Synthesis and Pharmacological Investigation of 5-Substituted-3-methylsulfanyl-1H-pyrazole-4-carboxylic Acid Ethyl Esters as New Analgesic and Anti-inflammatory Agents

Authors
P. D. Gokulan¹, B. Jayakar¹, V. Alagarsamy², V. Raja Solomon³

Affiliations
¹ Department of Pharmaceutical Chemistry, Vinayaka Mission’s College of Pharmacy, Sankari Main Road, Ariyanoor, Salem, India
² Department of Pharmaceutical Chemistry, MNR College of Pharmacy, Gr. Hyderabad, India
³ Medical & Process Chemistry Division, Central Drug Research Institute, Lucknow, India

Key words
pyrazole derivatives
analgesic
anti-inflammatory activity
ulcerogenic behaviour

Abstract

Purpose: To synthesize a new series of 5-substituted-3-methylsulfanyl-1H-pyrazole-4-carboxylic acid ethyl esters for their analgesic and anti-inflammatory activity.

Methods: The title compound synthesized by reacting the amino group of 5-amino-3-methylsulfanyl-1H-pyrazole-4-carboxylic acid ethyl ester with acid anhydrides, acid chlorides and phenyl dithiocarbamates. The synthesized compounds were characterized by IR, ¹H-NMR and mass spectral data; the purity of the compounds was determined by elemental analysis. The title compounds were investigated for analgesic, anti-inflammatory and ulcerogenic behaviour.

Results: The compound 5-benzoylamino-3-methylsulfanyl-1-phenyl-1H-pyrazole-4-carboxylic acid ethyl ester (4c) emerged as the most active compound and exhibiting imperative analgesic and anti-inflammatory activities. Interestingly the test compounds showed only mild ulcerogenic potential when compared to indomethacin.

Conclusion: The compound (4c) could serve as a lead molecule for further modification to obtain a clinically useful novel class of analgesic and anti-inflammatory agents.

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the choice treatment in various inflammatory diseases such as arthritis, rheumatisms and relieve body aches and pain of everyday life [1–4]. However, long-term use of the NSAIDs has been associated with gastrointestinal ulceration, bleeding and nephrotoxicity [1–4]. Therefore the discovery of new safer anti-inflammatory drugs represents a challenging goal for such a research area. The currently available NSAIDs belong to different chemical classes [5]. The pyrazole and substituted pyrazole derivatives have occupied a prominent place in medicinal chemistry because of their significant therapeutic properties in clinical applications [5–7]. In particular, it has been reported for diverse range of pharmacological activities including antihistamin, anti-arhythmic, anti-ulcer, leishmanicidal, HIV-RT inhibitor, anti-cancer, antimalarial, anti-microbial, and cytotoxicity [7–13]. In addition to this several pyrazole analogs were also reported to exhibit analgesic and anti-inflammatory activity [7,14–16]. However, tetra substituted pyrazole derivatives were not been studied towards analgesic and anti-inflammatory activities. Therefore, it was thought worthwhile to explore analgesic and anti-inflammatory activity of tetra substituted pyrazole derivatives. Accordingly the present work is concerned with the synthesis of tetra substituted pyrazole derivatives with the objective of discovering novel and potent analgesic and anti-inflammatory agents that might be devoid of gastrointestinal side effects. The synthesized compounds were tested for their analgesic, anti-inflammatory and ulcerogenic index behaviour.

Materials and Methods

Chemistry

Melting points (mp) were taken in open capillaries on Thomas Hoover melting point apparatus (Thomas Hoover, Philadelphia, USA) and are uncorrected. The IR spectra were recorded in film or in potassium bromide disks on a Perkin-Elmer 398 spectrometer (Bio Engineering, Wald, Switzerland). The ¹H NMR spectra were recorded on a DPX-300 MHz Bruker FT-NMR spectrometer (Pacific Northwest, Richland, Washington, USA). The chemical shifts were reported as parts per
Ethyl 2-cyano-3,3-bis(methylthio)acrylate (2)

To a solution of dimethylformamide (30 mL) and 1.13 g (0.01 mol) of ethyl 2-cyano-3,3-bis(methylthio)acetate, 0.01 mol of 10% sodium hydroxide was added drop wise at 10–15°C. The stirring was continued for further 3 h and the reaction mixture was poured into ice-water and filtered and dried; the precipitated was recrystallized from ethanol water (75:25) mixture. Yield 93%; m. p. 65–66°C; Rf 0.78 (CHCl₃ : CH₃OH, 9:1); IR (KBr, cm⁻¹): 3331 (Pyrazole NH Str), 3269 (Amide NH Str), 1690 (Ester C = O Str), 1245 (Ester C-O Str), 767 (CN deformation), 686 (Ethyl C-C Str); ¹H NMR (500 MHz, CDCl₃): δ 1.33–1.36 (t, 3H, CH₂CH₃), 2.60 (s, 3H, SCH₃), 2.75 (s, 3H, SCH₂), 4.26–4.30 (q, 2H, CH₂CH₃); MS (m/z) 217 (M⁺); Anal. Calcd for C₉H₁₂ClN₃O₃S: C, 38.89; H, 4.33; N, 15.11.

5-Amino-3-methylsulfanyl-1H-pyrazole-4-carboxylic acid ethyl ester (3a)

A mixture of compound 5-amino-3-methylsulfanyl-1H-pyrazole-4-carboxylic acid ethyl ester (3) (2.01 g, 0.01 mol) was dissolved in 0.02 mol of glacial acetic acid, to this chloroacetyl chloride (1.12g, 0.01 mol) was added and reaction mixture was refluxed for 1 h. The remaining procedure was the same as for compound 3a. Yield 82 % m. p. 115–117°C; IR (KBr, cm⁻¹): 3315 (Pyrazole NH Str), 3279 (Amide NH Str), 1690 (Ester C = O Str), 1664 (Amide C = O Str), 1585 (C = N Str), 1286 (Ester C = O Str), 781 (C = C Str); ¹H NMR (500 MHz, CDCl₃): δ 1.38–1.40 (t, 3H, CH₂CH₃), 2.27 (s, 3H, SCH₂), 4.22 (s, 2H, CICH₂CONH), 3.43–3.49 (q, 2H, CH₂CH₃), 9.68 (br s, 1H, NH); MS (m/z) 278 (M⁺); Anal. Calcd for C₉H₁₃N₂O₂S: C, 38.92; H, 4.36; N, 15.13; Found C, 38.89; H, 4.33; N, 15.11.

5-Chloroacetylamino-3-methylsulfanyl-1H-pyrazole-4-carboxylic acid ethyl ester (3b)

A mixture of compound 5-chloroacetylamino-3-methylsulfanyl-1H-pyrazole-4-carboxylic acid ethyl ester (3) (2.01 g, 0.01 mol) was dissolved in 5 mL of pyridine to this was added benzoyl chloride (0.013 mol) drop wise with stirring. After the completion of addition, the mixture was stirred for further 1 h and then the pyridine was removed by high vacuum rotavapor and the reaction mixture was dried to obtain the precipitated product. It was crystallized with chloroform-ethanol mixture. Yield 74%; m. p. 123–125°C; IR (KBr, cm⁻¹): 3041 (Pyrazole NH Str), 3020 (C = C-H), 1699 (Ester C = O Str), 1552 (Amide C = O Str), 1230 (Ester C = O Str), 784 (C = C Str); ¹H NMR (500 MHz, CDCl₃): δ 1.38–1.41 (t, 3H, CH₂CH₃), 2.38 (s, 3H, SCH₂), 4.28–4.32 (q, 2H, CH₂CH₃), 6.13 (br s, 2H, NH₂), 12.87 (br s, 1H, NH); MS (m/z) 277 (M⁺); Anal. Calcd for C₉H₁₃N₂O₃S: C, 41.78; H, 5.51; N, 18.38; Found C, 41.74; H, 5.48; N, 20.82.

5-Amino-3-methylsulfanyl-1H-pyrazole-4-carboxylic acid phenyl ester (4)

A mixture of 2.17g (0.01 mol) of ethyl 2-cyano-3,3-bis(methylthio)acetate (2) and 1.08 g (0.01 mol) of phenyl hydrazine in 30 mL ethanol was refluxed for 2 h. The remaining procedure was the same as for compound 3. Yield 85%; m. p. 95–97°C; IR (CHCl₃; CH₃OH, 9: 1); IR (KBr, cm⁻¹): 3331–3314 (NH₂ Str), 1669 (Ester C = O Str), 1608 (C = N Str), 1277 (Ester C = O Str), 687 (C = C Str); ¹H NMR (500 MHz, CDCl₃): δ 1.39–1.42 (t, 3H, CH₂CH₃), 2.54 (s, 3H, SCH₂), 4.32–4.36 (q, 2H, CH₂CH₃), 5.37 (br s, 2H, NH₂), 7.49–7.56 (m, 5H, Ar-H); MS (m/z) 277 (M⁺); Anal. Calcd for C₁₃H₁₅O₂S: C, 56.41; H, 5.37; N, 13.16; Found C, 56.38; H, 5.35; N, 13.14.

5-Acetylamino-3-methylsulfanyl-1H-pyrazole-4-carboxylic acid ethyl ester (3a)

A mixture of compound 5-acetylamino-3-methylsulfanyl-1H-pyrazole-4-carboxylic acid ethyl ester (3) (2.01 g, 0.01 mol) and acetic anhydride (0.05 mol) was placed in a round bottom flask and refluxed for 3 h. The reaction mixture was cooled and poured into ice-water and filtered. The precipitated product was filtered and washed with water and dried; the precipitated was recrystallized from ethanol water (75:25) mixture. Yield 80%; m. p. 150–152°C; IR (CHCl₃; CH₃OH, 9: 1); IR (KBr, cm⁻¹): 3344 (Pyrazole NH Str), 3269 (Amide NH Str), 1710 (Ester C = O Str), 1670 (Amide C = O Str), 597 (C = N Str), 1292 (Ester C = O Str), 705 (C = C Str); ¹H NMR (500 MHz, CDCl₃): δ 1.38–1.41 (t, 3H, CH₂CH₃), 2.26 (s, 3H, NHCOCH₃), 2.53 (s, 3H, SCH₂), 4.32–4.36 (q, 2H, CH₂CH₃), 9.67 (br s, 1H, NH); MS (m/z) 242 (M⁺); Anal. Calcd for C₉H₁₃N₂O₂S: C, 44.43; H, 5.39; N, 17.27; Found C, 44.4; H, 5.36; N, 17.24.

5-Benzyloamino-3-methylsulfanyl-1H-pyrazole-4-carboxylic acid ethyl ester (3c)

A mixture of compound 5-benzyloamino-3-methylsulfanyl-1H-pyrazole-4-carboxylic acid ethyl ester (3) (2.01 g, 0.01 mol) was dissolved in 5 mL of pyridine to this was added benzoyl chloride (0.013 mol) drop wise with stirring. After the completion of addition, the mixture was stirred for further 1 h and then the pyridine was removed by high vacuum rotavapor and the reaction mixture was dried to obtain the precipitated product. It was crystallized with chloroform-ethanol mixture. Yield 85% m. p. 95–97°C; IR (KBr, cm⁻¹): 3061 (Pyrazole NH Str), 3020 (C = C-H), 1700 (Ester C = O Str), 1552 (Amide C = O Str), 1230 (Ester C = O Str), 784 (C = C Str); ¹H NMR (500 MHz, CDCl₃): δ 1.38–1.41 (t, 3H, CH₂CH₃), 2.38 (s, 3H, SCH₂), 4.31–4.35 (q, 2H, CH₂CH₃), 7.48–7.54 (m, 5H, Ar-H), 8.47 (br s, 1H, NH), 9.41 (br s, 1H, NH); MS (m/z) 306 (M⁺); Anal. Calcd for C₁₃H₁₃N₂O₂S: C, 55.07; H, 4.95; N, 13.76; Found C, 55.04; H, 4.93; N, 13.73.

3-Methylsulfanyl-5-(3-phenyl-thioureido)-1H-pyrazole-4-carboxylic acid ethyl ester (3d)

A mixture of compound 5-methylsulfanyl-1H-pyrazole-4-carboxylic acid ethyl ester (3) (0.01 mol) and methyl-(N-phenyl)dithiocarbamate (0.01 mol) was dissolved in ethanol.
and a pinch potassium carbonate was added and refluxed for 9 h. The remaining procedure was the same as for compound 3a. Yield 81%; m. p. 217–221°C; IR (KBr, cm−1): 3388 (NH str), 1758 (C=O str), 1599 (C=N str), 1583 (ester C=O str), 1421 (C-N str), 1278 (ester C=O str). α-Methylbenzylamine (0.01 mol) was dissolved in ethanol and a pinch potassium carbonate was added and refluxed for 12 h. The remaining procedure was the same as for compound 3a. Yield 77%; m. p. 143–145°C; RF 0.41 (CHCl3:CH3OH, 9:1). α-Methylbenzylamine (0.01 mol) and methyl-(N-(4-methyl phenyl) dithiocarbamate (0.01 mol) was dissolved in ethanol and a pinch potassium carbonate was added and refluxed for 12 h. The remaining procedure was the same as for compound 3a. Yield 78%; m. p. 188–190°C; RF 0.41 (CHCl3;CH3OH, 9:1); IR (KBr, cm−1): 3342 (NH str), 1761 (Amide C=O str), 1685 (Amide C=O str), 1639 (C=N str), 1226 (ester C=O str), 632 (C(S)-C str); 1H NMR (500 MHz, CDCl3): δ 1.32–1.34 (t, 3H, CH2CH3), 2.34 (s, 3H, SCH3), 2.46–2.47 (q, 2H, CH2CH3), 7.15–7.17 (d, 2H, Ar-H), 7.31–7.32 (d, 2H, Ar-H), 7.39 (br s, 1H, NH), 8.44 (br s, 1H, NH), 9.17 (br s, 1H, NH); MS (m/z) 350 (M+); Anal. Calcld for C20H17N3O2S2: C, 58.23; H, 4.89; N, 13.58; Found. C, 58.2; H, 4.98; N, 13.09.

5-Benzoylamino-3-methylsulfanyl-1-phenyl-1H-pyrazole-4-carboxylic acid ethyl ester (4c)
A mixture of 5-aminomethylthiophenyl-1H-pyrazole-4-carboxylic acid ethyl ester (4) (2.77 g, 0.01 mol) was dissolved in 5 mL of pyridine to this was added benzoyl chloride (0.013 mol) drop wise with stirring. After the completion of addition, the mixture was stirred for further 1 h and then the pyridine was removed by suction and the reaction mixture was dried to obtain the precipitated product. It was crystallized with chloroform-ethanol mixture. Yield 79%; m. p. 152–155°C; IR (CHCl3): 3290.82 (Amide NH str), 1713 (ester C=O str), 1701 (Amide C=O str), 1589 (C=N str), 1236 (ester C=O str), 692 (C(S)-C str); 1H NMR (500 MHz, CDCl3): δ 1.22–1.25 (t, 3H, CH2CH3), 2.53 (s, 3H, SCH3), 4.27–4.31 (q, 2H, CH2CH3), 7.25–7.46 (m, 9H, Ar-H), 7.80 (br s, 1H, CONH); MS (m/z) 382(M+); Anal. Calcld for C29H19N3O2S2: C, 62.97; H, 5.02; N, 11.02; Found. C, 62.94; H, 4.98; N, 10.99.

5-Methylthio-4-carboxylate pyrazole-4-carboxylic acid ethyl ester (3b)
A mixture of compound 4b (0.01 mol) and methyl-(N-(4-methyl phenyl) dithiocarbamate (0.01 mol) was dissolved in ethanol and a pinch potassium carbonate was added and refluxed for 10 h. The remaining procedure was the same as for compound 3a. Yield 79%; m. p. 145–147°C; RF 0.46 (CHCl3;CH3OH, 9:1); IR (KBr, cm−1): 3306 (NH str), 1685 (Amide C=O str), 1639 (C=N str), 1226 (ester C=O str), 632 (C(S)-C str); 1H NMR (500 MHz, CDCl3): δ 1.30–1.33 (t, 3H, CH2CH3), 2.32 (s, 3H, SCH3), 2.37–2.38 (q, 2H, CH2CH3), 7.28–7.39 (m, 5H, Ar-H), 8.12 (br s, 1H, NH), 8.81 (br s, 1H, NH), 9.46 (br s, 1H, NH); MS (m/z) 336 (M+); Anal. Calcld for C6H12N2O2S2: C, 49.98; H, 4.75; N, 16.63.

5-Benzoylamino-3-methylsulfanyl-1-phenyl-1H-pyrazole-4-carboxylic acid ethyl ester (4d)
A mixture of compound 5-aminomethylthiophenyl-1H-pyrazole-4-carboxylic acid ethyl ester (4) (2.77 g, 0.01 mol) and acetic anhydride (0.05 mol) was placed in a RB flask and refluxed for 3 h. The remaining procedure was the same as in compound 3a. Yield 79%; m. p. 171–173°C; RF 0.48 (CHCl3;CH3OH, 9:1); IR (KBr, cm−1): 3388 (NH str), 1758 (C=O str), 1734 (Amide C=O str), 1599 (C=N str), 1239 (ester C=O str), 688 (C(S)-C str); 1H NMR (500 MHz, CDCl3): δ 1.40–1.43 (t, 3H, CH2CH3), 2.03 (s, 3H, NHCOCH3), 2.61 (s, 3H, SCH3), 4.31–4.34 (q, 2H, CH2CH3), 7.51–7.59 (m, 5H, Ar-H), 9.61 (br s, 1H, CONH); MS (m/z) 320 (M+); Anal. Calcld for C13H15N3O2S: C, 56.41; H, 5.37; N, 13.16; Found C, 56.38; H, 5.35; N, 13.14.

5-Chloroacetylaminomethylsulfanyl-1-phenyl-1H-pyrazole-4-carboxylic acid ethyl ester (4b)
A mixture of 5-amino-3-methylsulfanyl-1-phenyl-1H-pyrazole-4-carboxylic acid ethyl ester (4) (2.77 g, 0.01 mol) was dissolved in glacial acetic acid (0.02 mol), to this chloro acetylchloride (1.12 g, 0.01 mol) was added and the reaction mixture was refluxed for 1 h. The remaining procedure was the same as for compound 3a. Yield 89%; m. p. 161–163°C; RF 0.45 (CHCl3;CH3OH, 9:1); IR (KBr, cm−1): 3220 (Amide NH str), 1706 (ester C=O str), 1669 (Amide C=O str), 1559 (C=N str), 1234 (ester C=O str), 774 (C=Cl str), 657 (C(S)-C str); 1H NMR (500 MHz, CDCl3): δ 1.39–1.40 (t, 3H, CH2CH3), 2.57 (s, 3H, SCH3), 4.04 (s, 2H, CH2CONH), 4.31–4.36 (q, 2H, CH2CH3), 7.34–7.52 (m, 5H, Ar-H), 8.35 (br s, 1H, CONH); MS (m/z) 353 (M+); Anal. Calcld for C15H14ClN2O2S: C, 50.92; H, 4.56; N, 11.88; Found. C, 50.89; H, 4.54; N, 11.85.

Pharmacology
The synthesized compounds were evaluated for analgesic, anti-inflammatory and ulcerogenic index. The test compounds and the standard drugs were administered in the form of a suspension (using 1% carboxymethylcellulose as a vehicle) by oral route of administration for analgesic and anti-inflammatory. For ulcerogenicity studies the drug was administrated by intraperito-
neally as suspension in 10% V/V Tween 80. Each group consisted of six animals. The animals were procured from the Tetrex Biological Center, Madurai, India, and were maintained in colony cages at 25 ± 2°C, relative humidity 45–55%, under a 12 h light and dark cycle; they were fed standard animal feed. All the animals were acclimatized for a week before use. The Institutional Animal Ethics committee has approved the protocol adopted for the experimentation of animals.

**Analgesic activity**

Test for analgesic activity was performed by tail-flick technique using Wistar albino mice (25–35 g) of either sex selected by a random sampling technique [17–19]. The test compounds and pentazocine at a dose level of 5 mg kg⁻¹ was administered orally. The reaction time was recorded at 30 min and 1, 2 and 3 h after the treatment, and the cut-off time was 10 s. The percentage analgesic activity (PAA) was calculated by the following formula,

\[
PAA = \left( \frac{T_2 - T_1}{10 - T_1} \right) \times 100
\]

where \( T_1 \) is the reaction time (s) before treatment, and \( T_2 \) is the reaction time (s) after treatment.

**Anti-inflammatory activity**

Anti-inflammatory activity was evaluated by carrageenan-induced paw oedema test in rats [20]. The test compounds and Diclofenac sodium at a dose level of 5 mg kg⁻¹ was administered. The paw volumes were measured using the mercury displacement technique with the help of a plethysmograph immediately before and 1, 2 and 3 h after carrageenan injection. The percentage inhibition of paw oedema was calculated using (Table 2) the following formula

\[
\text{Percent inhibition} = 100 \left[1 - \frac{(a - x)}{(b - y)}\right]
\]

Where \( x \) is the mean paw volume of rats before the administration of carrageenan and test compounds or reference compound (test group), \( a \) is the mean paw volume of rats after the administration of carrageenan in the test group (drug treated), \( b \) is the mean paw volume of rats after the administration of carrageenan in the control group and \( y \) is the mean paw volume of rats before the administration of carrageenan in the control group.

**Evaluation of ulcerogenicity index**

Ulceration in rats was induced as described by reported protocol [21]. Albino rats of the Wistar strain weighing 150–200 g of either sex were divided into various groups each of 6 animals. The control groups of animals were administered only 10% V/V Tween 80 suspension intraperitoneally. One group were administered with indomethacin intraperitoneally at a dose of 5 mg kg⁻¹ once daily for 3 days. The remaining group of animals was administered with test compounds intraperitoneally at a dose of 5 mg kg⁻¹. On the fourth day, pylorus was ligated as per the method of Shay et al. [22]. Animals were fasted for 36 h before the pylorus ligation procedure. 4 h after the ligation, animals were sacrificed. The stomach was removed and opened along with

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**Fig. 1** Synthesis of pyrazole derivatives 3a–e and 4a–e.
the greater curvature. Ulcer index was determined by the method of Ganguly and Bhatnagar [23] and is recorded in Table 3.

Statistical analysis
Statistical analysis of the biological activity of the synthesized compounds on animals 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 Tukey’s test. A significance level of $p<0.05$ denoted significance in all cases. All values are expressed as mean±SD (standard deviations). For statistical analysis we have used GraphPad Prism version 3.0. (GraphPad Software, Inc. San Diego, CA 92130 USA).

Results and Discussion

Chemistry
The target compounds were synthesized according to steps outlined in Fig. 1. The precursor of ethyl 2-cyano-3,3-bis(methylthio)acrylate (1) was easily synthesized by treatment of ethyl cyanoacetate (1) with carbon disulphide and sodium hydroxide in DMF to give sodium dithiocarbamate, which was methylated with dimethyl sulphate to afford the dithiocarbamic acid methyl ester (2). The compound (2) reacted with hydrazine and substituted hydrazine that gives 5-amino-3-methylsulfanyl-1-substituted-1H-pyrazole-4-carboxylic acid ethyl ester (3 and 4) by 1,3-dipolar cyclo addition. The desired pyrazole derivatives (3a–e and 4a–e) were obtained by the reaction of 5-amino-3-methylsulfanyl-1-substituted-1H-pyrazole-4-carboxylic acid ethyl ester with different reagents and conditions (Fig. 1). The synthesized compounds were characterized by IR, $^1$H-NMR, and mass spectral data. Elemental analysis satisfactorily confirmed elemental composition and purity of the synthesized compounds.

Pharmacology
Test for analgesic activity was performed by tail-flick technique using Wistar albino mice [17–19]. The results of analgesic testing indicate that the test compounds exhibited moderate analgesic activity after 30 min of reaction time and an increase in activity after 1 h which reached a peak level after 2 h. Decline in activity was observed after 3 h (Table 1). According to the structure–activity relationship (SAR) studies, all the compounds have shown moderate analgesic activity when compared with reference drug pentazocine. Further results indicated that different substituents on the 5th position and N-1 pyrazole ring, exerted varied biological activity. Among the various amide (acetamide (3a), chloroacetamide (3b) and benzoyl amide (3c)) substitution on the 5th position, benzoyl amide substituted group (3c) showed significant activity. Compounds having 1-methyl-3-phenylthiourea (3d) and 1-methyl-3-p-tolythiourea substitution (3e) on the 5th position of pyrazole ring system leads to abolish the analgesic activity. The phenyl substitution on N-1 pyrazole ring system led to significant increases in analgesic activity when compared to unsubstituted compounds. Among the series, 5-benzoylamino-3-methylsulfanyl-1-phenyl-1H-pyrazole-4-carboxylic acid ethyl ester (4c) is found to equipotent with the reference drug pentazocine (Table 1). Anti-inflammatory activity was evaluated by carrageenan induced paw oedema test in rats [20]. The anti-inflammatory activity data (Table 2) indicated that all the test compounds protected rats from carrageenan-induced inflammation moderately after 30 min of reaction time with increased activity after 1 h that reached a peak level after 2 h. Decline in activity was observed after 3 h. The SAR studies indicated that different substituents on the 5th position and N-1 pyrazole ring, exerted varied protection effect from carrageenan-induced inflammation. The compound 5-benzoylamino-3-methylsulfanyl-1-phenyl-1H-pyrazole-4-carboxylic acid ethyl ester (4c) showed moderate anti-inflammatory activity when compared to the reference standard diclofenac sodium.

Table 2  Anti-inflammatory activity of synthesized compounds (3a–e and 4a–e) determined by carrageenan induced paw oedema test in rats.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>30 min</th>
<th>1h</th>
<th>2h</th>
<th>3h</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>5</td>
<td>23.61 ± 0.65 $^d$</td>
<td>38.00 ± 0.60 $^c$</td>
<td>57.24 ± 0.21 $^c$</td>
<td>16.07 ± 0.01 $^c$</td>
</tr>
<tr>
<td>3b</td>
<td>5</td>
<td>20.24 ± 0.66 $^a$</td>
<td>31.55 ± 1.01 $^c$</td>
<td>47.62 ± 0.58 $^a$</td>
<td>13.39 ± 0.80 $^c$</td>
</tr>
<tr>
<td>3c</td>
<td>5</td>
<td>33.00 ± 0.80 $^c$</td>
<td>39.32 ± 0.99 $^c$</td>
<td>58.43 ± 0.97 $^c$</td>
<td>22.52 ± 0.78 $^d$</td>
</tr>
<tr>
<td>3d</td>
<td>5</td>
<td>14.88 ± 0.50 $^a$</td>
<td>27.67 ± 0.50 $^c$</td>
<td>38.10 ± 0.68 $^a$</td>
<td>12.80 ± 0.79 $^d$</td>
</tr>
<tr>
<td>3e</td>
<td>5</td>
<td>13.89 ± 1.34 $^c$</td>
<td>28.77 ± 0.91 $^d$</td>
<td>39.25 ± 0.73 $^c$</td>
<td>12.33 ± 0.71 $^d$</td>
</tr>
<tr>
<td>4a</td>
<td>5</td>
<td>30.62 ± 1.11 $^d$</td>
<td>57.61 ± 1.26 $^c$</td>
<td>67.00 ± 1.12 $^c$</td>
<td>28.84 ± 1.11 $^d$</td>
</tr>
<tr>
<td>4b</td>
<td>5</td>
<td>27.68 ± 0.94 $^d$</td>
<td>46.73 ± 0.53 $^c$</td>
<td>63.99 ± 0.87 $^c$</td>
<td>21.13 ± 0.60 $^d$</td>
</tr>
<tr>
<td>4c</td>
<td>5</td>
<td>47.62 ± 0.87 $^c$</td>
<td>69.35 ± 1.10 $^c$</td>
<td>77.68 ± 1.23 $^c$</td>
<td>38.99 ± 0.71 $^c$</td>
</tr>
<tr>
<td>4d</td>
<td>5</td>
<td>20.24 ± 1.12 $^a$</td>
<td>36.31 ± 0.65 $^c$</td>
<td>50.00 ± 0.64 $^c$</td>
<td>17.56 ± 1.12 $^d$</td>
</tr>
<tr>
<td>4e</td>
<td>5</td>
<td>23.31 ± 0.96 $^a$</td>
<td>42.16 ± 1.16 $^c$</td>
<td>58.93 ± 1.23 $^c$</td>
<td>18.75 ± 1.18 $^d$</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>5</td>
<td>39.25 ± 0.79 $^c$</td>
<td>66.47 ± 0.73 $^c$</td>
<td>77.25 ± 0.43 $^c$</td>
<td>65.09 ± 0.71 $^c$</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>1.46 ± 0.69</td>
<td>2.27 ± 0.97</td>
<td>3.02 ± 0.51</td>
<td>2.09 ± 0.67</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD (n = 6). Significant difference relative to control at same time points and comparison were measured: $^p<0.001$, $^p<0.01$, $^p<0.05$, $^p<0.05$, $^p<0.01$, $^p<0.001$, $^p<0.05$.
Table 3 Evaluation of ulcerogenic behavior.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/Kg)</th>
<th>Volume of gastric juice (mL/4h)</th>
<th>pH</th>
<th>Total acid out put (mEq/L)</th>
<th>Free acid (mEq/L)</th>
<th>Ulcer index</th>
</tr>
</thead>
<tbody>
<tr>
<td>3c</td>
<td>5</td>
<td>4.34 ± 0.2</td>
<td>2.15 ± 0.3</td>
<td>29.54 ± 0.3</td>
<td>11.76 ± 0.5</td>
<td>1.84 ± 0.5</td>
</tr>
<tr>
<td>4c</td>
<td>5</td>
<td>4.53 ± 0.4</td>
<td>2.13 ± 0.2</td>
<td>29.65 ± 0.3</td>
<td>11.78 ± 0.5</td>
<td>1.68 ± 0.4</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>5</td>
<td>10.32 ± 0.4</td>
<td>2.04 ± 0.3</td>
<td>60.33 ± 0.5</td>
<td>24.90 ± 0.4</td>
<td>3.79 ± 0.3</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>2.12 ± 0.3</td>
<td>3.84 ± 0.5</td>
<td>13.32 ± 0.4</td>
<td>5.85 ± 0.2</td>
<td>0.06 ± 0.01</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD (n = 6). Significant difference relative to control at same time points and comparison were measured: *p<0.001, **p<0.01, *p<0.05

**Conclusion**

In the present study, synthesis of a new series of 5-substituted-3-methylsulfonyl-1H-pyrazole-4-carboxylic acid ethyl esters (3a–e and 4a–e) has been described. The results of the analgesic and anti-inflammatory activities of the 5-substituted-3-methylsulfonyl-1H-substituted)-pyrazole-4-carboxylic acid ethyl esters series showed that moderate enhancement of activity. The compound 5-benzoylamo-3-methylsulfonyl-1-phenyl-1H-pyrazone-4-carboxylic acid ethyl ester (4c) emerged as the most active compound. Hence this series could be developed as a novel class of analgesic and anti-inflammatory agents. Further structural modification is planned to obtain compounds with increased analgesic and anti-inflammatory activities with minimal ulcerogenic behaviour.

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**Conflict of Interest Statement**

The authors report no conflicts of interest to declare in connection with the contents of this manuscript.

**References**