-2-one), 7.355–7.378 (d, 1H of chromen-2-one), 8.014 (s,1H, 4-H of chromen-2-one); MS *m/z* (M^+): 396.16; *Anal*. Calcd for $C_{25}H_{20}N_2O_3$: C, 68.24; H, 5.03; N, 8.24. Found: C, 68.47; H, 5.05; N, 8.42.

General procedure for the synthesis of chromen-2-one analogs of isoxazole (3e). Anhydrous sodium acetate (0.01 M, 0.82 g) dissolved in a minimum amount of hot acetic acid was added to a solution of hydroxylamine hydrochloride (0.01 M, 0.695 g) in ethanol (15 mL). This solution was added to a solution of **2b** (0.01 M, 3.06 g) in ethanol (20 mL). The mixture was refluxed on an oil bath for 9 h, concentrated, and then poured onto crushed ice. The precipitated solid was filtered, dried, and recrystallised from acetone. The progress of the reaction was monitored by TLC, using methanol and chloroform (0.1:9.9) as the solvent system.

3-[3-(3-Methoxy-phenyl)-isoxazole-5-yl]-chromen-2-one (3e). State: Solid; Color: Brown; Yield: 69.59%; Melting Range: 111–113°C; IR (KBr, cm^{-1}): 1631.67 (C═N stretching), 1512.09 (C═C stretching), 1278.72 (C—O), 1382.87 (N—O); ^{1}H-NMR ($CDCl_3$, δ ppm): 2.074 (s, 3H, OCH_3), 7.095–7.103 (d, 1H, Ar—H), 7.195–7.204 (d, 1H of chromen-2-one), 7.261 (s, 1H, 4-H of chromen-2-one), 7.465–7.509 (t, 1H of Ar—H), 7.799–7.825 (t, 1H, 4-H of chromen-2-one); MS *m/z* (M + 1): 320.08; *Anal*. Calcd for $C_{19}H_{13}NO_4$: C, 61.47; H, 4.10; N, 4.39. Found: C, 61.46; H, 4.21; N, 4.28.

General procedure for the synthesis of chromen-2-one analogs of pyrazole (3f–g). A solution of **2a** (0.01 M, 3.10 g)/ **2b** (0.01 M, 3.06 g) and hydrazine hydrate (0.01 M, 0.49 mL) were dissolved in glacial acetic acid (25 mL). The reaction mixture was refluxed on a water bath at a temperature of 70–90°C for 4–5 h and then poured onto crushed ice. The precipitated solid was filtered, dried, and recrystallised from acetone. The progress of the reaction was monitored by TLC, using methanol and chloroform as the solvent system.

3-[5-(4-Chloro-phenyl)-2H-pyrazol-3-yl]-chromen-2-one (3f). State: Solid; Color: Light yellow; Yield: 92.26%; Melting Range: 168–170°C; IR (KBr, cm^{-1}): 1712.67 (Cyclic C═O stretching), 1600.81 (C═N stretching), 1487.01 (C═C stretching); ^{1}H-NMR ($CDCl_3$, δ ppm): 7.262–7.306 (d, 1H, Ar—H), 7.362–7.419 (t, 1H, Ar—H), 7.447–7.510 (d, 1H, Ar—H), 7.606–7.637 (d, 1H, of chromen-2-one), 7.728–7.796 (t, 1H of chromen-2-one), 8.611 (s, 1H, 4-H of chromen-2-one); MS *m/z* (M + 1): 323.17; *Anal*. Calcd for $C_{18}H_{11}ClN_2O_2$: C, 64.09; H, 3.44; N, 7.69. Found: C, 64.19; H, 3.38; N, 7.65.

***3-[5-(3-Methoxy-phenyl)-2H*-pyrazole-3-yl]-chromen-2-one (3g)**. State: Solid; Color: Light yellow; Yield: 76.36%; Melting Range: 128–130°C; IR (KBr, cm^{-1}): 1598.88 (C═N stretching), 1488.94 (C═C stretching), 1245.93 (C—O stretching); ^{1}H-NMR ($CDCl_3$, δ ppm): 2.078–2.174 (t, 3H, OCH_3), 7.138–7.164 (d, 1H, Ar—H), 7.263 (s, 1H of pyrazoline), 7.702–7.728 (d, 1H of chromen-2-one), 7.797–7.891 (d, 1H of chromen-2-one), 8.611 (s, 1H, 4-H of chromen-2-one); MS *m/z* (M + 1): 318.12; *Anal*. Calcd for $C_{19}H_{14}N_2O_3$: C, 71.99; H, 4.13; N, 8.16. Found: C, 71.93; H, 4.28; N, 8.52.

General procedure for the synthesis of chromen-2-one analogs of oxazine (3h–i). To a solution of **2a** (0.01 M, 3.10 g) and urea (0.03 mol, 1.3 g)/**2b** (0.01 mol, 3.06 g) in 20 mL ethanol, 5 mL alcoholic KOH (0.02 M, 1.12 g) was added. The reaction mixture was refluxed for 12–14 h and then poured in 50 mL of 10% cold HCl solution. The precipitated solid was filtered, washed with water until free from acid, and recrystallised from acetone. The progress of the reaction was monitored by TLC, using ethyl acetate and hexane (2:8) as the solvent system.

3-[2-Amino-4-(4-chloro-phenyl)-6H-[1,3]oxazin-6-yl]-chromen-2-one (3h). State: Solid; Color: Light yellow; Yield: 68.64%; Melting Range: 168–170°C; IR (KBr, cm^{-1}): 1598.88 (C═N stretching), 1488.94 (C═C stretching), 1245.93 (C—O stretching); ^{1}H-NMR ($CDCl_3$, δ ppm): 2.078–2.174 (t, 3H, OCH_3), 7.138–7.164 (d, 1H, Ar—H), 7.263 (s, 1H of pyrazoline), 7.702–7.728 (d, 1H of chromen-2-one), 7.797–7.891 (d, 1H of chromen-2-one), 8.611 (s, 1H, 4-H of chromen-2-one); MS *m/z* (M + 1): 318.12; *Anal*. Calcd for $C_{19}H_{13}ClN_2O_3$: C, 62.69; H, 3.71; N, 6.14. Found: C, 62.89; H, 3.74; N, 6.15.

3-[2-Amino-4-(3-methoxy-phenyl)-6H-[1,3]oxazin-6-yl]-chromen-2-one (3i). State: Solid; Color: Light yellow; Yield: 86.78%; Melting Range: 182–184°C; IR (KBr, cm^{-1}): 1693.38 (C═O stretching), 1598.88 (C═C stretching), 1257.50 (C—O stretching); ^{1}H-NMR ($CDCl_3$, δ ppm) 3.888 (s, 3H, OCH_3), 2.755 (s, 2H, NH_2), 6.672–6.636 (d, 1H, Ar—H), 6.844–6.917 (t, 1H of Ar—H), 7.243–7.255 (t, 1H of chromen-2-one), 7.493–7.511 (d, 1H of chromen-2-one). MS *m/z* (M + 1): 349.08; *Anal*. Calcd for $C_{20}H_{16}N_2O_4$: C, 68.96; H, 4.63; N, 7.04. Found: C, 69.04; H, 4.51; N, 7.17.

General procedure for the synthesis of chromen-2-one analogs of thiazine (3j). A solution of **2b** (0.01 M, 3.06 g) and thiourea (0.03 mol, 2.3 g) in 20 mL ethanol, 5 mL alcoholic KOH (0.02 M, 1.12 g) was added. The reaction mixture was refluxed for 15 h and then poured in 50 mL of 10% cold HCl solution. The precipitated solid was filtered, washed with water until free from acid, and recrystallised from acetone. The progress of the reaction was monitored by TLC, using ethyl acetate and hexane (1:9) as the solvent system.

3-[2-Amino-4-(3-methoxy-phenyl)-6H-[1,3]thiazin-6-yl]-chromen-2-one (3j). State: Solid; Color: Dark yellow; Yield: 46.43%; Melting Range: 155–157°C; IR (KBr, cm^{-1}): 1600.81 (C═N stretching), 1487.01 (C═C stretching), 1257.50 (C—O stretching); ^{1}H-NMR ($CDCl_3$, δ ppm) 3.877 (s, 3H, OCH_3), 2.091 (s, 2H, NH_2), 7.172–7.187 (d, 1H, Ar—H), 6.860–6.911 (t, 1H of Ar—H), 7.471–7.537 (t, 1H of chromen-2-one), 7.758–7.677 (d, 1H of chromen-2-one). MS *m/z* (M + 1): 365.12; *Anal*. Calcd for $C_{20}H_{16}N_2O_3S$: C, 70.92; H, 4.43; N, 7.09. Found: C, 70.76; H, 4.28; N, 7.10.

Anti-inflammatory activity [23–25]. Male albino rats weighing 100–225 g were used for the assessment of the anti-inflammatory activity of the synthesized chromen-2-one analogs. Animals were procured from the Animal House, Faculty of Pharmacy, Northern India Engineering College, Lucknow, Uttar Pradesh, India. The animals were housed in PP cages with steel net, in temperature-controlled room under

standard living conditions of; $25 \pm 5°C$ and relative humidity of; 55 ± 5 with regular 12 h light and 12 h dark cycles and allowed free access to standard laboratory food and water. All the animals were treated humanely in accordance with the guidelines laid down by the Institutional Animal Ethics Committee (IAEC). The anti-inflammatory activity was approved by the IAEC with protocol no. BBDGEI/IAEC/14/2011.

Male albino rats weighing 100–225 g were divided into 12 groups of five animals each. The animals were starved overnight. Group 1 (Control) received 1 mL of 0.5% carboxymethyl cellulose (CMC) suspension. Group 2 (standard), received Ibuprofen 100 mg/kg. Group 3 to 12 were treated with the suspension of the test compounds (**3a–j**), at a dose of 100 mg/kg b.w. After 1 h of oral dosing, sub-planter injection of 0.1 mL of 1% solution of carrageenan was given into the left hind paw of each animal. The paw edema volume was measured with a plethysmometer after 0, 1, 3, 5, and 7 h of carrageenan injection.

$$\text{Anti-inflammatory activity (\% edema inhibition)} = V_t - V_c / V_t * 100$$

where V_t and V_c are the volumes of edema in the drug-treated and the control groups.

All the values of the experimental results are expressed as mean ± SEM and analyzed by one-way analysis of variance followed by Dunnett's test for the possible significant ($P < 0.01$) identification between various groups. Statistical analysis was carried out using Graph pad prism 3.0 (Graph pad software, San Diego, CA, USA).

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REFERENCES AND NOTES

[1] Hiller, K. O.; Hodd, P. L.; Willson, R. L. Chem Biol Interact 1983, 47, 293.

[2] Song, Y.; Connor, D. T.; Sercel, A. D.; Sorenson, R. J.; Doubleday, R.; Unangst, P. C.; Roth, B. D.; Beylin, V. G.; Gilbertsen, R. B.; Chan, K.; Schrier, D. J.; Guglietta, A.; Bornemeier, D. A.; Dyer, R. D. J Med Chem 1999, 42, 1161.

[3] Dannhardt, G.; Kiefer, W. Eur J Med Chem 2001, 36, 109.

[4] Charlier, C.; Michaux, C. Eur J Med Chem 2003, 38, 645.

[5] Dadiboyena, S.; Nefzi, A. Eur J Med Chem 2010, 45, 4697.

[6] Khode, S.; Maddi, V.; Aragade, P.; Palkar, M.; Ronad, P. K.; Mamledesai, S.; Thippeswamy, A. H.; Satyanarayana, D. Eur J Med Chem 2009, 44, 1682.

[7] Tsai, W. J.; Shiao, Y. J.; Lin, S. J.; Chiou, W. F.; Lin, L. C.; Yang, T. H.; Teng, C. M.; Wu, T. S.; Yang, L. M. Bioorg Med Chem Lett 2006, 16, 4440.

[8] Piller, N. B. Br J Exp Pathol 1975, 56, 554.

[9] Piller, N. B.; Casley-Smith, J. R. Br J Exp Pathol 1975, 56, 439.

[10] Singh, P.; Kaur, M.; Holzer, W. Eur J Med Chem 2010, 45, 4968.

[11] Joshi, N. S.; Shaikh, A. A.; Deshpande, A. P.; Karale, B. K.; Bhirud, S. B.; Gill, C. H. Ind J Chem 2005, 44B, 422.

[12] Singh, O. M.; Devi, N. S.; Thokchom, D. S.; Sharma, G. J. Eur J Med Chem 2010, 45, 2250.

[13] Egan, D.; O'Kennedy, R.; Moran, E.; Cox, D.; Prosser, E.; Thornes, R. D. Drug Metab Rev 1990, 22, 503.

[14] Ghate, M.; Kusanur, R. A.; Kulkarni, M. V. Eur J Med Chem 2005, 40, 882.

[15] Fylaktakidou, K. C.; Hadjipavlou-Litina, D. J.; Litinas, K. E.; Nicolaides, D. N. Curr Pharm Des 2004, 10, 3813.

[16] Amir, M.; Kumar, H.; Khan, S. A. Bioorg Med Chem Lett 2008, 18, 918.

[17] Gilchrist, T. L. Heterocylic Chemistry II: Six-Membered Ring Compounds with two or More Heteroatoms; Pearson Education (Singapore) Pte. Ltd: Delhi, 2000.

[18] Nagaraj, A.; Reddy, C. S. J. Iran Chem Soc 2008, 5, 262.

[19] Levai, A.; Jeko, J.; Brahmbhatt, D. I. J. Heterocyclic Chem 2005, 42, 1231.

[20] Karabasanagouda, T.; Airody, V. A. Indian J Chem 2009, 48B, 430.

[21] Winter, C. A.; Risley, E. A.; Nuss, G. W. Proc Soc Exp Biol Med 1962, 111, 544.

[22] Kontogiorgis, C. A.; Hadjipavlou-Litina, D. J. J Med Chem 2005, 48, 6400.

[23] Bansal, E.; Srivastava, V. K.; Kumar, A. Eur J Med Chem 2001, 36, 81.

[24] Chandra, T.; Garg, N.; Lata, S.; Saxena, K. K.; Kumar, A. Eur J Med Chem 2010, 45, 1772.

[25] Isloor, A. M.; Kalluraya, B.; Sridhar Pai, K. Eur J Med Chem 2010, 45, 825.