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ORIGINAL ARTICLE

Synthesis and pharmacological evaluation of 5-methyl-2-phenylthiazole-4-substituted heteroazoles as a potential anti-inflammatory and analgesic agents

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Ulcerogenic potential

Abstract A series of novel 5-methyl-2-phenylthiazole-4-substituted-heteroazole derivatives (**6–15**) have been synthesized. The structures of these compounds were established by IR, ^{1}HNMR, Mass spectral data and elemental analyses. Compounds were evaluated for their anti-inflammatory and analgesic activities as well as gastric ulcerogenic effects. Derivatives **9**, **10**, **14** and **15** exhibited moderate to good anti-inflammatory and analgesic activities in carrageenan-induced rat paw edema and acetic acid-induced writhing in mice respectively, with low ulcerogenicity compared with the standard drug diclofenac sodium.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of pain and inflammation. Most currently used NSAIDs have limitations for therapeutic uses, since they cause gastrointestinal and renal side effects which are inseparable from their pharmacological activities. These compounds act via inhibition of the enzyme cyclooxygenase, thus preventing prostaglandin synthesis. In the early 1990s, it was discovered that the enzyme exists as two isomers, one constitutive (COX-1) and the other inducible (COX-2) (Hla and Neilson, 1992). COX-1 is an enzyme that is constitutively expressed and provides cytoprotection in the gastrointestinal (GI) tract; whereas inducible COX-2 mediates inflammation (Almansa et al., 2003). The traditional NSAIDs cause inhibition of both enzymes. In fact, most of them show greater selectivity for COX-1 than COX-2 (Jackson and Hawkey, 1999). Consequently long term therapy with nonselective NSAIDs may cause gastrointestinal complications ranging from stomach irritation to life-threatening GI ulceration and bleeding (Allison et al., 1992). Therefore, selective COX-2 inhibitors with better safety profile have been marketed as a new generation of NSAIDs (Tally et al., 2000). But careful prospective examination of coxibs has revealed unexpected cardiovascular adverse effects (Dogne and Pratico, 2005).

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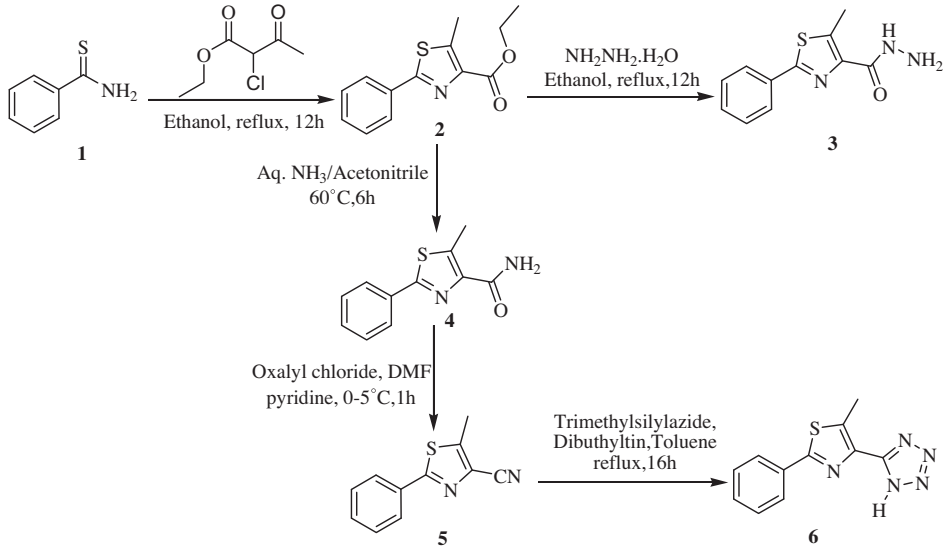
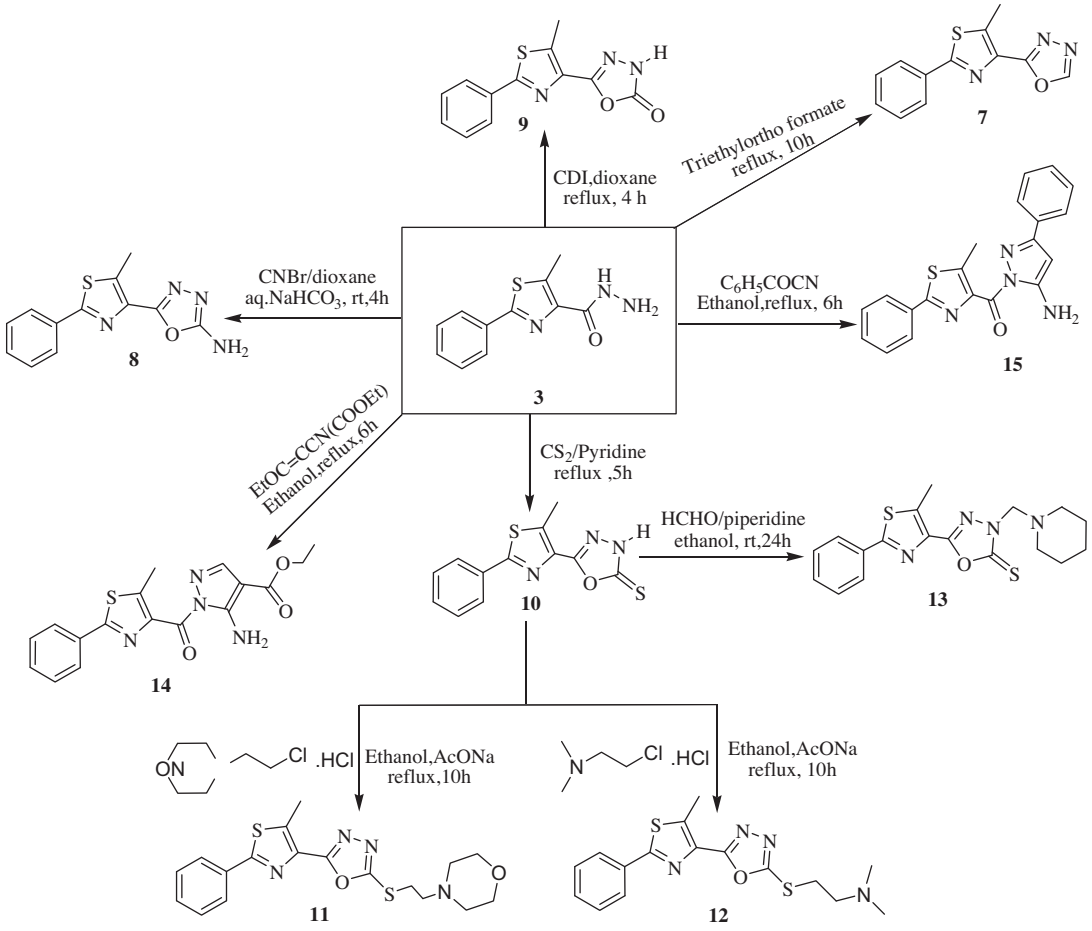


Scheme 1 Synthesis of intermediate **3** and target compound **6.**



Scheme 2 Synthesis of target compounds **7**–**15.**

Most of the clinical NSAIDs possess acidic carboxyl (COOH) group, which further cause GI irritation by direct contact of –COOH group in GIT at doses very close to anti-inflammatory ones. These serious side effects limit the use of NSAIDs as a safer drug for the treatment of inflammation. Several studies have described the derivatization of the carboxylate function of representative NSAID with less acidic azoles: thiazole (Giri et al., 2009), oxadiazole (Akhter et al., 2009),

thiadiazole (Aktay and Salgın-Göksen, 2007) etc. which resulted in an increased antiinflammatory activity with reduced ulcerogenicity. In our attempt to synthesize new, safer and potent agents for treatment of inflammatory diseases, we used 5-methyl 2-aryl-thiazole moiety. This heterocyclic system has found application in drug development for the treatment of inflammation. Fentiazac (2-phenyl-thiazole), Meloxicam (2-methyl-thiazole), Fanetizole (2-phenyl-thiazole), Meloxicam (2-methyl-thiazole), and Fanetizole (2-amino-thiazole) (Rehman et al., 2005; Potewar et al., 2007) are some examples of thiazole bearing anti-inflammatory products. Various non acidic (Amir et al., 2007) and acid bioesters of the type of 1,3,4-thiadiazole and 1,3,4-oxadiazole derivatives (Manjunathaa et al., 2010) were reported for anti-inflammatory activity. With the aim of obtaining new anti-inflammatory agents, we have replaced carboxylic acid group of 5-methyl-2-phenylthiazole scaffold with non acidic or less acidic heterocycles like 1,3,4-oxadiazole, 1,3,4-oxadiazolinone 1,3,4-oxadiazolinethione, tetrazole and pyrazole moiety at position 4 of thiazole.

The present work describes the synthesis of some new 5-methyl-2-phenylthiazole-4-substituted-heteroazole derivatives (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. General

The progress of reactions was monitored on aluminum silica gel 60 F_{254} (Merck) using chloroform–methanol (9:1 by volume) as an eluent. Iodine vapors and UV 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}HNMR spectra were recorded on a Bucker WM-400 (400 MHz FT NMR) spectrophotometer using $CDCl_3$ and DMSO-d_6 as solvents with TMS as an internal reference. Chemical shifts (δ) are expressed in ppm. 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. The chemicals were purchased from Aldrich and Merck. Reagents and solvents were of analytical grade.

2.2. Chemistry

2.2.1. Synthesis of 5-methyl-2-phenylthiazole-4-carboxylic acid ethyl ester(2) (Michael and Andrew, 2007)

Thiobenzamide **1** (4.0 g, 29.1 mmol) and ethyl 2-chloroacetoacetate (4.74 g, 29.1 mmol) were dissolved in 40 ml ethanol. The reaction mixture was refluxed for 12 h. The excess of ethanol was removed under reduced pressure to yield semisolid, which was purified by flash chromatography eluting with hexane–ethyl acetate (1:1) to provide yellow solid.

Yield: 6.0. g (83%); mp: 74–77 °C (74–75 °C lit.); IR (KBr, cm^{-1}): 3015 (Ar–CH), 1740 (C═O of ester); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.35 (t, 3H, CH_3), 2.65 (s, 3H, CH_3) 4.33 (q, 2H, CH_2)7.52–8.00 (m, 5H, Ar–H); MS (EI) m/z: 248 [M + 1].

2.2.2. Synthesis of 5-methyl-2-phenylthiazole-4-carbohydrazide (3) (Danil et al., 2008)

5-Methyl-2-phenylthiazole-4-carboxylic acid ethyl ester **2** (3.0 g, 12.0 mmol) was dissolved in 30 ml ethanol, to this hydrazine hydrate (1.81 g, 36.0 mmol) was added and refluxed for 12 h. The reaction mixture was cooled and solid appeared in the flask was filtered and recrystallized from ethanol to yield white crystalline product.

Yield: 2.4 g (85%); mp: 166–169 °C (168–169 °C lit.); IR (KBr, cm^{-1}): 3350, 3215 (NH_2), 3140 (NH), 1677 (C═O of amide); ^{1}H NMR (400 MHz, DMSO d_6) δ ppm: 2.73 (s, 3H, CH_3), 4.47 (bs, 2H, NH_2), 7.36–8.10 (m, 5H, Ar–H), 8.65 (bs, 1H, NH); MS (EI) m/z: 234 [M + 1].

2.2.3. Synthesis of 5-methyl-2-phenylthiazole-4-carboxamide (4)

5-Methyl-2-phenylthiazole-4-carboxylic acid ethyl ester **2** (1.0 g, 4.00 mmol) was dissolved in 10 ml of acetonitrile. To this, conc. ammonia (10 ml) was added drop wise and reaction mixture was heated on a water bath at 50–60 °C for 6 h. The reaction mixture was cooled and poured into ice water. The solid obtained was filtered, dried and recrystallized from ethanol.

Yield: 0.75 g (86%); mp:175–178 °C; IR (KBr, cm^{-1}): 3420, 3215 (NH_2), 3020 (Ar–CH), 1670 (C═O of amide); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.70 (s, 3H, CH_3), 4.92 (bs, 2H, NH_2), 7.41–8.14 (m, 5H, Ar–H); MS (EI) m/z: 219 [M + 1].

2.2.4. Synthesis of 5-methyl-2-phenylthiazole-4-carbonitrile (5)

To the solution of 5-methyl-2-phenylthiazole-4-carboxamide **4** (0.5 g, 2.2 mmol) in 10 ml dimethylformamide, oxalyl chloride (0.43 g, 3.44 mmol) was added at 0–5 °C and the reaction mixture stirred at 0-5 °C for 30 min. Then pyridine (0.54 g, 6.88 mmol) was added and stirred again for 10 min. The reaction mixture was poured on ice water and extracted with dichloromethane. The organic layer concentrated to get white crystals.

Yield: 0.34 g (76%); mp: 110–113 °C; IR (KBr, cm^{-1}): 3025 (Ar–CH), 2235 (CN); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.68 (s, 3H, CH_3), 7.46–8.12 (m, 5H, Ar–H); MS (EI) m/z: 201 [M + 1].

2.2.5. Synthesis of 5-methyl-2-phenyl-4-(1H-tetrazol-5-yl) thiazole (6)

5-Methyl-2-phenylthiazole-4-carbonitrile **5** (0.25 g, 1.25 mmol), trimethylsilyl azide (0.28 g, 2.50 mmol) and dibutyltin (20 mg) in 10 ml toluene were heated under reflux for 16 h. The excess of toluene was removed under reduced pressure and the residue was triturated with water. The solid product formed was filtered off and recrystallized from ethanol to yield white crystals.

Yield: 0.11 g (50%); mp: 190–193 °C; IR (KBr, cm^{-1}):3305 (NH), 3030 (Ar–CH), 1630 (C═N); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.72 (s, 3H, CH_3), 7.36–8.05(m, 5H, Ar–H); MS (EI) m/z: 244 [M + 1]; Anal. Cald. for $C_{11}H_9N_5S$: C, 54.31; H, 3.73; N, 28.79. Found: C, 54.30; H, 3.69; N, 28.74.

2.2.6. Synthesis of 2-(5-methyl-2-phenylthiazole-4-yl)-[1,3,4] oxadiazole (7)

A mixture of **3** (0.25 g, 1.0 mmol) and triethylorthoformate (5 ml) was refluxed for 10 h. The excess of the solvent was distilling off under reduced pressure and the solid product obtained was filtered and recrystallized from ethanol to yield white crystalline product.

Yield: 0.22 g (85%); mp: 210–213 °C; IR (KBr, cm^{-1}):3025 (Ar–CH), 1615 (C═N); ^1H NMR (400 MHz, DMSO d^6) δ ppm: 2.60 (s, 3H, CH_3), 7.34–7.97 (m, 5H, Ar–H),8.40 (s, 1H, oxadiazole-H); MS (EI) *m/z*: 244 [M + 1]. Anal. Cald. for $C_{12}H_9N_3OS$: C, 59.24; H, 3.73; N, 17.27. Found: C, 59.21; H, 3.72; N, 17.22.

*2.2.7. Synthesis of 5-(5-methyl-2-phenylthiazole-4-yl)-[1,3,4] oxadiazole-2-amine (**8**)*

Sodium bicarbonate (0.1 g, 1.2 mmol) in 5 ml of water was added to a solution of **3** (0.25 g, 1.0 mmol) in 10 ml of dioxane at room temperature. After 5 min, cyanogens bromide (0.136 g, 1.2 mmol) was added. After 4 h of room temperature stirring, the solid obtained was filtered and dried in vacuum. The solid was recrystallized from isopropyl alcohol to yield brown colored product.

Yield: 0.18 g (67%); mp: 222–225 °C; IR (KBr, cm^{-1}): 3385, 3130 (NH_2), 3020 (Ar–CH), 1620 (C═N); ^1H NMR (400 MHz, DMSO d_6) δ ppm: 2.67 (s, 3H, CH_3), 7.36 (bs, 2H, NH_2),7.52–7.97 (m, 5H, Ar–H); MS (EI) *m/z*: 259 [M + 1]. Anal. Cald. for $C_{12}H_{10}N_4OS$: C, 55.80; H, 3.90; N, 21.69. Found: C, 55.75; H, 3.91; N, 21.73.

*2.2.8. Synthesis of 5-(5-methyl-2-phenylthiazole-4-yl)-1,3,4-oxadiazole-2(3H)-one (**9**)*

A mixture of **3** (0.25 g, 1.0 mmol) and *N*,*N*′-carbonyldiimidazole (0.26 g, 1.61 mmol) in 10 ml dioxane was heated under reflux for 4 h. After cooling, the solvent was removed under reduced pressure and the residue was triturated with water. The solid product formed was filtered off and recrystallized from ethanol to yield colorless crystals.

Yield: 0.20 g (74%); mp: 141–144 °C; IR (KBr, cm^{-1}):3175 (NH), 3010 (Ar–CH), 1680 (C═O), 1620 (C═N);

^1H NMR (400 MHz, DMSO d_6) δ ppm: 2.71 (s, 3H, CH_3), 7.43–7.95 (m, 5H, Ar–H), 9.42 (s, 1H, NH); MS (EI) *m/z*: 260 [M + 1]. Anal. Cald. for $C_{12}H_9N_3O_2S$: C, 55.59; H, 3.50; N, 16.21. Found: C, 55.56; H, 3.51; N, 16.22.

*2.2.9. Synthesis of 5-(5-methyl-2-phenylthiazole-4-yl)-1,3,4-oxadiazole-2(3H)-thione (**10**)*

A mixture of **3** (0.5 g, 0.21 mmol) and carbon disulfide (3 ml) in 10 ml of pyridine was heated under reflux for 5 h with stirring. After cooling, the solvent was evaporated under reduced pressure and the residue was triturated with an ice-water mixture. The separated solid product was filtered, washed with water, dried and recrystallized from isopropyl alcohol to afford **10** as pale yellow crystals.

Yield: 0.48 g (81%); mp: 235–238 °C; IR (KBr, cm^{-1}): 3212 (NH), 3015 (Ar–CH), 1625 (C═N), 1310 (C═S); ^1H NMR (400 MHz, DMSO d_6) δ ppm: 2.67(s, 3H, CH_3), 7.53–8.00 (m, 5H, Ar–H),9.57 (s, 1H, NH); MS (EI) *m/z*: 276 [M + 1]. Anal. Cald. for: $C_{12}H_9N_3OS_2$: C, 52.35; H, 3.29; N, 15.26. Found: C, 52.33; H, 3.32; N, 15.22.

*2.2.10. Synthesis of 4-{2-[5-(5-methyl-2-phenylthiazole-4-yl)-1,3,4-oxadiazole-2-ylthio]-ethyl}-morpholine (**11**)*

A mixture of the oxadiazolinethione **10** (0.2 g, 0.72 mmol), fused sodium acetate (0.31 g, 3.8 mmol) and 4-(2-chloroethyl) morpholine hydrochloride (0.14 g, 0.72 mmol) in 10 ml of ethanol was heated under reflux for 10 h with stirring. The solvent was removed under reduced pressure and the residue was triturated with water. The solid product formed was filtered off and recrystallized from ethanol to yield white crystals.

Yield: 0.18 g (54%); mp: 134–137 °C; IR (KBr, cm^{-1}):3015 (Ar–CH), 1622(C═N);); ^1H NMR (400 MHz, DMSO d_6) δ ppm: 2.36–2.45 (m, 4H, NCH_2), 2.62 (s, 3H, CH_3), 2.78 (t, 2H, $SCH_2\underline{CH_2}N$), 3.17 (t, 2H, $S\underline{CH_2}CH_2N$), 3.43 (t, 4H, OCH_2), 7.39–8.10 (m, 5H, Ar–H); MS (EI) *m/z*: 389 [M + 1]. Anal. Cald. for: $C_{18}H_{20}N_4O_2S_2$: C, 55.65; H, 5.19; N, 14.42. Found: C, 55.64; H, 5.12; N, 14.46.

*2.2.11. Synthesis of dimethyl-{2-[5-(5-methyl-2-phenylthiazole-4-yl)-1,3,4-oxadiazole-2-ylthio]-ethyl}-amine (**12**)*

Prepared from compound **10** (0.2 g, 0.72 mmol), fused sodium acetate (0.31 g, 3.8 mmol) and 2-dimethylaminoethyl chloride hydrochloride (0.11 g, 0.72 mmol) by using the same procedure as mentioned in **11**.

Yield: 0.17 g (68%); mp: 131–134 °C; IR (KBr, cm^{-1}):3030 (Ar–CH),1628 (C═N); ^1H NMR (400 MHz, DMSO d_6) δ ppm: 7.49–8.13 (m, 5H, Ar–H), 3.28 (t, 2H, $S\underline{CH_2}CH_2N$), 2.82 (t, 2H, $SCH_2\underline{CH_2}N$), 2.65 (s, 3H, CH_3),2.20 (s, 6H, $N(CH_3)_2$); MS (EI) *m/z*: 347 [M + 1]. Anal. Cald. for: $C_{16}H_{18}N_4OS_2$: C, 55.47; H, 5.24; N, 16.17. Found: C, 55.44; H, 5.22; N, 16.12.

*2.2.12. Synthesis of 5-(5-methyl-2-phenylthiazole-4-yl)-3-(piperidin-1-yl-methyl)-1,3,4-oxadiazole-2(3H)-thione (**13**)*

To a solution of 1,3,4-oxadiazol-5-thione **10** (0.2 g, 0.72 mmol) in ethanol, a mixture of formaldehyde (0.2 g, 0.14 mmol) and a piperidine (0.065 g, 0.72 mmol) in ethanol was added with stirring. After complete addition, the stirring was continued overnight at room temperature. The separated white solid product was filtered, washed with water, dried and recrystallized from ethanol.

Yield: 0.15 g (60%); mp:251–254 °C; IR (KBr, cm^{-1}):3030 (Ar–CH), 1615 (C═N),1305 (C═S); ^1H NMR (400 MHz, DMSO d_6) δ ppm: 7.42–8.00 (m, 5H, Ar–H), 4.87 (s, 2H, N–CH_2–N), 3.32 (t, 4H, piperidine-CH_2), 2.20 (q, 2H, piperidine CH_2), 2.64 (s, 3H, CH_3),1.90 (q, 4H, piperidine-CH_2); MS (EI) *m/z*: 373 [M + 1]. Anal. Cald. for: $C_{18}H_{20}N_4OS_2$: C, 50.04; H, 5.41; N, 15.04. Found: C, 50.00; H, 5.38; N, 15.00.

*2.2.13. Synthesis of (5-amino-4-ethylester-1H-pyrazol-1-yl)(5-methyl-2-phenylthiazole-4-yl)-methanone (**14**)*

A mixture of **3** (0.25 g, 1.07 mmol) and ethyl(ethoxymethylene) cyanoacetate (0.18 g, 1.07 mmol) in 10 ml of ethanol was heated under reflux for 6 h. The reaction mixture was cooled and poured over crushed ice. The separated white solid was filtered, washed with water and recrystallized from ethanol.

Yield: 0.27 g (71%); mp: 182–185 °C; IR (KBr, cm^{-1}): 3350, 3185 (NH_2), 3030 (Ar–CH), 1720 (C═O), 1625 (C═N); ^1H NMR (400 MHz, DMSO d_6) δ ppm: 8.99 (s, 1H, pyrazole-H), 7.40 (bs, 2H, NH_2), 7.55–7.87 (m, 5H, Ar–H), 4.24 (q, 2H, CH_2), 2.68 (s, 3H, CH_3), 1.30 (t, 3H, CH_3); MS (EI) *m/z*: 357 [M + 1]. Anal. Cald. for: $C_{17}H_{16}N_4O_3S$: C, 57.29; H, 4.53; N, 15.72. Found: C, 57.27; H, 4.54; N, 15.70.

*2.2.14. Synthesis of (5-amino-3-phenyl-1H-pyrazol-1-yl)(5-methyl-2-phenylthiazol-4-yl)-methanone (**15**)*

Prepared from compound **3** (0.25 g, 1.07 mmol) and benzoyl acetonitrile (0.155 g, 1.07 mmol) by using the same procedure as mentioned in **14**.

Yield: 0.31 g (82%); mp: 210–213 °C; IR (KBr, cm^{-1}): 3378, 3212 (NH_2), 3020 (Ar–CH), 1695 (C═O), 1610 (C═N); ^1H NMR (400 MHz, DMSO d_6) δ ppm: 8.10 (s, 1H, pyrazole-H), 7.82–7.87 (m, 5H, Ar–H), 7.40–7.55 (m, 5H, Ar–H), 7.24 (bs, 2H, NH_2), 2.62 (s, 3H, CH_3); MS (EI) *m/z*: 361 [M + 1]; Anal. Cald. for $C_{20}H_{16}N_4OS$: C, 66.65; H, 4.47; N, 15.54. Found: C, 66.62; H, 4.41; N, 15.51.

3. Pharmacological evaluation

Synthesized compounds (**6–15**) were investigated for anti-inflammatory and analgesic activities and most active representatives (**9, 10, 14** and **15**) of the series were investigated for acute ulcerogenicity. Diclofenac sodium was used as a reference standard at a dose 25 mg/kg for anti-inflammatory analgesic and ulcerogenicity studies. The experiments were performed on Albino rats of Wistar strain of either sex, weighing 180–200 g for anti-inflammatory activity and 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.

3.1. 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 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 the percent of edema inhibition was obtained as follows:

$$\text{Percent inhibition} = (\text{Vt} - \text{Vc})\text{control} - (\text{Vt} - \text{Vc})\text{tested compound}/(\text{Vt} - \text{Vc})\text{control} \times 100$$

where Vt = volume of edema at specific time interval and Vc = volume of edema at zero time interval.

The results are presented in Table 1.

3.2. 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 % analgesic activity was expressed according to the formula:

$$\%\ \text{inhibition} = [n - n'/n \times 100]$$

where; n is the number of writhes in control group of mice and n' is the number of writhes in test and standard group of mice. The observations are tabulated as Table 2.

3.3. Evaluation of ulcerogenicity index

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 only 1% carboxymethylcellulose solution in water. One group was administered with diclofenac sodium at a dose of 25 mg/kg once daily for four days. The remaining group of animals was administered with test compounds at a dose of 25 mg/kg. On the fifth day, pylorus was ligated as per the method of (Shay et al., 1945). Animals were fasted for 24 h before

Table 1 Results of anti-inflammatory activity of title compounds (**6–15**) against carrageenan induced rat paw edema model in rats.

Compound	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	0.38 ± 0.03*	0.48 ± 0.04*	0.57 ± 0.09*	0.66 ± 0.04*	–	–	–
Diclofenac	0.27 ± 0.10*	0.33 ± 0.01 *	0.36 ± 0.10*	0.39 ± 0.08*	40	52.63	57.14
6	0.29 ± 0.04*	0.35 ± 0.04*	0.41 ± 0.10*	0.46 ± 0.01*	30	36.84	39.28
7	0.28 ± 0.06*	0.36 ± 0.07*	0.41 ± 0.08*	0.47 ± 0.11*	20	31.57	32.14
8	0.26 ± 0.06	0.34 ± 0.10*	0.40 ± 0.07*	0.45 ± 0.06*	20	26.31‘	32.14
9	0.31 ± 0.02*	0.38 ± 0.03*	0.42 ± 0.03*	0.46 ± 0.05*	30	42.10	53.57
10	0.33 ± 0.06*	0.38 ± 0.09*	0.42 ± 0.08*	0.44 ± 0.09*	50	52.63	60.71
11	0.35 ± 0.03*	0.42 ± 0.08*	0.47 ± 0.06*	0.52 ± 0.09*	30	36.84	35.71
12	0.37 ± 0.02*	0.44 ± 0.07*	0.50 ± 0.07*	0.55 ± 0.05*	30	31.57	35.71
13	0.30 ± 0.07*	0.37 ± 0.08*	0.41 ± 0.09*	0.46 ± 0.07*	30	42.10	42.85
14	0.34 ± 0.07*	0.40 ± 0.05*	0.42 ± 0.02*	0.44 ± 0.07*	40	57.89	64.28
15	0.39 ± 0.09*	0.45 ± 0.06*	0.49 ± 0.05*	0.53 ± 0.03*	40	47.36	50

Data analyzed by one-way ANOVA followed by Dunnett's test, (n = 6).

* $P < 0.05$ significant from control. Dose levels: test compounds and diclofenac sodium (25 mg/kg, b.w. p.o.).

Table 2 Analgesic activity of title compounds (**6–15**) against acetic acid induced writhing tests in mice.

Compound	No. of writhes in 15 min after treatment (mean ± SEM)	% Inhibition
Control	32.50 ± 0.94*	–
Standard	13.66 ± 0.50*	57.96
6	20.75 ± 0.62*	36.15
7	24.25 ± 0.45*	25.38
8	25.50 ± 0.61*	21.53
9	22.40 ± 0.95*	56.30
10	12.85 ± 0.94*	60.46
11	22.40 ± 0.72*	31.07
12	22.25 ± 0.91*	31.53
13	17.33 ± 0.78*	46.67
14	12.12 ± 1.00*	62.70
15	12.75 ± 0.73*	60.76

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).
* $P < 0.05$ significant from control.

Table 3 Evaluation of ulcerogenicity index.

Compound	Dose mg/kg/day (p.o.)	Time (days)	Ulcer index
Control	CMC 1%w/v	4	–
Diclofenac	25	4	3.32 ± 0.74*
9	25	4	1.11 ± 0.79*
10	25	4	0.77 ± 0.51*
14	25	4	0.79 ± 0.53*
15	25	4	0.86 ± 0.40*

Data analyzed by one-way ANOVA followed by Dunnett's test, ($n = 6$),
* $P < 0.05$ significant from control.

the pylorus ligation procedure. Four hours after the ligation, animals were sacrificed. The stomach was removed and opened along with the greater curvature. Ulcer index was determined by the method of (Ganguly and Bhatnagar, 1973) and is recorded in (Table 3).

3.4. 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 $P < 0.05$ 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).

4. Result and discussion

4.1. Chemistry

Synthesis of title compounds (**6–15**) is outlined in (Schemes 1 and 2). The parent compound 5-methyl-2-phenylthiazole-4-carboxylic acid ethyl ester (**2**) was synthesized by the reaction of thiobenzamide (**1**) with ethyl-2-chloroacetoacetate in ethanol. The compound (**2**) on reflux with conc. ammonia solution in the acetonitrile led to the formation of 5-methyl-2-phenyl-thiazole-4-carboxamide (4), which on further treatment with oxalyl chloride in DMF afforded 5-methyl-2-phenyloxazole-4-carbonitrile (**5**). The compound (**5**) possessess cyano group and can be conveniently converted into tetrazole derivative (**6**) by treatment with trimethylsilylazide in the presence of dibutyltin at reflux temperature.

The compound (**2**) on treatment with hydrazine hydrate afforded 5-methyl-2-phenylthiazole-4-carboxhydrazide (**3**). The acid hydrazide compound (**3**) was shown to be a key intermediate for the synthesis of several azoles. 1,3,4-Oxadiazole (**7**) was prepared by the reaction of acid hydrazide (**3**) with triethylorthoformate. The 2-amino-l,3,4-oxadiazoles (**8**) resulted from the action of cyanogens bromide and sodium bicarbonate on acid hydrazide (**3**).

The 1,3,4-oxadiazol-2-one (**9**) was obtained by treatment of the acid hydrazide (**3**) with 1,1′-carbonyldiimidazole (CDI) in refluxing dioxane. The acid hydrazide (**3**) was then subjected to cyclization with carbon disulfide in boiling pyridine to afford the corresponding 1,3,4-oxadiazol-2-thione (**10**). Alkylation of 1,3,4-oxadiazol-2-thione (**10**) with alkylating agents such as 4-(2-chloroethyl)morpholinyl hydrochloride or 2-dimethyaminoethyl chloride hydrochloride in boiling ethanol in the presence of fused sodium acetate gave independently required compounds which have been assigned the structures (**11**) and (**12**), respectively. The resultant 1,3,4-oxadiazol-2-thiones (**11**) were further converted into corresponding mannich bases (**13**) on aminomethylation with formaldehyde and piperidine.

Finally, acid hydrazide (**3**) on reaction with ethyl (ethoxymethylene) cyanoacetate in ethanol gave the corresponding 5-amino-4-ethylester pyrazole derivatives (**14**). The 5-amino-3-phenylpyrazole derivative (**15**) was isolated by the reaction of (**3**) with benzoylacetonitrile.

The structures of various synthesized compounds were assigned on the basis of spectral studies and it was reported in experimental protocols. The IR spectral characteristics of compounds (**6–15**) showed absorption bands ranging from 3130–3385 cm^{-1} for NH_2, 3212–3175 cm^{-1} for NH, 3015–3030 cm^{-1} for Ar–H, 1615–1630 cm^{-1} for C═N and 1305–1310 cm^{-1} for C═S. For 1,3,4-oxadiazole derivatives (**7–13**), the presence of C═N stretching band at 1615–1630 cm^{-1} is an evidence of ring closure. Oxadiazole (**9** and **10**) showed N–H stretching bands at 3175–3212 cm^{-1}. The absence of absorptions of the amide carbonyl in the region 1670 cm^{-1} and hydrazide in the region 3215–3350 cm^{-1} indicated the formation of azoles (**6–14**) from the corresponding acid amide (**4**) and acid hydrazide (**3**).

^1H NMR spectrum of compound (**2**) showed that triplet at δ 1.35 due to CH_3 protons of the ethyl group and quartet signals appeared at δ 4.33 due to the CH_2 protons of the ethyl group at position 4. The ^1H NMR spectra of compound (**2–15**) showed singlet within the region of δ 2.45–2.57 due to methyl thiazole proton and aromatic protons appeared as multiplets within the region of δ 7.34–8.14. For compound (**3**) a broad singlet peak at δ 4.47 for NH_2 and δ 8.65 for NH was observed. All other derivatives exhibited satisfactory chemical shift which confirmed the assigned structure of compounds.

4.2. Pharmacology

4.2.1. Anti-inflammatory activity

Anti-inflammatory activity was evaluated by the carrageenan-induced paw edema test in rats (Winter et al., 1962) at equimolar doses equivalent to 25 mg/kg (diclofenac sodium) body weight. The anti-inflammatory activity data (Table 1) indicated that all the test compounds protected rats from carrageenan-induced inflammation moderately at 1 h of reaction time with increased activity at 3 h. The entire compound in the series (**6–15**) exhibited the anti-inflammatory activity in the range of 20–50% after 1 h, 31–58% after 2 h and 32–64% after 3 h. The compounds (**10**, **14** and **15**) exhibited 50%, 40% and 40% anti-inflammatory activity, respectively, whereas standard diclofenac sodium showed 40% activity after 1 h of study. The same compounds showed 60.71%, 64.28% and 50% activity after 3 h, whereas standard diclofenac sodium exhibited 57.14% activity. It was observed that the thiazole derivatives **10** (60.71%) and **14** (64.28%) have shown the better activity than standard drug, diclofenac sodium (57.14%). Compounds **9** (53.57%) and **15** (50%) showed moderate activity. The structural correlation with anti-inflammatory activity showed that the 1,3,4-oxadiazole-2(3H)-one,1,3,4-oxadiazole-2(3H)-thione and pyrazole moiety are essential for activity. All the compounds (**9, 10, 14** and **15**) possessing these ring moieties exhibited activity in the range of 42.10–64.28% after 2 h and 3 h of studies.

4.2.2. Analgesic activity

Analgesic activity was evaluated using the acetic acid induced writhing method (Koster et al., 1959) at equimolar doses equivalent to 25 mg/kg (diclofenac sodium) body weight using wistar albino mice. The compounds possess significant analgesic activity in the range of 21.53–62.70% (Table 2). Among the derivatives, the compounds (**9, 10, 14** and **15**) showed analgesic activity around 56.30–62.70%, whereas standard diclofenac sodium, exhibited activity 57.96%. The compound **13** showed moderate activity (46.67%).

4.2.3. Gastric ulcerogenic studies

The compounds which showed significant anti-inflammatory and analgesic activities have been selected for acute ulcerogenicity studies. Ulcerogenic effects of substituted thiazole derivatives (**9, 10**, **14** and **15**) were evaluated in rat stress model at acute dose 25 mg/kg/day (Table 3). When compared to the reference standards diclofenac sodium (ulcer index 3.32 ± 0.74) the test compounds exhibited 23–33% of the ulcer index of the reference standards. The compounds (**10**, **14** and **15**) exhibited the lowest ulcer index of 0.77, 0.79 and 0.86 respectively among the tested compounds. Incorporation 1,3,4-oxadiazole-2(3H)-thione and pyrazole at position 4 resulted into most active and less ulcerogenic new chemical entities (NCEs) in the series.

5. Conclusion

Synthesized 5-methyl-2-phenylthiazole-4-substituted-heteroazole derivatives exhibited significant analgesic and anti-inflammatory activities. Compounds possessing 1,3,4-oxadiazole-2(3H)-one, 1,3,4-oxadiazole-2(3H)-thione (**9** and **10**) and pyrazole (**14** and **15**) at position 4 of thiazole exhibited most prominent and consistent anti-inflammatory activity than the standard drug. These compounds (**9, 10, 13, 14** and **15**) also showed significant analgesia in acetic acid induced writhing test. Interestingly tested compounds showed one-third of the ulcer index of the reference i.e. diclofenac. These series of compounds possess good potential and can be further developed into a potent lead.

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