Journal of Saudi Chemical Society (2016) **20**, 259–264

King Saud University

Journal of Saudi Chemical Society

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Novel ethyl-5-amino-3-methylthio-1H-pyrazole-4-carboxylates: Synthesis and pharmacological activity

S.N. Thore [a,*], Sunil V. Gupta [a], Kamalkishor G. Baheti [b]

[a] *Deogiri College, Padampura, Aurangabad 431005, India*
[b] *Y.B. Chavan College of Pharmacy, Rafiq Zakaria Campus, Aurangabad 431001, India*

Received 24 May 2012; accepted 29 June 2012
Available online 25 July 2012

KEYWORDS

NSAIDs;
Anti-inflammatory;
Analgesic;
Diclofenac sodium;
Pyrazole;
Ulcerogenic potential

Abstract A series of novel ethyl-5-amino-3-methylthio-1H-pyrazole-4-carboxylates **3a–j** were synthesized from condensation of various hydrazides **2a–j** with ketene dithioacetal. The synthesized compounds were screened for *in vivo* analgesic and anti-inflammatory activities using acetic acid writhing test in mice and carrageenan-induced paw edema test in rat, respectively. Diclofenac sodium was used as a standard drug for comparison. Compounds **3a**, **3c** and **3d** exhibited significant analgesic and anti-inflammatory activities at a dose of 25 mg/kg and showed quite less ulcerogenic index in the range of 0.9–1.12 whereas diclofenac sodium showed 3.10.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most useful clinical therapies for the treatment of pain, fever and inflammation (Singh and Triadafilopoulos, 1999). The major mechanism by which NSAIDs exert, their anti-inflammatory activity is by the inhibition of cyclooxygenase-derived prostaglandin synthesis. The chronic use of NSAIDs leads to gastrointestinal (Botting, 2006), renal (Schneider et al., 2006) and hepatic (Adebayo and Bjarnason, 2006) side effects. The prostaglandin synthase (cyclooxygenase), the key enzyme of inflammatory process and an important target of most of the currently used NSAIDs, exists in two isoforms (COX-1 and COX-2), COX-1 plays a cytoprotective role while COX-2 induced at the time of injury, causes inflammation, pain and fever (Weilin et al., 1992). The conventional NSAIDs cause inhibition of both enzymes, thus exhibit anti-inflammatory activity along with gastrointestinal (GI) toxicity on extended use. The association of COX-2 with induced inflammation has led to the hypothesis that selective inhibition of COX-2 over COX-1 might provide good anti-inflammatory activity with reduced side effects than classical NSAIDs. Therefore, selective COX-2 inhibitors (coxibs) with better safety profile have been marketed as a new generation NSAIDs but careful prospective examination of coxibs has revealed unexpected cardiovascular adverse effect (Dogne and Pratico, 2005).

The widely prescribed anti-inflammatory pyrazole derivatives such as Celecoxib, Deracoxib, Remifenazone and Kebuzone (Fig. 1) are COX inhibitors with reduced ulcerogenic side effects. The literature survey reveals that pyrazoles showed

* Corresponding author.
E-mail address: snthore@rediffmail.com (S.N. Thore).

Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jscs.2012.06.011

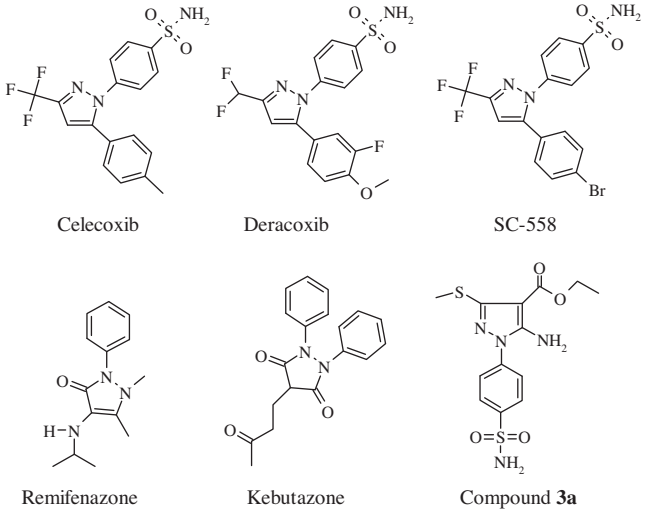


Figure. 1 Structure of marketed NSAIDs and synthesized compound **3a**.

excellent antiinflammatory, analgesic (Prokopp et al., 2006; Gyorgy et al., 2008; Morshed et al., 2009; Sung et al., 2011), antimicrobial (Pimerova and Voronina, 2001; Bekhit et al., 2008), antiviral, and anti-tumor (Park et al., 2005) activities.

Motivated by the aforesaid findings and pursuing our studies on pyrazole moiety, we have synthesized a new series of ethyl-1H-pyrazole-4-carboxylates **3a–j** (Schemes 1 and 2). Their antiinflammatory and analgesic activities were recorded. Synthesized compounds were characterized on the basis of their spectral data.

2. Experimental

2.1. Materials

The chemicals were purchased from Aldrich and Merck. Reagents and solvents were of analytical grade.

2.2. Instrumentation

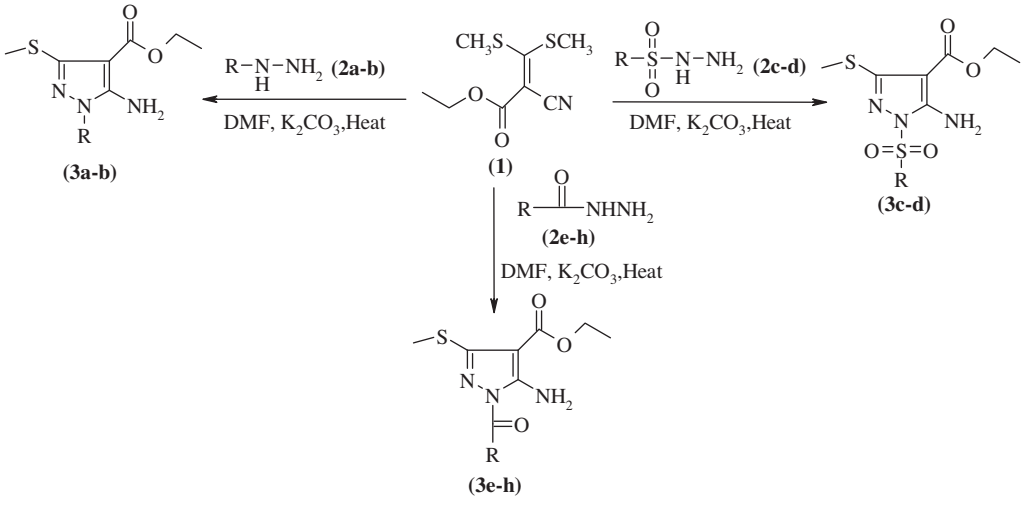
The progress of reactions was monitored on aluminum silica gel 60 F254 (Merck) using chloroform–methanol (9:1 by volume) as an eluent. Iodine vapors and U.V. light (wavelength 254 nm) were used as visualizing agents. Melting points were recorded on a Buchi capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 157 spectrometer using KBr pellets. The ^{1}H NMR spectra on a Bucker WM-400 (400 MHZ FT NMR) spectrophotometer were recorded using $CDCl_3$ and DMSO-d_6 as solvents with TMS as an internal reference. Chemical shifts (δ) are expressed in ppm and coupling constants (J) were measured in Hz. Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization. Elemental analyses were performed on Perkin Elmer Auto system 240c analyzer and were within $\pm 0.4\%$ of the theoretical values.

2.3. General method for the synthesis of ethyl-5-amino-3-methylthio-1H-pyrazole-4-carboxylate *3a–j*

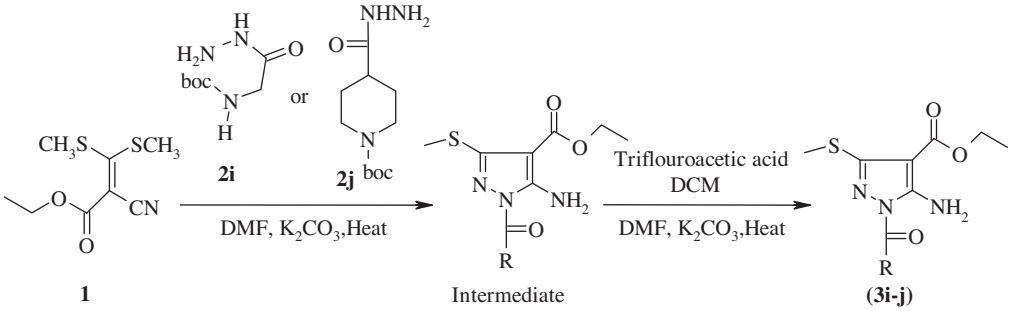
A mixture of **1** (Jensen and Henrikesen, 1968) (10 mmol) and hydrazides **2** (10 mmol) in DMF (5 ml) in the presence of catalytic amount of anhydrous K_2CO_3 was refluxed for 2–3 h. The reaction mixture was cooled and poured in ice cold water. The solid obtained was filtered, washed with water and recrystallized from ethanol to give **3**. The physical data of products are given in Table 1.

2.3.1. Ethyl-5-amino-3-methylthio-1-(4-sulfonamidophenyl)-1H-pyrazole-4-carboxylate (*3a*)

IR (KBr, cm^{-1}) 3454, 3329 (NH_2), 1730 (C=O), 1325, 1153 (S=O O). ^{1}H NMR (400 MHz, DMSO d^6) δ ppm: 1.26 (t, 3H, J = 6.94 Hz, CH_2CH_3), 2.48 (s, 3H, SCH_3), 3.85 (q, 2H, J = 7.0 Hz, $\underline{CH_2}CH_3$), 5.45 (bs, 2H, NH_2), 6.59 (bs, 2H, SO_2NH_2), 7.80 (d, 2H, J = 8.40 Hz, Ar–H), 7.94 (d, 2H, J = 8.40 Hz, Ar–H). MS (EI) m/z: 357 [M + 1], Anal. Cald. for $C_{13}H_{16}N_4O_4S_2$ (356.42): C, 43.81; H, 4.52; N, 15.72. Found: C, 43.80; H, 4.50; N, 15.69.

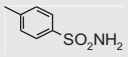
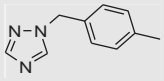
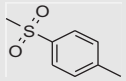
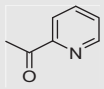
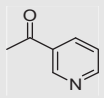
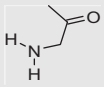
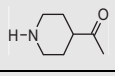


Scheme 1 Synthesis of Pyrazole derivatives (**3a–h**).



Scheme 2 Synthesis of Pyrazole derivatives (**3i** and **3j**).

Table 1 Physical data of synthesized compounds (**3a–j**).

Compound	R	M.P. (°C)	Yield (%)
3a		120–123	71
3b		175–178	78
3c		190–195	78
3d		202–205	81
3e		98–101	82
3f		130–133	87
3g		162–165	73
3h		180–183	75
3i		152–155	45
3j		142–145	52

*2.3.2. Ethyl-5-amino-3-methylthio-1-([4-(1, 2, 4-triazole-1-yl) methyl] phenyl)-1H-pyrazole-4-carboxylate (**3b**)*

IR (KBr, cm^{-1}) 3440, 3310 (NH_2), 1740 (C═O). ^1H NMR (400 MHz, DMSO d^6) δ ppm: 1.30 (t, 3H, J = 7.11 Hz, $CH_2\underline{CH_3}$), 2.57 (s, 3H, SCH_3), 3.94 (q, 2H, J = 7.15 Hz, $\underline{CH_2}CH_3$), 4.99 (s, 2H, CH_2), 5.60 (bs, 2H, NH_2), 7.15 (d, 2H, J = 8.00 Hz, Ar–H), 7. 24 (d, 2H, J = 8.00 Hz, Ar–H), 8.15 (s, 1H, 1,2,4-triazole–H), 8.23 (s, 1H, 1,2,4-triazole–H). MS (EI) m/z: 359 [M + 1], Anal. Cald. for $C_{16}H_{18}N_6O_2S$ (358.42): C, 53.62; H, 5.06; N, 23.45. Found: C, 53.56; H, 5.00; N, 23.44.

*2.3.3. Ethyl-5-amino-3-methylthio-1-phenylsulfonyl-1H-pyrazole-4-carboxylate **(3c)***

IR (KBr, cm^{-1}) 3420, 3190 (NH_2), 1750 (C═O), 1320, 1150 (S═O). ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 1.32 (t, 3H, J = 6.92 Hz, $CH_2\underline{CH_3}$), 2.42 (s, 3H, SCH_3), 4.26 (q, 2H, J = 6.98 Hz, $\underline{CH_2}CH_3$), 5.69 (bs, 2H, NH_2), 7.30–7.94 (m, 5H, Ar-H). MS (EI) m/z: 342 [M + 1], Anal. Cald. for $C_{13}H_{15}N_3O_4S_2$ (341.41): C, 45.74; H, 4.43; N, 12.31. Found: C, 45.73; H, 4.40; N, 12.28.

*2.3.4. Ethyl-5-amino-3-methylthio-1-(4-methylphenylsulfonyl)-1H-pyrazole-4-carboxylate (**3d**)*

IR (KBr, cm^{-1}): 3390, 3250 (NH_2), 1750 (C═O), 1310, 1140 (S═O) . ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 1.30 (t, 3H, J = 6.94 Hz, $CH_2\underline{CH_3}$), 2.40 (s, 3H, CH_3), 2.48 (s, 3H, SCH_3), 4.28 (q, 2H, J = 7.05 Hz, $\underline{CH_2}CH_3$), 5.60 (bs, 2H, NH_2), 7.34 (d, 2H, J = 8.26 Hz, Ar–H), 7.89 (d, 2H, J = 8.26 Hz, Ar–H). MS (EI) m/z: 356 [M + 1], Anal. Cald. for $C_{14}H_{17}N_3O_4S_2$ (355.44): C, 47.31; H, 4.82; N, 11.82. Found: C, 47.31; H, 4.80; N, 11.78.

*2.3.5. Ethyl-5-amino-1-(2-furanoyl)-3-methylthio-1H-pyrazole-4-carboxylate (**3e**)*

IR: (KBr, cm^{-1}): 3320, 3250 (NH_2), 1750 (C═O of ester), 1710 (C═O of ketone). ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 1.44 (t, 3H, J = 6.91 Hz, $CH_2\underline{CH_3}$), 2.55 (s, 3H, SCH_3), 4.38 (q, 2H, J = 7.02 Hz, $\underline{CH_2}CH_3$), 5.52 (bs, 2H, NH_2),6.60 (t, 1H, J = 5.33, Furan-H), 7.34 (d, 1H, J = 3.42 Hz, Furan-H), 7 .60 (d, 1H, J = 1.76 Hz, Furan-H). MS (EI) m/z: 296 [M + 1], Anal. Cald for $C_{12}H_{13}N_3O_4S$ (295.32): C, 48.81; H, 4.44; N, 14.23. Found: C, 48.78; H, 4.42; N, 14.21.

*2.3.6. Ethyl-5-amino-3-methylthio-1-(2-thiophenoyl)-1H-pyrazole-4-carboxylate **(3f)***

IR (KBr, cm^{-1}): 3380, 3190 (NH_2), 1740 (C═O of ester), 1715 (C═O of ketone). ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 1.40 (t, 3H, J = 6.94 Hz, $CH_2\underline{CH_3}$), 2.48 (s, 3H, SCH_3), 4.29 (q, 2H, J = 7.01 Hz, $\underline{CH_2}CH_3$), 5.62 (bs, 2H, NH_2),7.05 (t, 1H, J = 6.21, Thiophene-H), 7.61 (d, 1H, J = 4.94 Hz, Thiophene-H), 7.66 (d, 1H, J = 3.87 Hz, Thiophene-H). MS (EI) m/z: 312 [M + 1], Anal. Cald. for $C_{12}H_{13}N_3O_3S_2$ (311.38): C, 46.29; H, 4.21; N, 13.49. Found: C, 46.28; H, 4.20; N, 13.40.

*2.3.7. Ethyl-5-amino-3-methylthio-1-(2-pyridinoyl)-1H-pyrazole-4-carboxylate **(3g)***

IR (KBr, cm^{-1}): 3370, 3175 (NH_2), 1720 (C═O of ester), 1705 (C═O of ketone). ^1H NMR (400 MHz, DMSO d^6) δ ppm: 1.23 (t, 3H, 6.92 Hz,$CH_2\underline{CH_3}$), 2.48 (s, 3H, SCH_3), 4.20 (q, 2H, 6.98 Hz,$\underline{CH_2}CH_3$), 5.58 (bs, 2H, NH_2), 7.60 (t, 1H, J = 7.94, Pyridine-H), 8.26 (t, 1H, J = 7.33, Pyridine-H), 8 .76 (d, 1H, J = 4.95 Hz, Pyridine-H), 9 .09 (d, 1H, J = 3.90 Hz, Pyridine -H). MS (EI) m/z: 307 [M + 1], Anal. Cald. for $C_{13}H_{14}N_4O_3S$ (306.35): C, 50.97; H, 4.61; N, 18.29. Found: C, 50.80; H, 4.60; N, 18.21.

*2.3.8. Ethyl-5-amino-3-methylthio-1-(3-pyridinoyl)-1H-pyrazole-4-carboxylate **(3h)***

IR (KBr, cm^{-1}): 3375, 3170 (NH_2), 1740 (C═O of ester), 1705 (C═O of ketone). ^1H NMR (400 MHz, DMSO d^6) δ ppm: 1.30 (t, 3H, 6.94 Hz,$CH_2\underline{CH_3}$), 2.51 (s, 3H, SCH_3), 4.22 (q, 2H, 6.98 Hz, $\underline{CH_2}CH_3$), 5.65 (bs, 2H, NH_2), 7.64 (t, 1H, J = 7.70 Hz, Pyridine-H), 8.30 (d, 1H, J = 4.70 Hz, Pyridine-H), 8.79 (d, 1H, 4.64 Hz, Pyridine-H), 9.12 (s, 1H, Pyridine-H). MS (EI) m/z: 307 [M + 1], Anal. Cald. for $C_{13}H_{14}N_4O_3S$ (306.35): C, 50.97; H, 4.61; N, 18.29. Found: C, 50.85; H, 4.61; N, 18.23.

*2.3.9. Ethyl-5-amino-1-glycinoyl-3-methylthio-1H-pyrazole-4-carboxylate **(3i)***

IR (KBr, cm^{-1}): 3390, 3195 (NH_2), 1734 (C═O of ester), 1701 (C═O of ketone). ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 1.30 (t, 3H, 6.92 Hz, $CH_2\underline{CH_3}$), 2.45 (bs, 2H, glycinyl NH_2), 2.55 (s, 3H, SCH_3), 3.80 (s, 2H, CH_2 of glycinyl), 4.29 (q, 2H, 7.0 Hz, $\underline{CH_2}CH_3$), 5.70 (bs, 2H, NH_2). MS (EI) m/z: 259 [M + 1], Anal. Cald. for $C_9H_{14}N_4O_3S$ (258.30): C, 41.85; H, 5.46; N, 21.69. Found: C, 41.84; H, 5.42; N, 21.65.

*2.3.10. Ethyl-5-amino-3-methylthio-1-(4-piperidinoyl)-1H-pyrazole-4-carboxylate **(3j)***

IR (KBr, cm^{-1}): 3380, 3295 (NH_2), 3250 (NH), 1742 (C═O of ester), 1703 (C═O of ketone). ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 1.30 (t, 3H, 6.94 Hz, $CH_2\underline{CH_3}$), 1.91 (m, 4H, CH_2 of piperidinyl), 2.30 (bs, 1H, NH), 2.38 (m, 2H, CH_2 of piperidinyl), 2.47 (s, 3H, SCH_3), 2.80 (m, 4H, CH_2 of piperidinyl) 4.31 (q, 2H, 7.06 Hz, $\underline{CH_2}CH_3$), 5.70 (bs, 2H, NH_2). MS (EI) m/z: 313 [M + 1], Anal. Cald. for $C_{13}H_{20}N_4O_3S$ (312.39): C, 49.98; H, 6.45; N, 17.93. Found: C, 49.90; H, 6.42; N, 17.87.

2.4. Pharmacological evaluation

Synthesized compounds **3a–j** were investigated for anti-inflammatory and analgesic activities and most active representatives **3a, 3c**, and **3d** of the series were investigated for acute ulcerogenicity. Diclofenac sodium was used as a reference standard at a dose of 25 mg/kg for anti-inflammatory, analgesic and ulcerogenicity studies. The experiments were performed on Albino rats of Wistar strain of either sex, weighing 150–180 g for anti-inflammatory activity and Swiss Albino mice of either sex weighing 25–30 g for analgesic activity. The animals were divided into groups (control, reference and test groups) of 6 animals each.The tested compounds and the standard drugs were administered in the form of a suspension (using 1% carboxymethylcellulose) in distill water by oral route of administration for analgesic, anti-inflammatory and ulcerogenicity studies. The animals were maintained in colony cages at 25 ± 2 °C, relative humidity 45–55%, under a 12 h light–dark cycle; they were fed standard animal feed. All the animals were acclimatized for a week before use.

2.4.1. Analgesic activity

Analgesic activity was evaluated using acetic acid induced writhing method (Koster et al., 1959). After 50 min of the oral administration of test compound and standard drug, each animal was injected with 0.25 ml of 0.6% v/v acetic acid solution intraperitoneally. After 10 min of acetic acid injection, the numbers of muscular contractions (writhings) in mice were counted for a period of 15 min. A significant reduction in the number of writhing by any treatment as compared to control animals was considered as a positive analgesic response. The average number of writhes in each group of treated mice was compared with that of the control. The inhibition % was calculated and reported in Table 2.

2.4.2. Anti-inflammatory activity

Anti-inflammatory activity was evaluated using carrageenan induced rat paw edema method (Winter et al., 1962). Carrageenan solution (0.1% in sterile 0.9% NaCl solution) in a volume of 0.1 mL was injected subcutaneously into the subplantar region of the right hind paw of each rat. One group was kept as control and the animals of the other group were pretreated with

Table 2 Analgesic activity of title compounds (**3a–j**) against acetic acid induced writhing tests in mice.

Compounds	No. of writhes in 15 min after treatment (mean ± SEM)	% Inhibition
Control	30.4 ± 1.80**	–
Diclofenac sod.	11.2 ± 1.12**	63.15
3a	13.2 ± 0.66**	56.57
3b	16.6 ± 1.20**	45.39
3c	14.4 ± 0.86**	52.63
3d	13.8 ± 1.08**	54.60
3e	17.8 ± 0.72**	41.44
3f	17.2 ± 1.12**	43.42
3g	18.4 ± 1.11**	39.47
3h	18.8 ± 0.67**	38.15
3i	22.4 ± 0.78**	26.31
3j	23.2 ± 0.33**	23.68

Data analyzed by one-way ANOVA followed by Dunnett's test, (n = 6).
Dose levels: Test compounds and diclofenac sodium (25 mg/kg b.w. p.o).
** $P < 0.01$ significant from control.

Table 3 Results of anti-inflammatory activity of title compounds (**3a–j**) against carrageenan induced rat paw edema model in rats.

Compd.	Change in paw volume in (ml) after drug treatment (±SEM)				Anti-inflammatory activity (% inhibition)		
	0 h	1 h	2 h	3 h	1 h	2 h	3 h
Control	0.580 ± 0.29*	0.920 ± 0.27*	1.160 ± 0.30*	1.250 ± 0.32*	–	–	–
Diclofenac	0.576 ± 1.60*	0.792 ± 1.67*	0.888 ± 1.37*	0.974 ± 1.39*	36.47	46.20	40.56
3a	0.612 ± 1.36*	0.842 ± 1.52*	0.934 ± 1.32*	1.022 ± 1.41*	32.35	44.48	38.80
3b	0.575 ± 1.07*	0.842 ± 1.28*	0.963 ± 1.39*	1.035 ± 1.38*	21.47	33.10	31.34
3c	0.592 ± 1.57**	0.829 ± 1.90**	0.917 ± 1.33**	1.012 ± 1.40**	30.29	43.96	37.31
3d	0.621 ± 1.60*	0.860 ± 1.72*	0.948 ± 1.88*	1.039 ± 1.67*	29.70	43.62	37.61
3e	0.578 ± 1.58*	0.847 ± 1.28*	0.976 ± 1.18*	1.048 ± 1.21*	20.88	31.37	29.50
3f	0.620 ± 1.62*	0.879 ± 1.25*	1.010 ± 1.30*	1.080 ± 1.42*	23.82	32.75	31.34
3g	0.638 ± 1.35*	0.913 ± 1.27*	1.043 ± 1.38*	1.118 ± 1.46	19.11	30.17	28.35
3h	0.650 ± 1.60**	0.930 ± 1.65**	1.060 ± 1.67**	1.145 ± 1.39**	17.64	29.31	26.11
3i	0.590 ± 1.20*	0.887 ± 1.15*	1.007 ± 1.13*	1.093 ± 1.28*	13.23	28.44	25.22
3j	0.648 ± 1.60**	0.953 ± 1.55**	1.068 ± 1.59**	1.158 ± 1.64**	10.29	27.58	23.88

Data analyzed by one-way ANOVA followed by Dunnett's test, (n = 6).
Dose levels: Test compounds and diclofenac sodium (25 mg/kg b.w. p.o).
* $P < 0.05$.
** $P < 0.01$ significant from control.

the test drugs and standard drug 1 h before the carrageenan treatment. The paw volume of the all groups of rats was measured using the mercury displacement technique with the help of digital plethysmometer (UGO BASIL, ITALY) immediately before and 1, 2 and 3 h after carrageenan injection. The edema was expressed as a mean reduction in paw volume (ml) after treatment with tested compounds and anti inflammatory activity % was calculated and summarized in Table 3.

2.4.3. Ulcerogenicity

Ulceration in rats was induced as described by Goyal et al. (1985). Albino rats of the Wistar strain weighing 150–200 g of either sex were divided into various groups, each of six animals. Control group of animals was administered with only 1% carboxy methyl cellulose solution in water. One group of animals was administered with diclofenac sodium and remaining group was administered with test compounds at a dose of 25 mg/kg once daily for four days. On the fifth day, pylorus was ligated as per the method of Shay et al. (1945). Animals were fasted for 24 h before the pylorus ligation procedure. Four hours after the ligation, animals were sacrificed. The stomach was removed and opened along with the greater curvature and ulcer index was determined by the method of Ganguly and Bhatnagar (1973). The results are summarized in Table 4.

2.4.4. Statistical analysis

Statistical analysis of the biological activity of the synthesized compounds was evaluated using a one-way analysis of variance (ANOVA). In all cases, post hoc comparisons of the means of individual groups were performed using Dunnett's test. A significance level of $P < 0.05$ and $P < 0.01$ denoted significance in all cases. All values are expressed as mean ± -SEM. Statistical analysis was carried out using Graph Pad Prism (Graph Pad Prism 3.0 version).

Table 4 Evaluation of ulcer index.

Compound	Dose mg/kg/day (p.o.)	Time (days)	Ulcer index ± SEM
Control	CMC 1%w/v	4	–
Standard	25	4	3.10 ± 0.75**
3a	25	4	1.12 ± 0.62**
3c	25	4	0.95 ± 0.45**
3d	25	4	0.90 ± 0.53**

Data analyzed by one-way ANOVA followed by Dunnett's test, (n = 6).
** $P < 0.01$ significant from control.

3. Results and discussion

3.1. Chemistry

Compounds **3a–j** were synthesized from reactions of ethyl bismethylthio-2-cyanoacrylate (**1**) with hydrazides **2** (aromatic hydrazides **2a and 2b**, aromatic sulfonic acid hydrazides **2c and 2d,** heterocyclic carboxylic acid hydrazides **2e–h**, Boc-amino acid hydrazide **2i** and 1-Boc-piperidine-4-carboxylic acid hydrazide **2j**) in dimethylformamide in the presence of a catalytic amount of anhydrous potassium carbonate under reflux conditions for 2–3 h. Spectral data (IR, ^{1}H NMR and mass spectra) confirmed the structures of the synthesized compounds and the purity of these compounds was ascertained by elemental analyses. The IR spectra of compounds **3a–j** showed the presence of two absorption bands in the region of 3400–3150 cm^{-1} due to asymmetric and symmetric stretching of -NH_2 groups. The C═O stretching of ester groups appeared at 1750–1720 cm^{-1} as a sharp peak and the C═O stretching of ketone appeared at 1710–1700 cm^{-1}. The S═O stretching of sulphonyl group appeared as two absorption bands in the range of 1325–1140 cm^{-1}. ^{1}HNMR spectra of compounds **3a–j** showed that triplet signals at δ1.23–1.44 due to the CH_3 protons of the ethyl group and quartet signals at δ3.85–4.35 due to the CH_2 protons of the ethyl group. They also showed singlet signals within the region of δ 2.45–2.57 due to SCH_3 protons and broad distinguishable singlets at δ 5.45–5.70 corresponding to NH_2 protons. The aromatic protons appeared as multiplets within the region of δ 7.05–9.10.

3.2. Analgesic activity

The results of acetic acid induced writhing tests are given in Table 2. The data showed that all compounds exhibited anal-

gesic activity in the range of 23–57%. It was noticed that compounds **3a, 3c** and **3d** showed 56.57%, 52.63% and 54.60% reduction of writhing, respectively, after 1 h of treatment of tested compounds compared to 63.15% obtained with standard. The structure and analgesic activity correlation study showed that compounds with sulfonamidophenyl (**3a**) and aromatic suphonyl group (**3c** and **3d**) are the most active ones.

3.3. Anti-inflammatory activity

Anti-inflammatory activity was evaluated by carrageenan-induced paw edema test in rats. The activity of the newly synthesized compounds was measured before and at 1, 2 and 3 h after carrageenan injection. The anti-inflammatory activity data (Table 3) indicated that all the tested compounds protected rats from carrageenan-induced inflammation reached to peak level at 2 h then declining in activity was observed at 3 h. All the tested compounds showed a reasonable inhibition of edema size ranging from 23.88% to 38.80% compared 40.56% that obtained with diclofenac sodium after 3 h carrageenan injection.

A comparative study of the anti-inflammatory activity of tested compounds relative to the reference drug at different time intervals indicated the following: After 1 h compound **3a** was nearly as effective in inhibiting the paw edema (32.35%) compared to diclofenac sodium (36.47%). Taking the anti-inflammatory activity after 2 h time interval as a criterion for comparison, it was observed that compounds **3a**, **3c** and **3d** showed potent anti-inflammatory activity 44.48%, 43.96% and 43.62% respectively comparable with diclofenac sodium (46.20%). Compounds **3b**, **3e–g** displayed a good anti-inflammatory activity (30–33%).

Compounds **3a, 3c** and **3d** having sulfonyl group exhibited good anti-inflammatory activity. On the other hand, compounds **3i** and **3j** showed the least activity.

3.4. Ulcer index

The compounds which showed significant anti-inflammatory and analgesic activities have been selected for acute ulcerogenicity studies. Ulcerogenic effects of **3a, 3c** and **3d** were evaluated in rat stress model at acute dose of 25 mg/kg/day for 4 days (Table 4). When compared to the reference standards diclofenac sodium (ulcer index 3.10 ± 0.75), the tested compounds exhibited 29–35% of the ulcer index of the reference standards. The compounds **3c** and **3d** exhibited the lowest ulcer index of 0.95 and 0.90 respectively.

4. Conclusion

A series of novel 1H-pyrazole-4-carboxylates were synthesized and screened for analgesic and anti-inflammatory activities. Most compounds showed moderate to good analgesic and anti-inflammatory activities. Compounds **3a**, **3c** and **3d** exhibited significant analgesic and anti-inflammatory activities. Interestingly these compounds showed one-third of the ulcer index of the reference i.e. diclofenac sodium. Structure and biological activity relationship of the title compounds showed that the presence of sulfonamidophenyl and phenylsulphonyl groups at the N-1 position of pyrazole might be responsible for analgesic and anti-inflammatory activities.

Acknowledgments

The authors are thankful to Head, Department of Chemistry, Deogiri College, Aurangabad, Maharashtra-431 005 (India) for providing necessary facility for work. SIFC, Chandigarh University, Chandigarh, India for providing spectra of compounds.

References

Adebayo, D., Bjarnason, I., 2006. Is non-steroidal anti-inflammatory drug (NSAID) enteropathy clinically more important than NSAID gastropathy. Postgrad. Med. J. 82, 186–191.

Bekhit, A.A., Ashour, A.M.H., Bekhit, A.A.E.D., Baraka, A., 2008. Syntheses and biological evaluation of some thiazolyl and thiadiazolyl derivatives of 1H-pyrazole as antiinflammatory and antimicrobial agents. Eur. J. Med. Chem. 43, 456–463.

Botting, R.M., 2006. Cyclooxygenase: past, present and future. J. Therm. Biol. 31, 208–219.

Dogne, J.M., Pratico, D., 2005. Adverse cardiovascular effects of the coxibs. J. Med. Chem. 48, 2251–2257.

Ganguly, A.K., Bhatnagar, O.P., 1973. Effect of bilateral adrenalectomy on production of restraint ulcers in the stomach of albino rats. Can. J. Physiol. Pharmacol. 51, 748–750.

Goyal, R.K., Chakrabarti, A., Sanyal, A.K., 1985. The effect of biological variables on the antiulcerogenic effect of vegetable plantain banana. Planta Med. 29, 85–88.

Gyorgy, S., Janos, F., Klara, G., 2008. New celecoxib derivatives as anti-inflammatory agents. J. Med. Chem. 51, 142–147.

Jensen, K.A., Henrikesen, L., 1968. Studies of thioacids and their derivatives: reactions of carbon disulphide with active methylene compounds. Acta Chem. Scand. 22, 1107–1128.

Koster, R., Anderson, M., De Beer, E.J., 1959. Drug discovery and evaluation: pharmacological assays. Fed. Proc. 18, 412.

Morshed, A.C., Khaled, R.A., Mavanur, R.S., Edward, E.K., 2009. Synthesis of celecoxib analogues possessing a N-difluoromethyl-1,2-dihydropyrid-2-one 5-lipoxygenase. J. Med. Chem. 52, 1525–1529.

Park, H.J., Lee, K., Park, S., Ahn, B., Lee, J.C., Cho, H.Y., Lee, K.I., 2005. Identification of antitumor activity of pyrazole oxime ethers. Bioorg. Med. Chem. Lett. 15, 3307–3312.

Pimerova, E.V., Voronina, E.V., 2001. Syntheses of some new 1*H*-pyrazole, pyridazin-3(2*H*)-one, and oxazin-4-one derivatives. Pharm. Chem. 35, 18–20.

Prokopp, C.R., Rubin, M.A., Sauzem, P.D., Lourega, R.V., 2006. A pyrazolyl-thiazole derivative causes antinociception in mice. Braz. J. Med. Biol. Res. 39, 795–799.

Schneider, V., Hutchinson, T., Brophy, J.M., 2006. Association of selective and conventional nonsteroidal antiinflammatory drugs with acute renal failure. Am. J. Epidemiol. 164, 881–889.

Shay, M., Komarov, S.A., Meranze, D., Grunstein, H., Siplet, H., 1945. A simple method for the uniform production of gastric ulceration in the rats. Gastroenterology 5, 43–61.

Singh, G., Triadafilopoulos, G., 1999. Epidemiology of NSAID induced gastrointestinal complications. Rheumatology 56, 18–24.

Sung, H.H., Karen, M.W., Aaron, T.W., Bruce, D.H., 2011. Synthesis and structure activity relationship studies of urea containing pyrazoles as dual inhibitors of cyclooxygenase-2 and soluble epoxide hydrolase. J. Med. Chem. 54, 3037–3050.

Weilin, X., Donald, L.R., Daniel, L.S., 1992. Mitogen-inducible prostaglandin G/H synthase: a new target for nonsteroidal antiinflammatory drugs. Drug Dev. Res. 25, 249–265.

Winter, C.A., Risley, E.A., Nuss, G.N., 1962. Carrageenin-induced edema in hind paws of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. 111, 544–547.