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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of anti-inflammatory and analgesic activity of 3-[(5-substituted-1,3,4-oxadiazol-2-yl-thio)acetyl]-2H-chromen-2-ones

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Abstract A novel series of 3-[(5-substituted-1,3,4-oxadiazol-2-yl-thio)acetyl]-2H-chromen-2-one (**7a–i**) were synthesized by the condensation between the appropriately substituted 5-substituted-1,3,4-oxadiazolyl-2-thione (**4a–i**) derived from various existing NSAIDs and 3-(2-bromoacetyl)-2H-chromen-2-one (**6**) under reflux in the presence of sodium ethoxide. Structure of the synthesized compounds was established on the basis of physicochemical, elemental analysis, and spectral data. The title compounds were screened for in vivo acute anti-inflammatory and analgesic activities at a dose of 200 mg/kg bw. Among the series, four compounds **7c**, **7e**, **7f**, and **7h** were found to possess a significant anti-inflammatory and analgesic activity profile. In addition, these compounds were also found to possess a less degree of ulcerogenic potential as compared to standard NSAIDs.

Keywords 3-Acetyl coumarin · 1,3,4-Oxadiazole · NSAIDS · Anti-inflammatory · Analgesic

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Introduction

Large numbers of non-steroidal anti-inflammatory drugs (NSAIDs) are widely preferred class of drugs used in the treatment of pain, fever, inflammatory diseases, and rheumatoid arthritis (Tally *et al*., 2000; Palomer *et al*., 2002). The pharmacological activities of NSAIDs are related in the suppression of prostaglandin biosynthesis from arachidonic acid by inhibiting the enzyme prostaglandin endoperoxidase, popularly known as cyclooxygenase and 5-lipoxygenase (Smith *et al*., 1998; Warner *et al*., 1996). These enzymes catalyze the rate-limiting steps in the biosynthesis of prostaglandins and leukotrienes, respectively. Cyclooxygenase exists in two isoforms, COX-1 and COX-2, which are regulated and expressed differently. COX-1 provides cytoprotection in gastrointestinal tract, whereas inducible COX-2 selectively mediates inflammatory signals (Amgad *et al*., 2001).

Long-term usages of NSAIDs have been associated with dyspepsia, GI ulceration, and nephrotoxicity. The GI damage from NSAIDs is generally attributed to two factors: local irritation by the carboxylic acid moiety common to most NSAIDs (topical effect), and decreased tissue prostaglandin production, which determines the physiological role of cytoprotective prostaglandins in maintaining the GI health and homeostasis (Amir and Shika, 2007). The discovery of COX-2 isoforms has opened the possibility of developing selective COX-2 inhibitors to act as an effective NSAID without the gastric side effects. Previously marketed drugs like Celecoxib, Rofecoxib, and Etodolac, etc., act by inhibiting COX-2 enzyme. However, these COX-2 selective inhibitors have been reported to be associated with cardiovascular side effects (Bombardier *et al*., 2000). In order to overcome these side effects, most studies are being focused on synthesis and identification of

new chemical entities with minimal side effects and excellent anti-inflammatory properties. Inflammation is known not only as a symptom of great deal of common diseases but also as an early phase of some serious diseases such as cancer, heart vascular diseases, and Alzheimer's dementia. Thus, the discovery of novel anti-inflammatory drugs has been attracting a lot of interests.

The synthesis of coumarins and their derivatives has attracted considerable attention from organic and medicinal chemists for many years as a large number of natural and synthetic products contain this heterocyclic nucleus. Several coumarin derivatives have been reported to present multiple biological activities (Egan *et al*., 1990) and especially anti-inflammatory/antioxidant activities. Kontogiorgis and Hadjipavlou-Litina (2004) have reported the synthesis and in vivo/in vitro anti-inflammatory/antioxidant activities of several new coumarin derivatives with a 7-azomethine linkage.

The substituted 1,3,4-oxadiazoles are heterocyclic compounds, which serve both as biomimetic as well as reactive pharamacophores and are of considerable pharmaceutical interest. 1,3,4-oxadiazole is an imperative scaffold since several 1,3,4-oxadiazole derivatives are known to be associated with multiple biological activities, such as pesticidal, CNS stimulant, anti-inflammatory, hypotensive, bactericidal, hypoglycemic, analgesic, anticonvulsive, antiemetic, diuretic (Deshmukh *et al*., 1976). Recently, Bhandari *et al.* (2008) have reported the synthesis and evaluation of anti-inflammatory, analgesic, and ulcerogenicity studies of novel *S*-substituted phenacyl-1,3,4-oxadiazol-2-thione and schiff bases of diclofenac as non-ulcerogenic derivatives.

In view of the above facts and in continuation of our research on novel anti-inflammatory agents (Bolakatti *et al*., 2008; Khode *et al*., 2009; Ronad *et al*., 2008), we report herein the synthesis of 3-[(5-substituted-1,3,4-oxadiazol-2-yl-thio)acetyl]-2H-chromen-2-ones (**7a–i**), which have been found to possess an appealing profile of anti-inflammatory and analgesic activities. In this study, we have attempted to convert the carboxylic acid group of the well-known NSAIDs into 1,3,4-oxadiazole that is condensed with coumarin nucleus, which possess an interesting profile of anti-inflammatory activity along with significantly lower ulcerogenic potential.

Chemistry

The synthesis of 5-substituted-1,3,4-oxadiazolyl-2-thione (**4a–i**) derivatives was achieved through convenient synthetic route outlined in Scheme 1. The various well-known NSAIDs, viz., Ibuprofen, Aceclofenac, Diclofenac, Ketoprofen, Indomethacin, Flurbiprofen, Ketorolac etc., were refluxed for 8–10 h in presence of dry methanol and few drops of conc. sulfuric acid to yield methyl esters of NSAIDs (**2a–i**), which were further refluxed in absolute ethanol for 15–20 h in the presence of hydrazine hydrate, yielded the carbohydrazides of NSAIDs (**3a–i**). Further, 5-substituted-1,3,4-oxadiazolyl-2-thione (**4a–i**) were synthesized by refluxing a mixture of carbohydrazides of NSAIDs, in the presence of potassium hydroxide and carbon disulfide in methanol on a steam bath for 12 h.

Synthesis of 3-acetyl-2H-chromen-2-one (**5**) was carried out by reacting salicylaldehyde with ethyl acetoacetate in the presence of base piperidine as illustrated in Scheme 2. The reaction is an example of the Knoevenagel reaction (Knoevenagel, 1898), in which the active methylene compounds reacts with 2-hydroxy benzaldehyde, has been extensively used as the first step in the synthesis of 3-acetylcoumarin. Further, 3-acetyl-2H-chromen-2-one (**5**) was brominated using bromine in presence of chloroform to yield 3-(2-bromoacetyl)-2H-chromen-2-one (**6**).

The synthesis of 3-[(5-substituted-1,3,4-oxadiazol-2-yl-thio)acetyl]-2H-chromen-2-ones (**7a–i**) was carried out by the condensation of 5-substituted-1,3,4-oxadiazolyl-2-thione (**4a–i**) with 3-(2-bromoacetyl)-2H-chromen-2-one (**6**) under reflux in ethanol in presence of sodium ethoxide (see Scheme 3).

Pharmacological evaluation

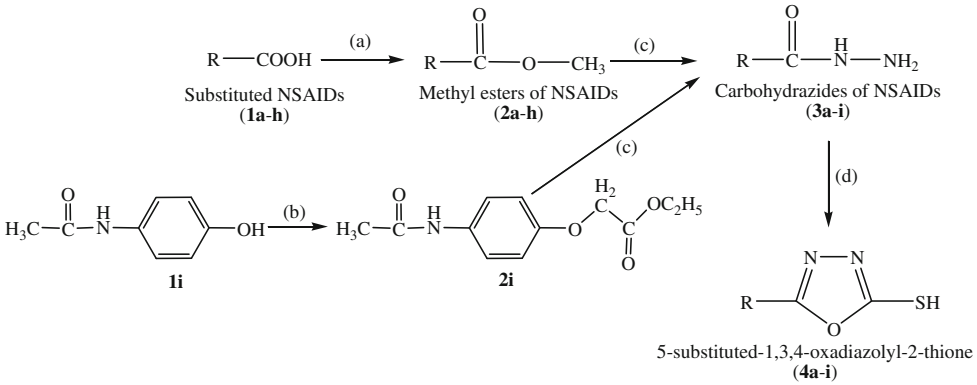
The acute toxicity test was carried out according to Organization for Economic Co-operation and Development (OECD) guidelines (OECD, Guidelines for Testing of Chemicals, 2008) to determine toxicity of all the synthesized compounds. The in vivo acute anti-inflammatory activity for all the test compounds was evaluated on male albino rats using carrageenan-induced rat paw edema model by adopting the earlier reported method of Winter *et al*. (1962). Further, analgesic activity and ulcerogenic studies were carried out for most active compounds **7c, 7e, 7f,** and **7h** by the earlier reported method of Koster and Anderson (1959) and Vogel (2002) (Drug Discovery and Evaluation: Pharmacological Assays), respectively.

Results and discussion

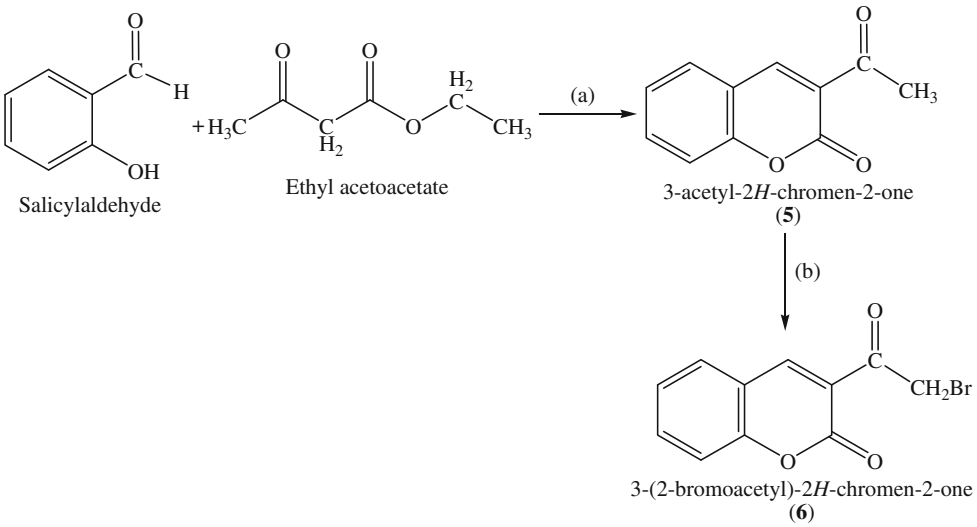
Synthetic and spectral studies

Structure of the title compounds (**7a–i**) was established on the basis of physicochemical, elemental analysis, and spectral data, which are summarized in experimental section. All the newly synthesized compounds gave satisfactory analysis for the proposed structures, which were confirmed on the basis of their IR and ^{1}H-NMR spectral

Scheme 1 Synthesis of 5-substituted-1,3,4-oxadiazol-2-thione (**4a–i**). Reagents and conditions: (A) Methanol, Conc. H_2SO_4, reflux, 8–10 h; (B) ethyl chloroacetate, dry acetone, reflux, 6 h; (C) Hydrazine hydrate, Absolute ethanol, reflux, 15–20 h; (D) KOH, Carbon disulfide in methanol, reflux, steam bath, 12 h



Scheme 2 Synthesis of 3-(2-bromoacetyl)-2H-chromen-2-one (**6**). Reagents and conditions: (A) Piperidine, stir, rt, 20 min; (B) Br_2, Chloroform, warm, 15–20 min



data. The IR spectra of these compounds showed moderately strong bands around 1711–1744 cm^{-1}, 1599–1618 cm^{-1}, and 2350–2367 cm^{-1}, characteristic of the C=O (lactone of coumarin), C=N and C–S groups, respectively. In the ^{1}H-NMR spectra, a characteristic singlet signal due to the fourth proton of coumarin ring appeared at δ 8.29–8.73. The most informative signal due to the –S–CH_2–C=O protons appeared as singlet at δ 4.24–4.7. The signals due to the aromatic protons appeared as multiplets at δ 6.8–8.3.

Pharmacological screening

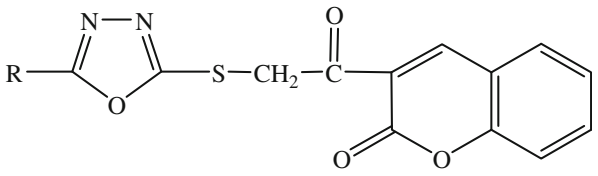
Acute toxicity study

From the preliminary toxicity studies, it was observed that, all the test compounds have revealed good safety profile till the uppermost dose (2,000 mg/kg). No mortality of animals observed even after 24 h but there were few changes in the behavioral response like alertness, touch response, and restlessness. Therefore, 1/10th of the maximum tolerated dose, i.e., 200 mg/kg bw. was chosen as therapeutic dose for the various pharmacological evaluations.

In vivo acute anti-inflammatory activity

Table 1 reveals the in vivo acute anti-inflammatory activity of a novel series of 3-[(5-substituted-1,3,4-oxadiazol-2-yl-thio)acetyl]-2H-chromen-2-one (**7a–i**) at a dose of 200 mg/kg in carrageenan-induced paw edema method. As shown in Table 1, the entire investigated compounds exhibited moderate-to-good anti-inflammatory activity with the percentage inhibition of edema formation ranging from 37.5–81.3 and 26.3–71.3, while the reference drug diclofenac (13.5 mg/kg) showed 79.5% and 75.0% inhibition at third and fifth hour, respectively. Compounds **7e** (74.9 and 61.6%), **7f** (65.4 and 60.9%), and **7h** (67.4 and 71.3%) showed good inhibitory activity at 3rd and 5th hour, respectively, while the most active compound **7c** (81.3 and 64.5%) among the series, exhibited the excellent inhibitory activity at 3rd and 5th hour, respectively. The anti-

Table 1 In vivo acute anti-inflammatory activity of a novel series of 3-[(5-substituted-1,3,4-oxadiazol-2-yl-thio)acetyl]-2H-chromen-2-one (**7a–i**) derivatives by carrageenan-induced paw edema



(**7a-i**)

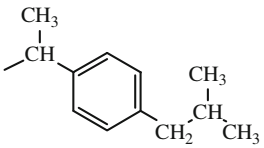
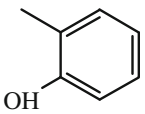
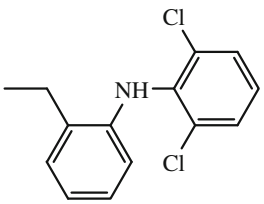
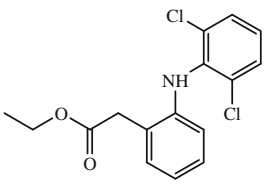
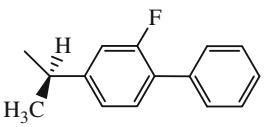
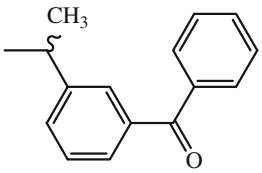
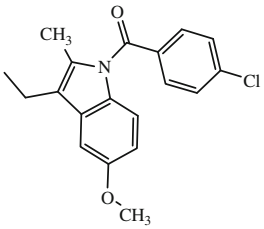
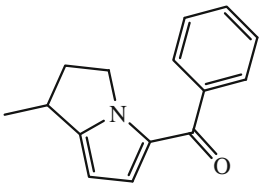
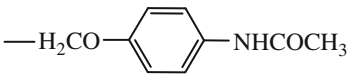
Compound	R	Anti-inflammatory activity[a] (%) Inhibition of edema (±SEM)	
		3rd h	5th h
Control		–	–
7a		45.0 (± 0.054)*	46.1 (± 0.028)*
7b		46.3 (± 0.039)*	48.7 (± 0.040)*
7c		81.3 (± 0.010)**	64.5 (± 0.040)**
7d		37.5 (± 0.045)*	26.3 (± 0.069)
7e		74.9 (± 0.015)**	61.6 (± 0.040)**

Table 1 continued

Compound	R	Anti-inflammatory activity[a] (%) Inhibition of edema (±SEM)	
		3rd h	5th h
7f		65.4 (± 0.047)**	60.9 (± 0.010)**
7g		56.9 (± 0.005)*	51.3 (± 0.008)*
7h		67.4 (± 0.016)**	71.3 (± 0.018)**
7i		56.3 (± 0.035)*	43.4 (± 0.008)*
Diclofenac	–	79.5 (± 0.013)†	75.0 (± 0.017)†

[a] Inhibitory activity on carrageenan-induced rat paw edema. The results are expressed as mean ± SEM; Significance was calculated by using one-way ANOVA with Dunnet *t*-test. The difference in results was considered significant when $p < 0.05$. * $p < 0.05$ vs. control at 200 mg/kg bw.; ** $p < 0.01$ vs. control at 200 mg/kg bw.; † $p < 0.01$ vs. control at 13.5 mg/kg bw

inflammatory activity of those compounds was comparable to that of the standard drug diclofenac sodium. Compounds that showed significant anti-inflammatory activity profile were further evaluated for analgesic activity.

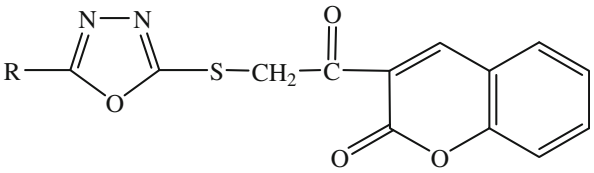
Analgesic activity

Abdominal construction response induced by acetic acid is a sensitive procedure to establish of peripherally acting analgesics. The compounds **7c, 7e, 7f**, and **7h** were tested for analgesic activity at 200 mg/kg bw. in mice. The results of analgesic activity indicated that all the tested compounds exhibited good analgesic activity (Table 2). Compounds **7c** (68%), **7e** (60%), **7f** (73%), and **7h** (54%) have shown almost comparable activity to that of reference drug acetylsalicylic acid (69%) in peripheral analgesic activity model.

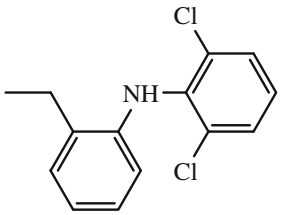
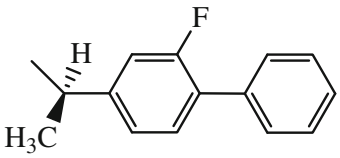
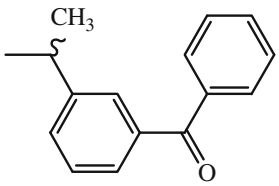
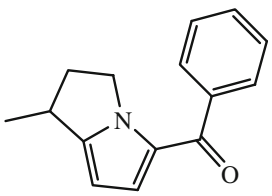
Ulcerogenic activity

The major drawback of NSAIDs is their gastric ulcer formation due to gastric irritation. The extent of ulcerogenic effect was evaluated for compounds **7c**, **7e, 7f**, and **7h** in rat stress model at the therapeutic dose (i.e., 200 mg/kg bw.). The gastric ulcerogenic potential was evaluated by calculating the ulcer index in treated and control animals. Results are given in Table 3 that indicates the ulcer index range of compounds **7c** (1.7–2.1), **7e** (2.3–2.8), **7f** (3.2–3.5), and **7h** (4.4–5.1) as compared to their standard reference drugs Diclofenac (5.5–6.4), Flurbiprofen (5.3–6.0), Ketoprofen (4.6–5.4), and Ketorolac (4.9–5.7), respectively. Therefore, the test compounds cause less gastric ulceration and disruption of gastric epithelial cells at the above mentioned oral dose. However, test compound **7h** induces ulcer, which is almost equipotent to its standard drug.

Table 2 Analgesic activity of compounds **7c**, **7e**, **7f**, and **7h** by acetic acid-induced writhing method



(**7c**, **7e**, **7f** and **7h**)

Compound	R	No. of wriths in 15 min (mean ± SEM)	% Protection
Control	–	44.9 ± 0.21	–
7c		14.2 ± 0.59**	68.0
7e		18.1 ± 0.23*	60.0
7f		12.3 ± 0.35**	73.0
7h		20.5 ± 0.46*	54.0
Acetylsalicylic acid	–	13.8 ± 0.57†	69.0

The results are expressed as mean ± SEM ($n = 6$). Significance was calculated by using one-way ANOVA with Dunnet *t*-test. The difference in results was considered significant when $p < 0.05$. * $p < 0.05$ vs. control at 200 mg/kg bw.; ** $p < 0.01$ vs. control at 200 mg/kg bw.; † $p < 0.001$ vs. control at 135 mg/kg bw

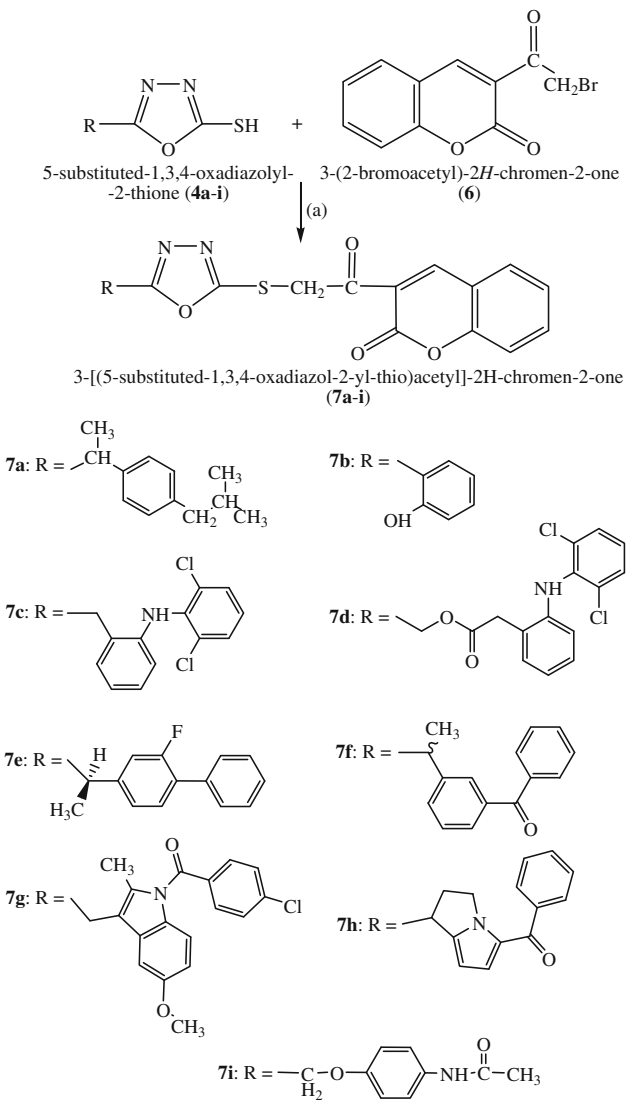
Conclusion

In this paper, we report the synthesis and pharmacological evaluation of a novel series of 3-[(5-substituted-1,3,4-oxadiazol-2-yl-thio)acetyl]-2H-chromen-2-one (**7a–i**). These novel compounds containing both 1,3,4-oxadiazole and coumarin ring systems were prepared by the condensation reaction between the appropriately 5-substituted-1,3,4-

Table 3 Ulcerogenic activity of compounds **7c**, **7e**, **7f** and **7h** in comparison with their standard NSAIDs

Compound	Ulcer index range
Control	1.2–1.6
7c	1.7–2.1
7e	2.3–2.8
7f	3.2–3.5
7h	4.4–5.1
Diclofenac	5.5–6.4
Flurbiprofen	5.3–6.0
Ketoprofen	4.6–5.4
Ketorolac	4.9–5.7

The results are expressed as ulcer index values from minimum to maximum range



Scheme 3 Synthetic route of 3-[(5-substituted-1,3,4-oxadiazol-2-yl-thio)acetyl]-2H-chromen-2-one (**7a–i**). Reagents and conditions: (A) absolute ethanol, sodium ethoxide, reflux, 25–30 h

oxadiazolyl-2-thione (**4a–i**) derived from various NSAIDs and 3-(2-bromoacetyl)-2H-chromen-2-one (**6**) as depicted in Schemes 1–3.

The title compounds did not show toxic effects at doses up to 2,000 mg/kg bw in acute toxicity experiments. The in vivo acute anti-inflammatory effects of the test compounds were assessed using the functional model of carrageenan-induced rat paw edema. Four compounds **7c**, **7e**, **7f**, and **7h** were found to possess a promising and significant anti-inflammatory profile. These compounds were also found to have significant analgesic activity in the acetic acid-induced writhing model. In addition, the tested compounds were also found to possess less degree of ulcerogenic potential as compared to standard NSAIDs.

Experimental

All the research chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA) or Lancaster Co. (Ward Hill, MA, USA) and used as such for the reactions. Various NSAIDs used in this study were received as a gift sample from Fourrts (India) Lab. Pvt. Ltd. Chennai (Tamilnadu, India). Solvents except laboratory reagent grade were dried and purified, when necessary, according to the literature. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel plates from Merck (Darmstadt, Germany).

Melting point of synthesized compounds was determined in Thermonik melting point apparatus (Thermonik, Mumbai, India) and is uncorrected. IR spectrum was recorded on Thermo Nicolet IR200 FT-IR Spectrometer (Madison, WI, USA) by using KBr pellets. The ^{1}H-NMR spectra were recorded on Bruker Avance II [400 MHz (Bruker, Rheinstetten/Karlsruhe, Germany)] using $CDCl_3$ and DMSO-d6 as solvent. Chemical shift are given in δ ppm units with respect to TMS. The purity of the compounds was examined by TLC on silica gel G plate using n-hexane and ethyl acetate (6:4) as mobile phase and iodine vapors as visualizing agent. The compounds were purified using flash chromatography SP-01 (Biotage, Sweden). The anti-inflammatory activity was carried out using digital plethysmometer (Ugo-Basile, Italy).

General procedure for the synthesis of methyl esters of NSAIDs (**2a–h**)

To a mixture of 0.05 mol of NSAIDs (**1a–h**) and 20-ml absolute methanol, few drops of Conc. sulfuric acid were added. The reaction mixture was refluxed for 8–10 h (progress of reaction was constantly monitored by TLC). Then, reaction mixture was cooled, and the solvent was distilled

off on a rotary evaporator (Buchii). The residue was taken into separating funnel containing 100 ml of distilled water and was successively extracted with diethyl ether. The methyl esters NSAIDs (**2a**–**h**) were separated rapidly at the bottom of the separatory funnel. The lower layer of methyl esters NSAIDs (**2a**–**h**) was carefully collected and was dried over anhydrous sodium sulfate. Finally, the excess of diethyl ether was distilled off. The product was collected and recrystallized from appropriated solvent.

General procedure for the synthesis of **2i**

A mixture of *p*-acetamido phenol (**1i**, 0.01 mol) and ethyl chloroacetate (0.01 mol) was refluxed using dry acetone in the presence of anhydrous potassium carbonate for 6 h. The reaction mixture was cooled and poured into crushed ice. The solid obtained was filtered, dried, and recrystallized from glacial acetic acid (IIango *et al*., 2009).

General procedure for synthesis of carbohydrazides of NSAIDs (**3a–i**)

Compound **2a**–**i** (0.01 mol) and hydrazine hydrate (99%, 0.02 mol) were refluxed in absolute ethanol for 15–20 h (monitored by TLC). The mixture was concentrated, cooled, and poured the in ice cold water. The solid thus separated was filtered, dried, and recrystallized from appropriated solvent.

General procedure for synthesis of 5-substituted-1,3,4-oxadiazol-2-thione (**4a–i**)

A mixture of one of the carbohydrazides of NSAIDs (**3a**–**i**) (0.05 mol), KOH (0.05 mol), and carbon disulfide (5 ml) in methanol was refluxed on a steam bath for 12 h. The solution was then concentrated, cooled, and acidified with dil. HCl. The solid mass that separated out was filtered, washed with ethanol, dried, and recrystallized from the appropriated solvent (Amir and Shikha, 2004).

General procedure for synthesis of 3-acetyl-2H-chromen-2-one (**5**)

To a cold mixture of salicylaldehyde (0.2 mol) and ethyl acetoacetate (0.2 mol), 2 ml of piperidine was added by rapid stirring. After 20 min, the yellowish solid separated was filtered off subsequently washed with ethanol and was recrystallized from water:ethanol (3:7) (Khode *et al*., 2009). Yield = 79.3%, mp 120°C. IR spectra (KBr cm^{-1}): 3075 (C–H str of Ar), 2929 (C–H str of CH_3), 1740 (C=O str of coumarin), 1695 (C=O str of acetyl). ^1H NMR ($CDCl_3$, δ, ppm): 8.51 (s, 1H, fourth proton of coumarin), 7.65–7.68 (d, 2H, Ar–H), 7.32–7.37 (d, 2H, Ar–H), 2.73 (s, 3H, $–CH_3$).

General procedure for synthesis of 3-(2-bromoacetyl)-2H-chromen-2-one (**6**)

To a solution of 3-acetyl-2H-chromen-2-one (**5**, 0.25 mol) in 200 ml of ethanol-free chloroform was added bromine (0.25 mol) in 25 ml chloroform with intermittent shaking and warming. The mixture was heated for 15 min on water bath to expel most of the hydrogen bromide, then cooled, and filtered. The solid was washed with ether and recrystallized from glacial acetic acid gave colorless needles (Koelsch, 1950). Yield = 62%, mp 160–163°C. IR spectra (KBr cm^{-1}): 3060 (C–H str of Ar), 2895 (C–H str of CH_2), 1737 (C=O str of coumarin), 1685 (C=O str of acetyl). ^1H NMR ($CDCl_3$, δ ppm): 8.43 (s, 1H, fourth proton of coumarin), 7.54–7.6 (d, 2H, Ar–H), 7.41–7.45 (d, 2H, Ar–H), 4.73 (s, 2H, $–CH_2Br$).

General procedure for synthesis of 3-[(5-substituted-1,3,4-oxadiazol-2-yl-thio)acetyl]-2H-chromen-2-one (**7a–i**)

A mixture of 5-substituted-1,3,4-oxadiazol-2-thione (**4a**–**i**, 0.01 mol) was dissolved in ethanol (5 ml) and 1 ml of sodium ethoxide (0.01 mol) and heated under reflux for 4 h. To the above reaction mixture, a solution of 3-(2-bromoacetyl)-2H-chromen-2-one (**6**, 0.01 mol) in ethanol was added and refluxed further for 25–30 h. (The reaction was monitored by TLC). The reaction mixture was poured into ice-cold water and the precipitate of 3-[(5-substituted-1,3,4-oxadiazol-2-yl-thio)acetyl]-2H-chromen-2-one (**7a**–**i**) was collected by filtration after washing with water and dried. Further, the title compounds were purified by flash chromatography. The physicochemical and spectral data of each compound are given below.

*3-[2-{5-(1-(4-Isobutylphenyl)ethyl)-1,3,4-oxadiazol-2-ylthio}acetyl]-2H-chromen-2-one (**7a**)*

Yield = 54%, mp 178–180°C. IR spectra (KBr, cm^{-1}): 3033 (C–H str of Ar), 2947 (C–H str of CH_3), 2822 (C–H str of CH_2), 2361 (C–S str), 1729 (C=O str of coumarin), 1688 (C=O str of $S–CH_2–C=O$ group), 1607 (C=N str), 1518 (C=C str). ^1H NMR ($CDCl_3$, δ, ppm): 8.5 (s, 1H, fourth proton of coumarin), 7.08–7.7 (m, 8H, Ar–H), 4.7 (s, 2H, $S–CH_2–C=O$ group), 4.2 (q, 1H, –CH of Ibuprofen), 2.72 (d, 2H, $–CH_2$ of Ibuprofen), 1.93 (m, 1H, –CH, isobutyl group), 1.41 (d, 3H, $–CH_3$ of Ibuprofen), 1.16 (dd,

6H, $-CH_3$ of isobutyl group). Elemental (CHN) analysis calculated for $C_{25}H_{24}N_2O_4S$: C, 66.86; H, 5.44; N, 6.21 (Found: C, 66.83; H, 5.50; N, 6.28).

*3-[2-{5-(2-Hydroxyphenyl)-1,3,4-oxadiazol-2-yl-thio}acetyl]-2H-chromen-2-one (**7b**)*

Yield = 83%, mp 204–206°C. IR spectra (KBr cm^{-1}): 3503 (br, O–H str), 3082 (C–H str of Ar), 2890 (C–H str of CH_2), 2361 (C–S str), 1711 (C=O str of coumarin), 1693 (C=O str of S–CH_2–C=O group), 1599 (C=N str), 1552 (C=C str). ^{1}H NMR ($CDCl_3$, δ, ppm): 8.36 (s, 1H, fourth proton of coumarin), 6.75–7.5 (m, 8H, Ar–H), 5.29 (s, br, 1H, OH), 4.24 (s, 2H, S–CH_2–C=O group). Elemental (CHN) analysis calculated for $C_{19}H_{12}N_2O_5S$: C, 60.05; H, 3.15; N, 7.40 (Found: C, 59.97; H, 3.20; N, 7.44).

*3-[2-{5-(2-(2,6-Dichlorophenylamino)benzyl)-1,3,4-oxadiazol-2-ylthio}acetyl]-2H-chromen-2-one (**7c**)*

Yield = 70%, mp 138–140°C. IR spectra (KBr cm^{-1}): 3294 (N–H str), 3073 (C–H str of Ar), 2915 (C–H str of CH_2), 2363 (C–S str), 1724 (C=O str of coumarin), 1682 (C=O str of S–CH_2–C=O group), 1605 (C=N str), 1566 (C=C str). ^{1}H NMR ($CDCl_3$, δ, ppm): 8.88 (s, 1H, N–H of diclofenac), 8.52 (s, 1H, fourth proton of coumarin), 6.93–7.85 (m, 11H, Ar–H), 4.37 (s, 2H, S–CH_2–C=O group), 2.24 (s, 2H, CH_2 of diclofenac). Elemental (CHN) analysis calculated for $C_{26}H_{17}C_{l2}N_3O_4S$: C, 58.04; H, 3.18; N, 7.83 (Found: C, 58.09; H, 3.21; N, 7.84).

*[5-2-Oxo-2-(2-oxo-2H-chromen-3-yl)ethylthio)-1,3,4-oxadiazol-2-yl)methyl 2-(2(2,6-dichlorophenylamino}phenyl]acetate (**7d**)*

Yield = 77%, mp 140–142°C. IR spectra (KBr cm^{-1}): 3290 (N–H str), 3040 (C–H str of Ar), 2897 (C–H str of CH_2), 2350 (C–S str), 1730 (C=O str of coumarin), 1689 (C=O str of S-CH_2-C=O group), 1607 (C=N str), 1565 (C=C str). ^{1}H NMR (DMSO, δ, ppm): 9.26 (s, 1H, N–H of aceclofenac), 8.57 (s, 1H, fourth proton of coumarin), 6.84–7.77 (m, 11H, Ar–H), 4.44 (s, 2H, S–CH_2–C=O group), 3.61 (s, 2H, –OCH_2–Het), 2.67 (s, 2H, –CH_2–C=O of aceclofenac). Elemental (CHN) analysis calculated for $C_{28}H_{19}C_{l2}N_3O_6S$: C, 56.40; H, 3.22; N, 7.04 (Found: C, 56.39; H, 3.24; N, 7.04).

*3-[2-{5-(1-(2-Fluorobiphenyl-4-yl)-1,3,4-oxadiazol-2-ylthio}acetyl]2H-chromen-2-one (**7e**)*

Yield = 64%, mp 176–178°C. IR spectra (KBr cm^{-1}): 3070 (C–H str of Ar), 2910 (C–H str of CH_2), 2359 (C–S str), 1729 (C=O str of coumarin), 1682 (C=O str of S–CH_2–C = O group), 1610 (C=N str), 1557 (C=C str). ^{1}H NMR ($CDCl_3$, δ, ppm): 8.6 (s, 1H, fourth proton of coumarin), 7.0–7.7 (m, 12H, Ar–H), 4.58 (q, 1H, –CH of flurbiprofen), 4.36 (s, 2H, S–CH_2–C=O group), 1.25 (t, 3H, CH_3 of flurbiprofen). Elemental (CHN) analysis calculated for $C_{27}H_{19}FN_2O_4S$: C, 66.65; H, 3.95; N, 5.75 (Found: C, 66.69; H, 3.94; N, 5.74).

*3-[2-{5-(1-(3-Benzoylphenyl)ethyl)-1,3,4-oxadiazol-2-ylthio}acetyl]-2H-chromen-2-one (**7f**)*

Yield = 70%, mp 170–172°C. IR spectra (KBr cm^{-1}): 3024 (C–H str of Ar), 2952 (C–H str of CH_3), 2902 (C–H str of CH_2), 2363 (C–S str), 1744 (C=O str of coumarin), 1710 (C=O str of ketoprofen), 1695 (C=O str of S–CH_2–C=O group), 1605 (C=N str), 1566 (C=C str). ^{1}H NMR (DMSO, δ, ppm): 8.47 (s, 1H, fourth proton of coumarin), 6.9–7.8 (m, 13H, Ar–H), 4.49 (s, 2H, S–CH_2–C=O group), 1.33 (t, 3H, CH_3 of ketoprofen). Elemental (CHN) analysis calculated for $C_{28}H_{20}N_2O_5S$: C, 67.72; H, 4.05; N, 5.65 (Found: C, 67.79; H, 4.04; N, 5.67).

*3-[2-{5-((1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)methyl)-1,3,4-oxadiazol-2-yl-thio}acetyl]-2H-chromen-2-one (**7g**)*

Yield = 64%, mp 118–120°C. IR spectra (KBr cm^{-1}): 3055 (C–H str of Ar), 2970 (C–H str of CH_3), 2892 (C–H str of CH_2), 2363 (C–S str), 1738 (=N–C=O str of indomethacin), 1717 (C=O str of coumarin), 1679 (C=O str of S–CH_2–C=O group), 1600 (C=N str), 1555 (C=C str). ^{1}H NMR ($CDCl_3$, δ, ppm): 8.43 (s, 1H, fourth proton of coumarin), 6.8–8.3 (m, 11H, Ar–H), 4.81 (s, 2H, CH_2 of indomethacin), 4.64 (s, 2H, S–CH_2–C=O group), 3.82 (s, 3H, OCH_3 of Indomethacin), 1.9 (s, 3H, CH_3 of indomethacin). Elemental (CHN) analysis calculated for $C_{31}H_{22}ClN_3O_6S$: C, 62.07; H, 3.73; N, 6.98 (Found: C, 62.09; H, 3.78; N, 7.03).

*3-[2-{5-(5-Benzoyl-2,3-dihydro-1H-pyrrolizin-1-yl)-1,3,4-oxadiazol-2-ylthio}acetyl]-2H-chromen-2-one (**7h**)*

Yield = 61%, mp 184–186°C. IR spectra (KBr cm^{-1}): 3080 (C–H str of Ar), 2878 (C–H str of CH_2), 2360 (C–S str), 1726 (C=O str of coumarin), 1705 (C=O str of ketorolac), 1688 (C=O str of S–CH_2–C=O group), 1602 (C=N str), 1568 (C=C str). ^{1}H NMR ($CDCl_3$, δ, ppm): 8.29 (s, 1H, fourth proton of coumarin), 7.4–8.1 (m, 9H, Ar–H), 7.1–7.3 (dd, 2H, pyrrzolo protons), 4.5 (s, 2H, S–CH_2–C=O group), 3.52 (t, 1H, CH of ketorolac), 3.18–3.24 (dd, 2H, CH_2 of

ketorolac), 2.83–2.9 (dd, 2H, CH_2 of ketorolac). Elemental (CHN) analysis calculated for $C_{19}H_{13}N_3O_5S$: C, 57.75; H, 3.30; N, 10.67 (Found: C, 57.79; H, 3.28; N, 10.73).

*3-(2-{(5-(4-Hydroxyphenylamino)-1,3,4-oxadiazol-2-yl}thio)acetyl)-2H-chromen-2-one (**7i**)*

Yield = 72%, mp 224–226°C. IR spectra (KBr cm^{-1}): 3433 (N–H str), 3014 (C–H str of Ar), 2862 (C–H str of CH_2), 2362 (C–S str), 1734 (C=O str of coumarin), 1689 (C=O str of S–CH_2–C=O group), 1618 (C=N str), 1538 (C=C str). ^{1}H NMR (DMSO, δ, ppm): 9.60 (s, 1H, N–H of paracetamol), 8.73 (s, 1H, fourth proton of coumarin), 6.87–7.85 (m, 8H, Ar–H), 5.22 (s, 2H, O–CH_2 of paracetamol), 4.69 (s, 2H, S–CH_2–C=O group), 1.96 (s, 2H, O=C–CH_3). Elemental (CHN) analysis calculated for $C_{27}H_{19}N_3O_5S$: C, 65.16; H, 3.85; N, 8.45 (Found: C, 65.19; H, 3.88; N, 8.47).

Pharmacology

Animals

Albino mice of either sex weighing 20–25 g were used for acute toxicity studies and analgesic activity. Healthy male albino adult rats weighing 170–220 g were used for various pharmacological screening. Animals were procured from Venkateshwara Enterprises, Bangalore, India (245/CPCSEA) and housed individually in polypropylene cages, maintained under standard conditions of alternating 12-h light/dark cycles at a constant temperature (25 ± 2°C) and 35–60% relative humidity). The pharmacological evaluations were conducted after obtaining ethical clearance from Animal Ethics Committee of KLES College of pharmacy, Hubli (India). Animals were fed with standard rat pellet diet (Hindustan Lever Ltd, Mumbai, India) and water ad libitum.

Acute toxicity

The acute toxicity test was carried out according to the OECD guidelines to establish the therapeutic dose of test compounds. Albino mice of each six each weighing between 20 and 25 g were grouped into nine groups of six animals each, starved for 24 h with water and libitum prior to test. On the day of experiment, animals were administered with different compounds to different groups in an increase dose of 10, 100, 200, 1000, and 2000 mg/kg body weight orally. The animals were observed continuously for 3 h for general behavioral, neurological, and autonomic profiles, and then every 30 min for next 3 h, and finally for next 24 h or till death.

Acute anti-inflammatory activity

In vivo acute anti-inflammatory activity was evaluated using carrageenan-induced rat paw edema assay model of inflammation by adopting the method of Winter *et al.* (1962) for the compounds listed in Table 1. Male albino rats (170–220 g) were fasted with free access to water at least 12 h prior to experiments and were divided randomly into 11 groups of six each. Control group received 1 ml of 0.5% sodium carboxymethyl cellulose (sodium CMC), standard group received 13.5 mg/kg of diclofenac and test groups received 200 mg/kg of synthesized compounds (**7a–i**). The rats were dosed orally, 1 h later; a subplantar injection of 0.05 ml of 1% solution of carrageenan in sterile distilled water was administered to the left hind footpad of each animal. The paw edema volume was measured with a digital plethysmometer at 0, 1st, 3rd, and 5th h after carrageenan injection. Paw edema volume was compared with vehicle control group, and percent reduction was calculated as 1 − (edema volume in the drug-treated group/edema volume in the control group) × 100.

Analgesic activity

Twenty-four hours prior to actual testing a large number of mice (20–25 g) received intraperitoneally 10 ml/kg 0.6% glacial acetic acid. Animals were observed for writhing movements. Only those showing one or other type of writhing movements (positive responders) were chosen for the test on the next day. On the test day, the responders received compounds half an hour prior to glacial acetic acid challenge. Compounds **7c, 7e, 7f**, and **7h** were orally administered at a dose of 200 mg/kg as a suspension in 0.5% sodium CMC. Standard drug used was acetylsalicylic acid at a dose of 30 mg/kg as a suspension in 0.5% sodium CMC. Each mouse was then observed for the total number of stretching episodes or writhing for 15 min following glacial acetic acid injection. The mean value for each group was calculated.

Ulcerogenic activity

Albino rats of either sex were divided into control, standard, and different test groups of six animals each group (170–220 g). They were starved for 48 h (water ad libitum) before drug administration. Control group received only 0.5% sodium CMC solution, standard group was orally administered with acetylsalicylic acid in sodium CMC solution and test compounds **7c**, **7e, 7f**, and **7h** were administered orally at the dose of 200 mg/kg bw. All the animals were sacrificed after 7 h of drug administration. Stomach was removed and placed on saline-soaked filter paper until inspection. A longitudinal incision along the greater curvature was made with fine scissors. The stomach

was everted over the index finger, and the presence or absence of gastric irritation was determined. The ulcer index range for each group was determined according to a previously reported method by counting the number of lesions (x) in each of five size classes (y). The classes were defined as $y = 1$ (pinpoint lesion), $y = 2$ (lesions < 1 mm diameter), $y = 3$ (lesions 1–2 mm diameter), $y = 4$ (lesions 2–4 mm diameter) and $y = 5$ (lesions > 4 mm diameter). The ulcer index was calculated using $\Sigma 5_{i=1}\, x_i y_i$.

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. The significance of difference was accepted at $p < 0.05$.

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